



Vol. 6
2025

Romanian Journal of Horticulture





**ROMANIAN JOURNAL
OF HORTICULTURE
VOL. 6**



Academy of Agricultural and Forestry Sciences – “*Gheorghe Ionescu-Șișești*”
Horticulture Section



ROMANIAN JOURNAL OF HORTICULTURE

Vol. 6

Editura PIM
- 2025 -

Publisher**Academy of Agricultural and Forestry Sciences – “Gheorghe Ionescu-Șișești”****Horticulture Section**

Address: 61 Marasti, District 1, 011464, Bucharest, Romania

Phone: 021-318.44.72; 021-318.44.54-55/164; Email: horticultura@asas.ro

Webpage: www.romanianjournalofhorticulture.ro

Editorial Board**General Editor:**

Dorin Ioan SUMEDREA - National Research & Development Institute for Biotechnology in Horticulture Stefanesti-Arges - NRDIBH; member of the Academy of Agricultural and Forestry Sciences Gheorghe Ionescu-Șișești (AAFS)

Executive Editor:

Gheorghe GLĂMAN– President of Horticulture Section- AAFS

Secretary:

Elena Ioana CUCU – Scientific Secretary - Section of Horticulture – AAFS

Alina Constantina FLOREA – NRDIBH

Members:

Adrian ASĂNICĂ- University of Agronomic Sciences and Veterinary Medicine of Bucharest

Marian-Iancu BOGOESCU- Vice-President - AAFS

Valentina BOZHKOVA - Fruit Growing Institute, Plovdiv, Bulgaria

Ioan BURZO–member – AAFS

Maria CANTOR - University of Agronomic Sciences and Veterinary Medicine Cluj-Napoca

Mihail COMAN - Research Institute for Fruit Growing Pitesti

Valeriu V. COTEA- University of Agricultural Sciences and Veterinary Medicine Iași, member - AAFS

Sina COSMULESCU - University of Craiova - Faculty of Horticulture

Constantin DADU - Institute of Scientific and Practical Horticulture and Food Technologies Chisinau, Moldova

Liviu Coriolan DEJEU- member - AAFS

Boris GAINA–Academy of Sciences, Moldova

Károly HROTKÓ - Szent István University, Faculty of Horticultural Science Budapest, Hungary

Mihail IANCU - member - AAFS

Marian ION - Research and Development Institute for Viticulture and Oenology – Valea Calugareasca

Darko JEVREMOVIĆ - Fruit Research Institute, Čačak, Serbia

Victor LĂCĂTUȘ- member - AAFS

George MANGANARIS - Cyprus University of Technology, Limassol

Mihail MAREȘ – Vice – President of Romanian Society of Horticulturist

Ifigeneia MELLIDOU - Institute of Plant Breeding and Genetic Resources, Thessaloniki, Greece

Liu MENGJUN–The Hebei Agricultural University, China

Georgios MERKOUROPOULOS - Institute of Olive Tree, Subtropical Crops and Viticulture Athens, Greece

Viorel MITRE - University of Agronomic Sciences and Veterinary Medicine Cluj-Napoca

Silvana NICOLA – Torino University of, Department of Agricultural, Forest and Food, Italy

Michael OBERHUBER –Laimburg Research Centre for Agriculture and Forestry, Italy

Cristian PALTINEANU – Research and Development Institute for Soil Science, Agro-chemistry and Environmental Protection, Bucharest

Carmen Florentina POPESCU - National Research & Development Institute for Biotechnology in Horticulture Stefanesti-Arges

Laura RUSTIONI - University of Salento, Lecce, Italy

Ion SCURTU – Research and Development Institute for Vegetable and Flower Growing – Vidra

Vice-President - Horticulture Section – AAFS

Florin STĂNICA - University of Agronomic Sciences and Veterinary Medicine of Bucharest, Vice-President of Horticulture Section - AAFS

Adrian ȘERDINESCU- member - AAFS

Costel VÎNĂTORU - Research and Development Station for Vegetables, Buzau

Marian VINTILĂ- Institute for Processing and Marketing of Horticultural Products Bucharest

Valentin VLĂDUȚ – National Research – Development Institute for Machines and Installations Designed to Agriculture and Food Industry, Bucharest, Romania

Georgios TSOKTOURIDIS - Institute of Plant Breeding& Genetic Resources, Thessaloniki, Greece

Yüksel TÜZEL - Ege University, Agriculture Faculty, Department of Horticulture, Izmir, Turkey; President of the International Society for Horticultural Science (ISHS)

ISSN-L 2734 – 7656

Print ISSN 2734 – 7656

On line ISSN 2734 – 8083

CD-ROM ISSN 2734 – 7664

CONTENT

I. BIOTECHNOLOGY	7
1. INNOVATIVE GENOMIC AND BIOTECHNOLOGICAL TOOLS FOR THE MANAGEMENT AND ENHANCEMENT OF VEGETABLE GENETIC RESOURCES	9
<i>T. Cardi and S. Esposito</i>	
2. MOLECULAR CHARACTERIZATION AND RELATIONSHIPS AMONG SOME FIG (<i>FICUS CARICA</i> L.) GENOTYPES FROM THE COLLECTION OF THE FACULTY OF HORTICULTURE, BUCHAREST, USING SIMPLE SEQUENCE REPEAT (SSR) MARKERS	33
<i>E. Moisescu, S. Teoman Duran, Y. Insel, B. Oler, A. Peticilă and F. Stănică</i>	
3. EFFECT OF ALTERNATIVE CARBOHYDRATES ON MICROPROPAGATION IN PLANT SPECIES WITH SPECIAL REFERENCE TO <i>VANILLA PLANIFOLIA</i> JACKS	41
<i>C. Córdova-Nieto, L.G. Iglesias-Andreu, J.C. Noa-Carrazana, A. Alonso-López, G. Hernández -Zárate, J.C. López-Collado and C. Garcias-Morales</i>	
4. RESULTS OF HIGHLIGHTING GENES ASSOCIATED WITH ETHYLENE PRODUCTION IN APPLE GENOTYPES CULTIVATED IN ROMANIA	49
<i>A.F. Iancu, M. Militaru and S. Cosmulescu</i>	
5. GRAPEVINE VIRUSES – A GLOBAL CHALLENGE AND BIOTECHNOLOGICAL SOLUTIONS TO OBTAIN HEALTHY MATERIAL	57
<i>E. C.Buciumeanu, I. C. Guță* and D.I. Sumedrea</i>	
6. THE EFFECT OF A MODIFIED DKW NUTRIENT MEDIUM AND ANTIOXIDANT-ADSORBENT SYNERGY ON THE <i>IN VITRO</i> REGENERATION OF <i>LIRIODENDRON TULIPIFERA</i> L.	75
<i>R.R. Khaydaraliev-and Kh.Kh. Kushiev</i>	
7. BIOTECHNOLOGICAL POTENTIAL OF AQUATIC PLANT ECDYSTEROIDS	83
<i>A. J. Jumaniyozova and N.A. Khojamshukurov</i>	
II. VEGETABLE, AROMATIC AND MEDICINAL PLANTS	91
8. GROWTH AND YIELD PERFORMANCE OF CUCUMBER (<i>CUCUMIS SATIVUS</i>) UNDER DIFFERENT INTERCROPPING PATTERNS WITH WATER SPINACH (<i>IPOMOEA AQUATICA</i>)	93
<i>R. Agustina, A.F. Indrazatmiko, S. Budi and E.S. Redjeki</i>	
9. REVIEW ON ETHNOBOTANICAL KNOWLEDGE AND SOCIO-ECONOMIC VALUE OF ANCHOTE (<i>COCCINIA ABYSSINICA</i>) CULTIVATION AND PROCESSING IN MAJOR GROWING AREAS OF ETHIOPIA	101
<i>B.T. Abebe and A.F. Dejenu</i>	
10. CONTROL OF DAMAGE AGENTS ON CABBAGE AND CAULIFLOWER CROPS UNDER HIGH PLASTIC TUNNELS	115
<i>A.E. Cenușă, S.Ș. Hoge, G. Șovărel and M. Costache</i>	
III. POTATO GROWING	121
11. POTATO (<i>SOLANUM TUBEROSUM</i> L.) GROWTH AND YIELD RESPONSE TO INFLORESCENCE REMOVAL AND SEED TUBER SIZE IN GIRAR JARSO DISTRICT, CENTRAL ETHIOPIA	125
<i>B.Tamiru Abebe and G.Megersa Geleta</i>	
12. TOTAL PHENOLIC CONTENT IN TEN POTATO CULTIVARS WITH DIFFERENT RESISTANCE TO POTATO VIRUS S(PVS)	133
<i>C.L. Bădărău, M. Cioloca, N. Bărăscu, A.I. Moja, and R.A. Munteanu Ichim</i>	

IV. FRUIT GROWING	141
13. PHENOLOGICAL DYNAMICS OF SOME FRUIT SPECIES FROM THE NORTHEASTERN AREA OF ROMANIA IN THE CONTEXT OF CLIMATE CHANGE	143
<i>I. Mineață, I. Perju, S. Sîrbu, I.E. Golache, I.V. Ungureanu and E. Iurea</i>	
14. A NEW ROMANIAN <i>PRUNUS DOMESTICA</i> L. CULTIVAR EXHIBITING HIGH TOLERANCE TO NATURAL INFECTIONS TO PLUM POX VIRUS	149
<i>L.A. Zagrai and I. Zagrai</i>	
15. THE INFLUENCE OF SOIL MANAGEMENT PRACTICES ON BIOCHEMICAL CHARACTERISTICS OF SOIL IN PLUM ORCHARD	159
<i>M. Butac, I. Grafu, M. Militaru, E. Măreș, G. Togan, and L. Popa</i>	
16. TRANSNATIONAL EVOLUTION OF <i>MYRISTICA FRAGRANS</i> FROM THE BANDA ISLANDS TO GLOBAL SPICE MARKETS	167
<i>B. Damoah</i>	
V. VITICULTURE AND OENOLOGY	181
17. CONFIRMED VOCATIONS TOWARDS HIGH QUALITY OENOLOGICAL ACHIEVED STANDARDS ASCERTAINED IN REGARD TO SOME VINE KINDS CREATED AT THE DRAGASANI VINEYARD	183
<i>A. Popa and F. Tutulescu</i>	
18. AGROBIOLOGICAL EVALUATION OF SEEDLESS GRAPEVINE CULTIVARS FOR IMPROVING THE GRAPEVINE ASSORTMENT	191
<i>S. Tofan and L. Yuşhan</i>	
19. IMPACTS OF CLIMATE CHANGE ON SELECTED AGROBIOLOGICAL AND TECHNOLOGICAL TRAITS OF THE 'FETEASCĂ NEAGRĂ' CULTIVAR: A COMPARATIVE STUDY OF THE MURFATLAR VINEYARD (ROMANIA) AND THE CODRU WINE REGION (REPUBLIC OF MOLDOVA)	199
<i>A. Ranca, E. Alexandrov, A. Tănase, I. Dina, V. Artem, M. Kwon and T.S. Cosma</i>	
20. CLIMATE CHANGE IMPACT ON GRAPE RIPENING IN MURFATLAR VINEYARD	207
<i>I.C. Ciobanu, V. Artem and I. Dina</i>	
21. INFLUENCE OF IRRIGATION ON THE GROWTH AND QUALITY OF GRAPEVINE PROPAGATION MATERIAL	215
<i>D.G. Dinu, A.C. Florea, D.I. Sumedrea and A.M. Dumitru</i>	
22. OIL CONTENT IN GRAPEVINE SEEDS OF INTRA- AND INTERSPECIFIC PROVENANCE GENOTYPES	223
<i>E. Alexandrov</i>	
VI. FLORICULTURE, ORNAMENTAL PLANTS, LANDSCAPE ARCHITECTURE	251
23. STUDIES ON THE PROPAGATION AND BEHAVIOR OF SOME BIOTYPES OF <i>MIRABILIS JALAPA</i> L.	231
<i>E. Hărșan, Hărșan E., I. Moldovan, L. Draghia and M. Cantor</i>	
24. REDUCING OF WATER CONSUMPTION OF SEASHORE PASPALUM USING A HYDROGEL COMPOSITE	241
<i>M. A. E. Elashwah and W. F. Abobatta</i>	
VII. POSTHARVEST	249
25. CHITOSAN-BASED MELATONIN COATING EFFECTS ON APRICOT POSTHARVEST LIFE	251
<i>E. Bal</i>	
26. THE DEVELOPMENT AND INDUSTRIAL IMPLEMENTATION OF TECHNOLOGY FOR PRODUCING BIOLOGICALLY ACTIVE SUPPLEMENTS	259
<i>L. Yushan</i>	
27. SUPERCRITICAL CO₂ EXTRACTION: METHODOLOGICAL APPROACHES AND PRACTICAL APPLICATIONS FOR VAPORIZING AGRO-INDUSTRIAL SECONDARY PRODUCTS	267
<i>L. Yushan, T. Andrusenko, N. Taran and E. Zyryanova</i>	



Biotechnology

Innovative genomic and biotechnological tools for the management and enhancement of vegetable genetic resources

T. Cardi* and S. Esposito

CNR-IBBR, Institute of Biosciences and BioResources, via Università 133, 80055 Portici, Italy

*Corresponding author email: teodoro.cardi@cnr.it

ABSTRACT

Vegetables represent a diverse and significant category of specialty crops, which show high nutritional and economic value compared to the limited occupation of arable land. Changes in lifestyles and dietary habits are stimulating the development of horticultural products with high service content, while climate change is posing new challenges to food production, causing new abiotic and biotic stresses, with negative repercussions on productivity and product quality. A wise and extensive use of vegetable genetic resources conserved worldwide in Gene Banks can help develop varieties adapted to new consumer needs and climate change. This review describes the latest developments in genomic and biotechnological tools that can help manage and improve vegetable genetic resources for sustainable use in breeding efforts. Furthermore, it discusses the use of such tools for collection management, pre-breeding and gene discovery, and breeding. Finally, it highlights the technological challenges and regulatory landscape, as well as prospects underlying a broader implementation of 'omics' sciences and biotechnology to increase the effectiveness and efficiency of vegetable genetic resources management and use.

Keywords: Genomics, 'Omics' sciences, Biotechnology, New Genomic Techniques, Gene Banks

INTRODUCTION

Vegetables comprise a rather large group of specialty crops, very diverse in terms of botanical features, geographical origin, reproductive characteristics, cultivation systems, product types, and uses (Rajasekharan, 2019). In general, they exhibit high nutritional and economic value, particularly when compared to limited cropland occupation (Ebert, 2020; van Zonneveld *et al.*, 2023). Fresh vegetables are generally perceived by consumers as healthy and environmentally sustainable foods. In 2022, they occupied only 1.9% of the total EU agricultural land, but their production accounted for around 7.6% of the total value of agricultural products (<https://ec.europa.eu/eurostat/en/web/products-eurostat-news/w/ddn-20240301-1>).

In industrialized countries, due to a greater focus on psycho-physical well-being linked to nutrition, food production must respond not only to traditional food requirements, but also to complementary needs such as health, wholesomeness, eco-sustainability of products, sensory experience, flexibility of use, and cultural connection between territory and consumer. Changes in lifestyles and eating habits are stimulating the development of horticultural products with a high service content, such as those ready-to-eat or ready-to-cook, with new pre- and post-harvest needs, especially related to the control of pathogens and chemical contaminants, and the maintenance of the organoleptic/nutritional quality and

healthiness characteristics of products during processing, transport and storage. Furthermore, climate change is posing new challenges to food production, causing new abiotic and biotic stresses, with negative repercussions on productivity and product quality. In a global scenario, current forecasts indicate the achievement of a demographic plateau, about 9.5 billion people, in the second half of this century (<https://population.un.org/wpp/Graphs/DemographicProfiles/Line/900>), and a concomitant qualitative and quantitative reduction in the availability of natural resources (soil and water) for agriculture (Yang *et al.*, 2024). Population growth, mainly located in developing and emerging countries, will be accompanied by an increase in urbanization and by social transformations that are already leading to significant changes in dietary styles in various areas of the world. This will not only lead to a growing demand for food in terms of calories, but also to the need for a qualitative improvement of nutrition that will have to deal, on the one hand, with the lack of micronutrients and vitamins (*hidden hunger*), on the other hand with the emergence also in these countries of non-communicable diseases that until now have been typical of richer countries.

The continuous and fast development of new varieties is essential to address these challenges, aiming for higher production of better quality with less use of resources (*more with less*). Wise and extensive use of vegetable genetic resources (VGR) conserved worldwide in Gene Banks can help develop varieties adapted to new consumers' needs and climate change. In this article, we will review the latest developments in genomic and biotechnological tools that can help manage and improve vegetable genetic resources for their sustainable use in breeding efforts.

Vegetable Genetic Resources, Domestication, and Breeding

Vegetable genetic resources (about 518,000 holdings) total about 7% of the 7.4 million accessions of PGRFA (Plant Genetic Resources for Food and Agriculture) globally maintained *ex situ* in Gene Banks. This figure approaches one million if also accessions of crops used partially as vegetables are considered (Ebert, 2020). VGR, including crop wild species (CWR), landraces and heirloom varieties, are adapted to specific environments, often characterized by stressful conditions, and/or present valuable quality characteristics, thus representing a rich reservoir of genes and traits for current and future threats to crop cultivation.

However, first during the domestication process and then during the intentional selection of elite genotypes, along with improvement in agronomic performance, the genetic diversity of various crops has progressively reduced, with a general loss of genes linked to traits different from yield (e.g. nutritional and organoleptic quality) or potentially useful to support the challenges derived from old and emergent abiotic and biotic stresses (Figure 1). After a long domestication process, initiated with the development of agriculture and progressively integrated with intuitive breeding of crops for food or other uses, prompted by the rediscovery of Mendel's laws at the beginning of the last century, various methods have been developed to breed, with improved efficiency, new varieties. In this process, novel breeding approaches integrated scientific and technological knowledge related to *in vitro* culture of tissues, molecular biology tools, and high-performance sequencing technologies. The latter two, together with advances in computing capacity and (bio)informatics tools, marked a significant change in approaches, because through their use it is now possible to look directly at the genotype, instead of inferring the genotype through the observation of the phenotype.

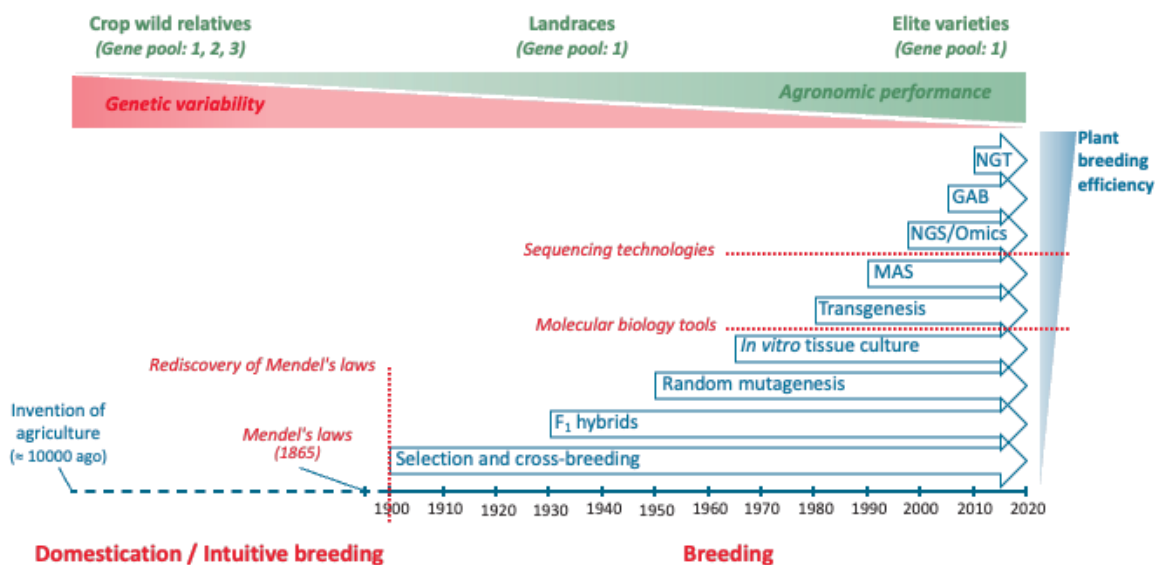


Figure 1. Domestication and breeding of crop plants. MAS, Marker Assisted Selection; NGS/Omics, Next Generation Sequencing/Omics sciences; GAB, Genomics Assisted Breeding; NGT, New Genomic Techniques (modified from Fricano *et al.*, 2016).

‘Omics’ and Biotechnological Tools

‘Omics’ technologies

The phenotype of crop plants depends on the interrelated expression of multiple genes present in their genomes. Such expression occurs at various levels with the production of different molecules, such as RNA transcripts, proteins, metabolites, and the external effect of the environment and crop management procedures, eventually affecting the expression of phenotypic traits.

In the last decades, various genome-scale technologies (‘omics’ technologies, from the Greek ‘whole’) have been developed to find out the relationships between the genotype and the phenotype and how they are influenced by the interaction with the natural and agronomic environment (Figure 2). Genomics, transcriptomics, proteomics, and metabolomics analyze the structure and function of DNA, RNA, proteins, and metabolites, respectively, while phenomics investigates the expression of traits (phenotypes) of interest. Further ‘omics’ technologies have been developed for the analysis of the epigenome, the ionome, the microbiome, and other data. All such technologies produce large datasets that can be analyzed and integrated in multi-omics approaches, using bioinformatics and artificial intelligence tools (Scossa *et al.*, 2021; Yang *et al.*, 2021; Zenda *et al.*, 2021; Zhang *et al.*, 2022; Chao *et al.*, 2023).

Genomics studies the structure and organization of genomes, looking at how genes are assembled and are similar/diverse among different organisms or species. Structural genomics aims to characterize sequence polymorphisms and chromosomal organization. Such information can be used to make physical and genetic maps for gene discovery, mapping, and breeding applications. On the other hand, through various approaches (reverse genetics, T-DNA tagging, targeted mutagenesis), functional genomics aims to uncover gene functions governing the expression of traits of interest (Yang *et al.*, 2021).

The development of high-throughput sequencing technologies in the 2000s, so-called second- and third-generation sequencing technologies (Table 1), reduced the cost of

sequencing a complete genome, thereby exponentially increasing the number of genomes sequenced in various crops, including vegetables (Scossa *et al.*, 2021; Sun *et al.*, 2022; Chao *et al.*, 2023).

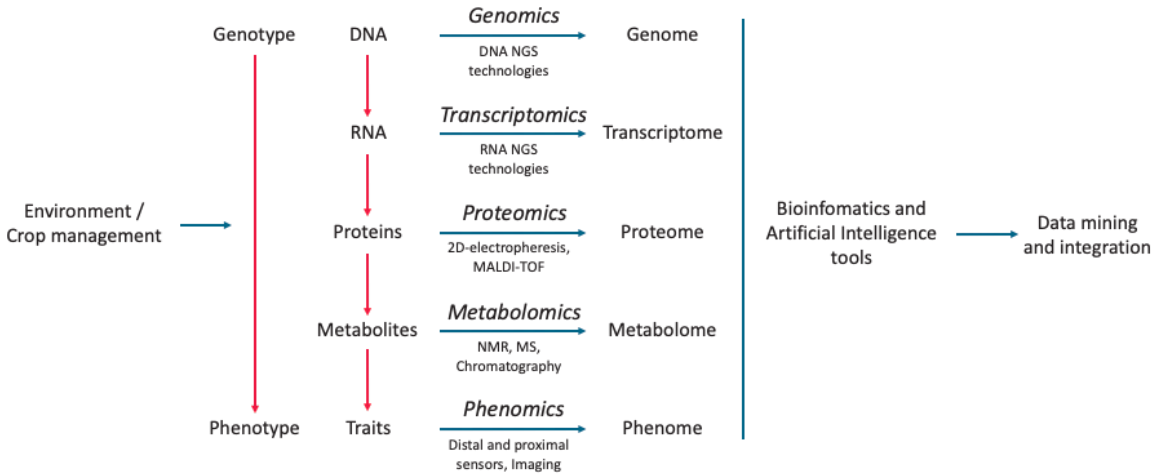


Figure 2. Omics technologies for the genome-scale analysis of different molecules and traits to find out genotype-to-phenotype relationships. Examples of some analytical techniques used are also indicated for each ‘omics’ science.

Table 1. DNA sequencing technologies that are used in plant genetics and breeding.

Technology	Generation	Cost	Read Length	Error Rate	Applications
Sanger Sequencing	I	\$3–\$10 per kilobase	500 – 1000 bp	<0.1%	Validation, targeted sequencing, mutation analysis
Illumina (NGS)	II	\$10–\$50 per gigabase	50 – 300 bp	0.1–1%	Whole-genome sequencing (WGS), RNA-Seq, metagenomics
PacBio (SMRT)	III	\$250–\$1000 per gigabase	10,000–50,000 bp	5–13% (0.1% with HiFi reads)	De novo assembly, epigenetics, structural variants
Oxford Nanopore	III	\$50–\$1000 per gigabase	10,000 – >1 Mb	~5–20%	Long-read assembly, portable sequencing, RNA sequencing

The availability of such information allows for physical genome maps in which genes and regulatory sequences can be positioned. Since each variation in nucleotide sequence can be used as a marker, single-nucleotide polymorphisms (SNPs) gave a strong impulse to mapping studies, using either linkage or association approaches, and subsequent association of genotypic and phenotypic variation (Zenda *et al.*, 2021). Third-generation sequencing approaches, such as PacBio and ONT technologies, recently ~~often~~ been coupled with other technologies (e.g. “Bionano Optical Genome Mapping”, “Hi-C High-throughput Chromosome Conformation Capture”), producing long stretches of sequenced DNA molecules (long-read sequencing) and have been recognized as the “Method of the Year” in 2022 (Garg *et al.*, 2024). They had important outcomes as concerns the development of telomere-to-telomere assemblies and haplotype phasing (i.e., the separation of homologous sequences in heterozygous or polyploid species based on parental origin) as well as the production of pangenomes (Tao *et al.*, 2019; Khan *et al.*, 2020; Scossa *et al.*, 2021; Pazhamala *et al.*, 2021; Yang *et al.*, 2021; Schreiber *et al.*, 2024; Garg *et al.*, 2024).

The assembly and analysis of pangenomes (pangenomics) is based on the comparison of *de novo* assembled genomes of different genotypes/species, allowing the discovery of new genomic variation besides that derived from SNPs and short InDels (insertions and deletions <50 base pairs). Indeed, in many crops, it was demonstrated that additional polymorphism depends on structural variants (SVs, > 50 bp), which include large-scale deletions, duplications, rearrangements, presence/absence variants (PAVs), and copy number variants (CNVs). Linked to such variation was the discovery that some genes are present in all related species (core genome), whereas some genes are present only in some species or genotypes (dispensable genome), suggesting their loss during evolution and breeding of elite varieties (Tao *et al.*, 2019; Khan *et al.*, 2020; Scossa *et al.*, 2021; Pazhamala *et al.*, 2021; Yang *et al.*, 2021; Zenda *et al.*, 2021; Schreiber *et al.*, 2024) (Figure 3). Dispensable genes are often related to resistance/tolerance to biotic or abiotic stresses, and so they are an important target for breeding. The possibility to compare pangenomes is also important for deeper phylogenetic and evolutionary relationships within cultivated gene pools and between crops and their wild relatives.

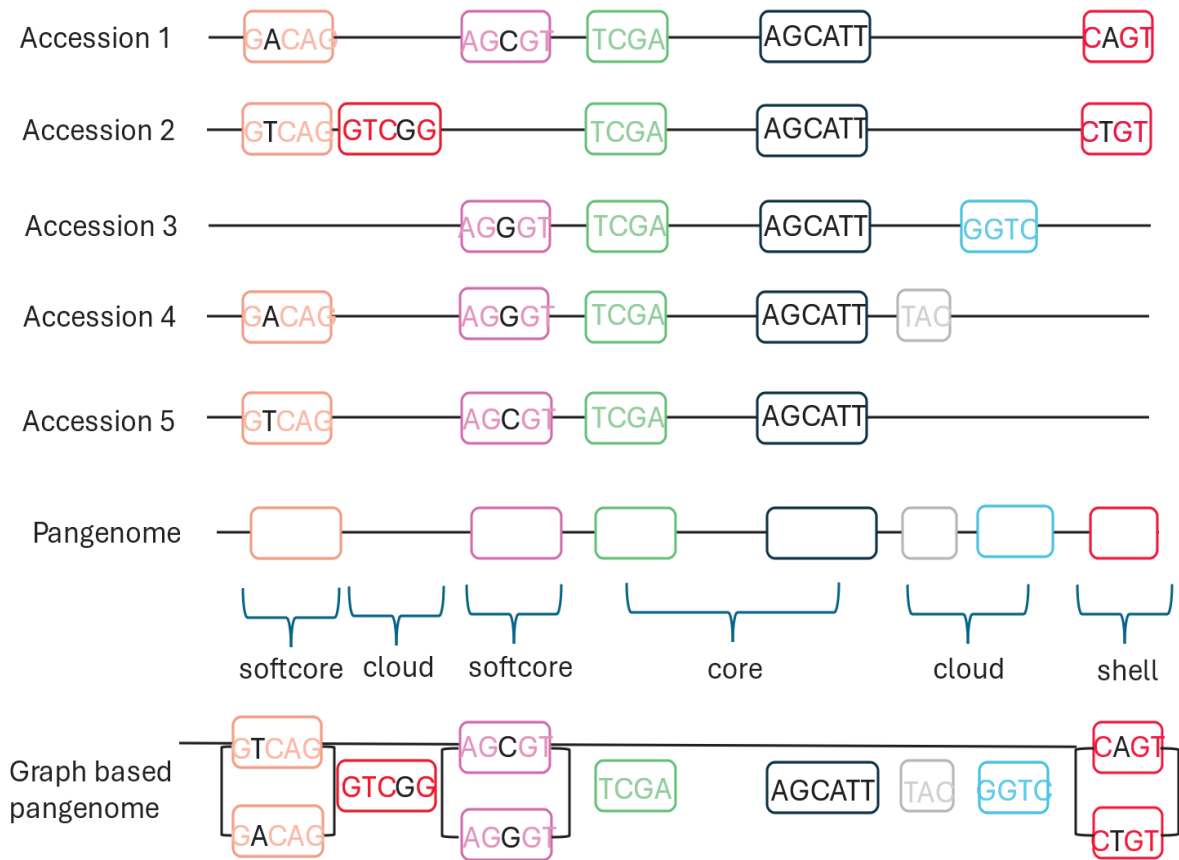


Figure 3. Schematic representation of a pangenome. The core includes genes that are present in all genomes analyzed, softcore in the large majority, shell in a few, and cloud in only one accession.

High-throughput sequencing technologies have also facilitated the analysis of the transcriptome, allowing studies on gene expression at the whole-genome level. Comparative transcriptomics using RNAs isolated in contrasting genotypes and/or differential growth/stress conditions, and consequent gene expression profiles, allowed us to dissect molecular mechanisms underlying specific biological phenomena and to identify, based on

their differential expression, candidate genes involved in the response to environmental conditions or developmental stages (Pazhamala *et al.*, 2021; Yang *et al.*, 2021; Zenda *et al.*, 2021). Transcriptomic analyses also evidenced the regulatory role of non-coding RNAs.

Proteomics and metabolomics aim to analyze the next layers of expression pathways in plants as well as other organisms, profiling the total protein (proteome) or metabolite (metabolome) content linked to specific developmental stages/tissues/environmental conditions. Using a range of techniques (mass spectrometry and derived methods among the most important), proteomic and metabolomic studies clarify the structure, function, and expression of proteins or metabolites, and their within-class (protein-protein, metabolite-metabolite) or between-class (protein-metabolite) interactions, during growth and in response to biotic and abiotic stresses, dissecting the molecular factors underlying biological networks. Besides elucidating the role of specific proteins and metabolites in metabolic pathways and their association with traits of interest in plants, the assessment of their quantitative and qualitative content is often positively or negatively linked to the nutritional value of food (Scossa *et al.*, 2021; Yang *et al.*, 2021; Zenda *et al.*, 2021).

Accurate plant phenotyping is essential for the proper characterization of natural genetic resources and induced mutants, the selection of the best genotypes in breeding populations, and the discovery of new genes in mapping populations. However, due to the amount of labour and/or expertise required, and the cost, plant phenotyping is often a limiting step, giving rise to the so-called “phenotyping bottleneck” (Tripodi *et al.*, 2018). This reflects a lag in technology implementation compared to genomics (sequencing) techniques applied to plant genetics and breeding. Recently, various phenotyping technologies, characterized by a diverse degree of resolution and processivity, have been developed (Li *et al.*, 2014; Visakh *et al.*, 2024). With either shared or differential issues between phenotyping in the field or a controlled environment, such technologies involve different sensors, platforms, and data processing software.

Based on the differential reaction of plant tissues and cellular components to electromagnetic radiation with different wavelengths (each component has wavelength-specific absorbance, reflectance, and transmittance properties), proximal or remote sensors carried by (automated) platforms allow the non-destructive high throughput phenotyping (HTP) of multiple samples per day highlighting differences in plant structure and morphology, response to abiotic and biotic stresses, and elemental and molecular composition. HTP is thus based on digital imaging carried out with visible light, thermal (IR thermography), spectral, fluorescence, and three-dimensional (3D) technologies. Various vegetation (performance) indices, including NDVI (normalized difference vegetation index) as one of the most used, can be calculated based on scoring with multiple wavelengths in multi/hyper spectra. Tomographic technologies provide 3D imaging and include NMR/MRI (Magnetic Resonance Imaging, which uses a combination of radio waves and magnetic fields), PET (Positron Emission Tomography, which uses γ -rays), X-rays CT (Computed Tomography, based on X-rays), which have a high resolution, but a limited level of processivity (low-medium throughput). More 3D technologies include LiDAR (Light Detection and Ranging, based on laser light), Time-of-flight (TOF) cameras, Terahertz spectroscopy, Microwave resonator, Ultrasonic sensors, and others (Li *et al.*, 2014; Tripodi *et al.*, 2018; Visakh *et al.*, 2024). These sensing technologies can be used either in the open field or in controlled environments, such as greenhouses or growth chambers. In the field, sensors can be stationary (e.g., mounted on phenopoles) or mobile, carried by either ground (carts, phenomobiles) or aerial vehicles (drones, small planes, satellites). The type of platform affects the resolution power and the extension of the surface covered. In growth chambers and greenhouses, either plant-to-sensor systems, which utilize conveyors to move the plants to the sensors, or sensor-to-plant systems, which move the sensors to the plants, can be used (Coppens *et al.*, 2017; Araus *et al.*, 2022). Whatever the platform used, HTP

produces a large amount of data, which must be managed and stored properly for further integration (Coppens *et al.*, 2017; Tripodi *et al.*, 2018; Araus *et al.*, 2022; Visakh *et al.*, 2024). While each omics discipline individually provides valuable data, the integration of multi-omics is particularly intriguing given the close relationship between each omics layer. The integration of omics data, derived from genomics, transcriptomics, proteomics, metabolomics, and phenomics, enhances a comprehensive understanding of complex biological systems, enabling researchers to uncover the intricate interactions between genes, proteins, metabolites, and phenotypic traits (Yadav *et al.*, 2022). Using different multi-omics techniques, essential key factors involved in senescence, stress response, and harvest index of many economically important crops have been identified (McLoughlin *et al.*, 2018; Peng *et al.*, 2020; Uchida *et al.*, 2020; Raza *et al.*, 2021; Ma *et al.*, 2021). Multi-omics could also support gene and protein annotations by integrating information from multiple layers, supporting the identification of biomarkers and key players involved in stress tolerance or susceptibility (Choudhary *et al.*, 2025).

However, the integration of these datasets remains challenging due to variations in preprocessing steps, data scales, and biases (e.g., high variance). Different strategies have been proposed, including vertical, horizontal, and diagonal integrations. Vertical integration connects data across molecular layers, such as linking genomic variations to transcriptomic and proteomic changes, to reveal hierarchical regulatory networks. Horizontal integration focuses on combining datasets of the same 'omic' type, such as transcriptomic profiles from different populations or conditions, to identify shared or distinct patterns. Diagonal integration, on the other hand, combines multiple 'omic' layers across varying conditions or genotypes, providing a dynamic and multidimensional view of biological processes. Given the vast amount of raw data, data integration often involves advanced computational tools, including machine learning and artificial intelligence, to identify biomarkers, understand gene-environment interactions, and accelerate crop improvement strategies. By bridging the gaps between 'omic' layers and leveraging advanced computational tools, researchers will uncover new biological insights and design more effective strategies for crop improvement and stress resilience. Overcoming current challenges, such as data heterogeneity and batch effects, will be critical to fully realize the transformative power of multi-omics integration in both research and practical applications.

Biotechnologies

Since the appearance of the first transgenic plants, based on the random integration in the host genome of a gene isolated from an unrelated species/organism, several technologies have been developed to increase the precision of genetic modifications and/or avoid the insertion of genetic material from species outside the breeder's genepool (Cardi and Stewart, 2016). The increasing knowledge in the structure and function of genomes and genes in cultivated crops and related genetic resources prompted the development of New Genomic Techniques (NGT), which include cisgenesis and targeted mutagenesis.

Cisgenesis involves the isolation and transfer of genetic material derived from the same or a sexually compatible species. A cisgene includes its own regulatory sequences and is transferred in sense orientation. In addition, no marker genes must be present in the final cisgenic plant. Following approaches for genetic transformation, by *Agrobacterium* or other methods, the cisgene is usually integrated randomly into the recipient's genome. However, if it is transferred by adopting genome editing approaches, it can be precisely inserted in a pre-determined genomic region (targeted cisgenesis) (Cardi, 2016). Therefore, cisgenesis is very similar to backcrossing, but, in comparison, it is faster and does not show linkage drag, *viz.*, the co-transfer of unwanted sequences/genes linked to the gene of interest (Schneider *et al.*, 2023).

Genomic information, coupled with the development of precise genome editing methods, made targeted approaches for mutagenesis or gene transfer a reality (Cardi, 2016). Genome editing approaches include various techniques that allow editing, deleting, replacing, or inserting target genomic sequences in a pre-defined site. Although various modules can be used for targeting specific sequences, the CRISPR/Cas system, developed in 2013, is presently the most used due to its versatility and simplicity (Cardi *et al.*, 2023). Based on the use of different effectors, various kinds of modifications can be induced. If an endonuclease is linked to CRISPR, or other targeting modules such as ZFN or TALE, a double cut in the DNA molecule (double-strand break, DSB) is induced, determining a repair process that leads to random indels or precise modifications, from single nucleotide variations to large substitutions/integrations, depending on the absence or presence of a template molecule (Li *et al.*, 2024a). The use of modified nucleases (with a nickase activity) together with other effectors, such as deaminase or reverse transcriptase, also allows efficient and precise genetic modifications, including single-base substitutions or small indels, via base editing or prime editing (Li *et al.*, 2024a). Large DNA fragments can be mobilized by inducing insertions, deletions, inversions, or translocations (chromosome engineering) using methods relying either on the generation of multiple DSBs or the use of additional enzymes like reverse transcriptases, transposases, or recombinases (Capdeville *et al.*, 2023; Li *et al.*, 2024a). The induction of chromosomal mutations can affect positively or negatively the frequency of genetic recombination at meiosis, and so the possibility of recovering useful recombinants in segregating populations. To have a stable mutated progeny, editing reagents (targeting modules and effectors) must be delivered into plant cells, and the induced mutation must eventually be recovered in the germinal lines. To achieve that, conventional procedures (e.g., *Agrobacterium*-mediated transformation, virus-induced genome editing, biolistics, PEG-mediated delivery) or emerging technologies (e.g., *in planta de novo* induction of meristems, haploid induction, grafting, nanotechnologies) can be used (Prestwich *et al.*, 2023; Li *et al.*, 2024a).

Management and Enhancement of Vegetable Genetic Resources

Vegetable genetic resources are of fundamental importance to enable agriculture to address old and new challenges that, exacerbated by population growth and climate change in various areas of the world, require increased production of high-quality food, increased productivity to reduce land use for agriculture, improved sustainability of farming systems, and increased crop resilience. Many potentially high-value VGRs are preserved as *ex situ* collections in Gene Banks but are largely uncharacterized and underutilized. The availability of reference genomes and, more recently, of pangenomes in multiple species of various vegetable families (Hao *et al.*, 2020) (Table 2) has prompted the development of 'Omics' sciences and new biotechnologies (NGT), which can not only help preserve but also more efficiently utilize conserved genetic variability. Below, we report some examples of plant genetic resources valorization using the above-mentioned technologies for collection management, gene discovery and pre-breeding, and breeding.

Collections management

Accessions in Gene Banks are generally identified by passport data, including taxonomy and origin details. Such information, however, is often not sufficient to have a clear and comprehensive picture of the genetic variability stored. Conventional passport data can now be integrated with high-throughput sequence data that form the basis for molecular passports. Although first-generation molecular markers (RAPD) have been proposed for such a purpose in *Brassica oleracea* (Phippen *et al.*, 1997), sequence-derived marker

variation, now available for most vegetable crops, can be used with significantly higher efficiency and efficacy to analyze population structures, reconstruct ancestry and phylogeny, identify relationships among the accessions maintained, sort out redundancies, identify collection gaps, and prioritize holdings management. Furthermore, sequence data can help face additional challenges of Gene Banks collections, such as discovering duplicates and monitoring gene integrity (Van Hintum and Kntipffer, 1995; Mascher *et al.*, 2019; Bohra *et al.*, 2022; Wambugu *et al.*, 2024).

However, for operational and economic reasons, there is a need to reduce and prioritize the management and evaluation of accessions maintained, especially when their phenotypic value must be assessed in different environments and for several agronomic traits. For such a purpose, it is necessary to build up core collections (CC) that include a reduced number of accessions with minimum redundancy, representing adequately the genetic variability stored (D'Agostino and Tripodi, 2017). Several methods have been proposed for the establishment and evaluation of core collections (Odong *et al.*, 2013; Gu *et al.*, 2023), and they have been adopted in various vegetable crops, e.g. (Zewdie *et al.*, 2004; Taranto *et al.*, 2016; Miyatake *et al.*, 2019; Guijarro-Real *et al.*, 2020; Esposito *et al.*, 2020; Tripodi *et al.*, 2021). The availability of extensive molecular data, more recently including also novel variation evidenced by pangenome analysis, will allow us to better understand phylogenetic relationships among germplasm accessions and constitute CC more easily and efficiently.

To make customized collections that maximize the probability of displaying specific adaptive traits, molecular data can be integrated with eco-geographic data in an approach called FIGS (Focused Identification of Germplasm Strategy) (D'Agostino and Tripodi, 2017; Bohra *et al.*, 2022). So far, no examples of the use of FIGS have been reported for vegetables, and the incorporation of refinements has been advocated for mining some specific traits, e.g., insect resistance (Stenberg and Ortiz, 2021). Nevertheless, it has been used with remarkable success to identify abiotic and biotic stress-tolerant accessions in cereals and grain legumes (Khazaei *et al.*, 2013; D'Agostino and Tripodi, 2017; Bohra *et al.*, 2022). The efficiency and predictive ability of FIGS can also be improved by Machine Learning approaches (Bohra *et al.*, 2022).

Considering the relatively high cost of phenotyping, high-throughput molecular data can be leveraged to predict the phenotypic value of germplasm accessions (Mascher *et al.*, 2019, 2024; Varshney *et al.*, 2021). Indeed, genomic prediction approaches have been first assessed in large Gene Bank collections of sorghum, wheat, and soybean (Jarquin *et al.*, 2016; Crossa *et al.*, 2016; Yu *et al.*, 2016) and only recently applied in vegetables, e.g. carrots, where valuable germplasm accessions could be identified without the need for extensive field evaluations (Corak *et al.*, 2023).

Gene discovery and pre-breeding

Although germplasm accessions represent a rich reservoir of diversity for genetic improvement and varietal innovation, breeders tend not to use CWR and landraces to obtain the desired phenotypes, due to the difficulty of introgressing genes encoding novel traits in elite backgrounds and the time required to get rid of unwanted genes transferred along with the wished ones (linkage drag). Thus, gene discovery and pre-breeding activities are necessary to make new materials and genes/QTLs from exotic germplasm amenable to further breeding. Such activities have recently been boosted by the implementation of genomic tools and NGTs. The genomic resources available in some important vegetable crops, including molecular markers, mapping populations, genetic maps, genome sequences, mapped genes and QTLs, have been recently reviewed with an outlook to the future (Simko *et al.*, 2021).

Starting from the '80s of the last century, many kinds of DNA molecular markers have been developed in plants for various genetics/pre-breeding, and breeding purposes. Based on the techniques used, from hybridization to PCR to sequencing, the throughput and number of loci analyzed increased during the years (Tripodi, 2023). Indeed, DNA markers have been instrumental in making genetic maps with thousands of markers available, enabling linkage mapping of genes and QTL in various vegetable crops (Kulwal, 2018; Simko *et al.*, 2021). To this purpose, a range of experimental populations, usually derived by biparental crosses and including, among others, RIL (Recombinant Inbred Lines), BIL (Backcross Inbred Lines), NIL (Isogenic Lines), can be used (D'Agostino and Tripodi, 2017). A special kind of isogenic lines is represented by ILs (Introgression Lines), based on the introgression of exotic chromosomal segments in an elite genetic background (Bohra *et al.*, 2022). Such genomic libraries, for the production of which the time required can be reduced by Speed (pre)-Breeding approaches (Mascher *et al.*, 2019; Bohra *et al.*, 2022), allow pinpointing genomic regions involved in the control of agronomic traits, narrowing the search for relevant genes derived from CWRs (Szymański *et al.*, 2020). Among vegetables, they have been developed first in tomato, for which segments from various wild relatives have been introgressed in processing or fresh market backgrounds (Eshed *et al.*, 1992; Chetelat and Meglic, 2000; Monforte and Tanksley, 2000; Barrantes *et al.*, 2014; Chetelat *et al.*, 2019; Chen *et al.*, 2023), and then also in other species (Jeuken and Lindhout, 2004; Perpiñá *et al.*, 2016; Gramazio *et al.*, 2017; Pereira *et al.*, 2021; Gu *et al.*, 2025).

Due to limited genetic variation and mapping resolution produced by linkage mapping, alternative methods based on linkage disequilibrium and historical recombination in natural populations (Genome Wide Association Studies, GWAS) or on multiparental experimental populations (e.g., Multiparent Advanced Generation InterCross, MAGIC; Nested Association Mapping, NAM) have been developed; however, they also show some limitations to consider (D'Agostino and Tripodi, 2017; Kulwal, 2018; Hao *et al.*, 2020). Association mapping by GWAS, in which hundreds of genotypes must be genotyped and accurately phenotyped to find out correlations among genotypic and phenotypic data, has been largely employed in vegetables, identifying candidate genes and SNPs linked to the target traits, either causally involved or in linkage disequilibrium with the causal variant (Hao *et al.*, 2020; Simko *et al.*, 2021; Nandi *et al.*, 2024). The availability of a reference genome and the relatively low cost of resequencing have pushed the development of alternative methods, such as BSA-seq, QTL-seq and similar, that associate the phenotype and the SNP frequency in bulks of contrasting individuals, identifying functional genes and loci associated with quantitative traits in various vegetables (Hao *et al.*, 2020). Potentially useful variation among alleles of validated candidate genes can then be searched by screening germplasm collections (allele mining).

Additionally, untapped genetic variability present in exotic germplasm can be captured by pangenome analysis. In all vegetable families investigated so far, genes encoding agronomically relevant traits have been found associated, often determining gene expression changes, with structural variation among accessions and the dispensable portion of the genome (Table 2). For instance, among Solanaceae, a non-reference allele in tomato, with a substitution in the *TomLoxC* gene promoter, determining the increase of some volatile compounds responsible of fruit flavor, was found in the wild relative *S. pimpinellifolium* (Gao *et al.*, 2019), while a functional form of the gene *Sgal12g015720*, determining biomass and yield increase, was found in nine wild but not the cultivated species, where it is pseudogenized due to a 244 bp deletion (Li *et al.*, 2023). In pepper, deletions in or close to *pvr6*, *pun1*, *CCS*, and *FA* genes have been found associated with virus resistance, pungency, fruit colour, and shape, respectively (Ou *et al.*, 2018; Lee *et al.*, 2022). In potato, the *Y* gene, responsible for yellow flesh colour, has been found associated with a 5.8 Mb insertion, while the *IT1* tuber identity gene showed several small SVs in the

upstream regulatory region (Tang *et al.*, 2022). Many insertions or deletions (Indels) have been found in genes responsible for differential leaf and flower development at the basis of morphotype variability in *B. oleracea* and *B. rapa* (Golicz *et al.*, 2016; Cai *et al.*, 2021; Guo *et al.*, 2024; Li *et al.*, 2024b). In Cucurbitaceae, the presence of spines or warts on cucumber fruits, an important quality trait, has been found to vary according to SVs affecting the presence or expression of several genes, e.g., *CsTu* and *CsGSL3* (Li *et al.*, 2022). SVs associated with *TST2* and *LCYB* genes, correlated to fruit sugar content and flesh colour, as well as others linked to disease resistance and other agronomically relevant genes, have been identified by PAV-GWAS in watermelon (Zhang *et al.*, 2024b). Similarly, PAV-GWAS, carried out in *Lactuca spp.*, identified new QTL for *Bremia lactucae* resistance in regions absent in the reference lettuce genome (van Workum *et al.*, 2024).

Table 2. Examples of pangenomes constructed in vegetable species.

Crop species	Accessions (no.) ^a	Variable genome (%)	Phenotypes associated to SV (variable genome) ^b	Reference
Lettuce	474 (4 species)	50.0 ^c	Seed colour, anthocyanin content, disease resistance	(van Workum <i>et al.</i> , 2024)
Radish	11	83.7 ^c	Disease resistance and metabolic processes	(Zhang <i>et al.</i> , 2021)
<i>Brassica rapa</i>	3	13.0 ^c	Plant morphology and development	(Lin <i>et al.</i> , 2014)
	162	44.3 ^c	Leafy head domestication	(Cai <i>et al.</i> , 2021)
<i>Brassica oleracea</i>	10	18.7 ^c	Disease resistance, flowering time, glucosinolate metabolism, vitamin biosynthesis	(Golicz <i>et al.</i> , 2016)
	11	56.3 ^c	Environmental response, specific organogenesis	(Guo <i>et al.</i> , 2024)
	27	76.8 ^c	Domestication traits, developmental morphotypes	(Li <i>et al.</i> , 2024b)
	12	20.0 ^c	Plant and fruit development, flowering time, root growth	(Li <i>et al.</i> , 2022)
Watermelon	27 (7 species)	57.2 ^d	Fruit bitterness and sweetness, flesh colour, disease resistance	(Zhang <i>et al.</i> , 2024b)
Tomato	725 (4 species)	25.8 ^c	Defence response, fruit firmness and flavour	(Gao <i>et al.</i> , 2019)
	100 (4 species)	- ^e	Disease resistance, fruit quality and size, yield	(Alonge <i>et al.</i> , 2020)
	838 (2 species)	62.0 ^d	Flavonoid content, soluble solid content, flavour	(Zhou <i>et al.</i> , 2022)
	13 (11 species)	46.0 ^d	Plant architecture, yield, flavour, fruit metabolite content	(Li <i>et al.</i> , 2023)
Potato	6	21.2 ^c	Defence responses, response to hormones (auxin)	(Hoopes <i>et al.</i> , 2022)
	45 (22 species)	74.5 ^c	Disease resistance, tuber development, carotenoid content in tubers	(Tang <i>et al.</i> , 2022)
	296 (60 species)	82.6 ^c	Photosynthesis, flowering, tuberization, adaption to the environment, defence response	(Bozan <i>et al.</i> , 2023)
Pepper	383 (4 species)	44.3 ^c	Carotenoid content, capsaicin content	(Ou <i>et al.</i> , 2018)
	12	51.3 ^c	Potyvirus resistance, fruit color, pungency, fruit shape and orientation	(Lee <i>et al.</i> , 2022)
	3 (3 species)	- ^e	Flowering time, flavor, resistance to biotic and abiotic stresses. fruit size, shape and pungency	(Liu <i>et al.</i> , 2023)

^a Number of genotypes used to build graph-based and/or super pangenomes

^b SV, Structural variants

^c Based on gene loci (pan-loci)

^d Based on whole genome sequences

^e Not specified

Modern approaches for targeted mutagenesis are a powerful method to validate novel genes/alleles identified in VGR through genomic approaches and clarify the molecular bases of phenotypic variation. For instance, a candidate major gene for promoting tuberization in potato, pinpointed by whole-genome alignment of sequences from tuber-bearing and non-tuber-bearing *Solanum* species, has been knocked out using genome editing tools in a diploid clone, inhibiting the formation of tubers in edited plants. This permitted naming the new gene *IT1* (*Identity of Tuber 1*) (Tang *et al.*, 2022). Knockout tomato mutants for two candidate genes encoding a kinase (*SIDPK27* and *SIDPK26*), identified by GWAS and sequence analysis, recently confirmed the role of the two genes in the phosphorylation and consequent degradation of sucrose synthase in fruits of modern varieties, which show, in comparison with undomesticated-early domesticated genotypes, limited sugar accumulation (Zhang *et al.*, 2024a). Previously, tomato SVs were functionally linked to major domestication and breeding traits after integrating genome editing tools with information from pangenome analysis, *de novo* assemblies, and expression data. Indeed, genome editing of the *SIKLUH* gene produced isogenic genotypes with a variable number of functional gene copies, contributing to clarify the effect on fruit weight of *SIKLUH* dosage variation and, indirectly, the role on the same trait of a duplication of the corresponding genomic region (*fw3.2*), found only in modern accessions with large fruits (Alonge *et al.*, 2020).

Comparative genomic studies in crops and wild relatives have evidenced several major genes controlling traits involved in crop domestication. Such genes (domestication genes) can be introgressed in crop progenitors or in non-domesticated/orphan species to achieve redomestication or *de novo* domestication, respectively. Although either traditional breeding or genome editing can be theoretically used for such a purpose, the latter has been proven to have several advantages, and its feasibility has been demonstrated (Fernie and Yan, 2019). In tomato, genes controlling meristem size, plant architecture, insensitivity to day length, flower and fruit development, nutrient content, have been edited to redomesticate the wild progenitor *S. pimpinellifolium*. Efforts to *de novo* domesticate the orphan Solanaceae species *Physalis pruinosa* (groundcherry), distantly related to tomato, have been carried out by editing orthologs to genes of the cultivated species and with an effect on domestication and improvement traits, such as plant architecture, flower production, and fruit size (Fernie and Yan, 2019). Other possible attempts in vegetables have been discussed by Gasparini *et al.*, (Gasparini *et al.*, 2021). Although outcomes of redomestication/domestication efforts can be useful in trait mapping and introgression breeding, and resultant semi-domesticated genotypes combine domestication traits with resilience features that have been lost in elite modern varieties, it is expected that such genotypes require further improvement, aiming to improve quantitative traits controlled by multiple genes with minor effects (Mascher *et al.*, 2024).

Systems genomics approaches, based on the analysis and integration of data from multiple levels of organization, and requiring innovative computational and modelling tools for their analysis, have been advocated to discover novel genes responsible for agronomic traits, bridge the gap between genotype and phenotype, and promote advanced breeding strategies (Purugganan and Jackson, 2021; Zenda *et al.*, 2021).

Breeding

VGRs have a tremendous potential not only in gene discovery and pre-breeding efforts, but also for their direct or indirect use in breeding and agriculture. However, while landraces and heirloom varieties can be used either directly, after limited breeding attempts, or indirectly, as a source of sequence information/genes, CWR usually require more complex breeding efforts to transfer useful genetic information into elite cultivated backgrounds (Cardi, 2016). New scientific and technological improvements, based on the advancement of

genotyping and phenotyping platforms as well as speed breeding approaches, have driven the development of novel breeding strategies, progressively moving from phenotypic selection (PS) to marker-assisted selection (MAS) to genomics-assisted breeding (GAB).

After crosses involving exotic germplasm resources, MAS can be employed to accelerate breeding either by tracking major genes underlying simple qualitative traits with high heritability or by discarding unwanted exotic sequences to more quickly recover the cultivated genetic background. Over the years, however, molecular marker usage shifted from those in linkage disequilibrium with target traits, developed without much sequence information (e.g., RFLP, RAPD, AFLP), to those based on extensive information about sequence variation often causally linked to target phenotypes (e.g., SNP, CAPS, KASP). MAS approaches have been largely used in vegetable crops (Hao *et al.*, 2020; Simko *et al.*, 2021).

The recent proliferation of genome-wide information on the structure and function of genes for many agronomic traits and the concomitant development of various CRISPR/Cas-derived technologies have driven the implementation of genome editing approaches in applied plant breeding (Cardi *et al.*, 2023). Major genes involved in quality traits, abiotic/biotic stress tolerance/resistance, or other agronomically important traits have been precisely modified in various vegetables (Hao *et al.*, 2020) (Table 3). Trait modifications by editing approaches, however, are more easily achievable in the case of negative regulators, where relevant genes can be inactivated by knock-out approaches, than for positive regulators, which require more difficult knock-in approaches (Cardi *et al.*, 2023). Results of genome editing studies aiming to validate gene function can be easily transferred to breeding applications to produce improved genotypes, as recently exemplified in tomato mutants for genes controlling fruit sugar content (Zhang *et al.*, 2024a). The various challenges and opportunities of genome editing for crop improvement have been discussed in recent reviews (Gao, 2021; Puchta *et al.*, 2022; Wada *et al.*, 2022; Tang and Zhang, 2023; Cardi *et al.*, 2023; Li *et al.*, 2024a).

In case of complex quantitative traits with low heritability, derived by the interaction of multiple minor genes, often not identified individually, MAS or GE have limited power. Larger gains are expected by genomic selection methods, which capitalize on the low cost of genotyping compared to phenotyping. They, in fact, are based on extensive genotyping and phenotyping of a training set of genotypes, and subsequent prediction of the phenotypic value of a target population, which needs only to be genotyped but not phenotyped (Varshney *et al.*, 2021; Bohra *et al.*, 2022). Prediction accuracy can be improved by incorporating information on structural variation recently made available after pangenome analyses (Schreiber *et al.*, 2024; Mascher *et al.*, 2024). However, notwithstanding the increased availability of genomic resources in various vegetable crops, the implementation of GS has been limited (Simko *et al.*, 2021).

Pangenomic studies can assist breeding efforts in several ways (Tao *et al.*, 2019; Khan *et al.*, 2020; Schreiber *et al.*, 2024). They provide detailed information about the taxonomy and phylogeny of accessions maintained in Gene Banks, facilitating their choice for use in breeding schemes. In addition, variation in specific genomic regions and the presence of arrangements preventing or limiting their exploitation are evidenced. Finally, novel genes present in the dispensable portion of the genome or structural variants affecting gene expression, revealed in various vegetable families, are now available for breeding purposes. Among these, some aim to “rewild” crops by transferring genes that improve resilience in unfavorable environmental conditions (Palmgren *et al.*, 2015). However, the exploitation of desirable genetic sequences from CWR in breeding is not straightforward. Depending on the relatedness of parental genotypes, transfer into an elite background can be difficult due to pre- or post-fertilization crossing barriers. In addition, undesirable traits can be transferred together with wished ones because of pleiotropy or linkage drag. The removal of “hitchhiked” genes encoding negative traits, however, is often hampered by low

recombination between parental genomes (Prohens *et al.*, 2017). Some new findings and technologies, however, can help overcome such limitations in vegetables as well as in other crops.

Cisgenesis can be used to avoid linkage drag by isolating and transferring beneficial genes from CWR (Cardi, 2016; Schneider *et al.*, 2023). Cisgenic potato plants resistant to late blight were obtained by transferring two broad-spectrum resistance genes, *Rpi-sto1* and *Rpi-vnt1.1*, from the wild relatives *S. stoloniferum* and *S. venturii*, sexually compatible with the common potato (Jo *et al.*, 2014). Deleterious variants transferred in elite genotypes can be more easily removed by increasing recombination frequency by manipulating genes that suppress crossing-overs at meiosis (Bohra *et al.*, 2022; Epstein *et al.*, 2023). Indeed, an EMS-induced tomato mutant with a premature stop codon in the *SIRECQ4* gene recently showed a 2.7-fold increase in recombination compared to the wild type (Mieulet *et al.*, 2018). New genome editing techniques can also be used to inactivate the same or other similar genes.

Table 3. Recent examples (2022-2024) of applications of genome editing in vegetables.

Crop species	Accessions (no.) ^a	Variable genome (%)	Phenotypes associated to SV (variable genome) ^b	Reference
Lettuce	474 (4 species)	50.0 ^c	Seed colour, anthocyanin content, disease resistance	(van Workum <i>et al.</i> , 2024)
Radish	11	83.7 ^c	Disease resistance and metabolic processes	(Zhang <i>et al.</i> , 2021)
<i>Brassica rapa</i>	3	13.0 ^c	Plant morphology and development	(Lin <i>et al.</i> , 2014)
	162	44.3 ^c	Leafy head domestication	(Cai <i>et al.</i> , 2021)
<i>Brassica oleracea</i>	10	18.7 ^c	Disease resistance, flowering time, glucosinolate metabolism, vitamin biosynthesis	(Golicz <i>et al.</i> , 2016)
	11	56.3 ^c	Environmental response, specific organogenesis	(Guo <i>et al.</i> , 2024)
	27	76.8 ^c	Domestication traits, developmental morphotypes	(Li <i>et al.</i> , 2024b)
Cucumber	12	20.0 ^c	Plant and fruit development, flowering time, root growth	(Li <i>et al.</i> , 2022)
Watermelon	27 (7 species)	57.2 ^d	Fruit bitterness and sweetness, flesh colour, disease resistance	(Zhang <i>et al.</i> , 2024b)
Tomato	725 (4 species)	25.8 ^c	Defence response, fruit firmness and flavour	(Gao <i>et al.</i> , 2019)
	100 (4 species)	- ^e	Disease resistance, fruit quality and size, yield	(Alonge <i>et al.</i> , 2020)
	838 (2 species)	62.0 ^d	Flavonoid content, soluble solid content, flavour	(Zhou <i>et al.</i> , 2022)
	13 (11 species)	46.0 ^d	Plant architecture, yield, flavour, fruit metabolite content	(Li <i>et al.</i> , 2023)
Potato	6	21.2 ^c	Defence responses, response to hormones (auxin)	(Hoopes <i>et al.</i> , 2022)
	45 (22 species)	74.5 ^c	Disease resistance, tuber development, carotenoid content in tubers	(Tang <i>et al.</i> , 2022)
	296 (60 species)	82.6 ^c	Photosynthesis, flowering, tuberization, adaption to the environment, defence response	(Bozan <i>et al.</i> , 2023)
Pepper	383 (4 species)	44.3 ^c	Carotenoid content, capsaicin content	(Ou <i>et al.</i> , 2018)
	12	51.3 ^c	Potyvirus resistance, fruit color, pungency, fruit shape and orientation	(Lee <i>et al.</i> , 2022)
	3 (3 species)	- ^e	Flowering time, flavor, resistance to biotic and abiotic stresses. fruit size, shape and pungency	(Liu <i>et al.</i> , 2023)

^a Number of genotypes used to build graph-based and/or super pangenomes

^b SV, Structural variants

^c Based on gene loci (pan-loci)

^d Based on whole genome sequences

^e Not specified

Genome editing has been used to induce chromosomal rearrangements, such as translocations and inversions (Capdeville *et al.*, 2023), that can prevent recombination after interspecific crosses, as, for example, between the cultivated tomato and the wild relatives *S. chilense* and *S. pimpinellifolium* (Verlaan *et al.*, 2011; Palomba *et al.*, 2024). Finally, genome editing could be used to inactivate or delete unwanted genes associated with beneficial ones (Gao, 2021; Varshney *et al.*, 2021) or produce novel gene variants in resistance genes showing pleiotropic effects (Sha *et al.*, 2023).

Challenges and Perspectives

Although VGRs have a great potential for breeding programs, breeders tend to reduce their use as they move from gene pool 1 (landraces, traditional and elite varieties) to gene pool 3 (wild relatives of crops). Paradoxically, the latter are the ones most likely to contain useful genes to address old and new agricultural challenges. To facilitate and increase the use of such genetic resources in plant breeding, “introgressiomics” and similar approaches have been proposed, based on focused or unfocused strategies for CWR selection, hybridization with the target crop, development of pre-breeding introgressiomics populations, creation of dedicated repositories and databases, and moving the new materials into breeding pipelines (Prohens *et al.*, 2017). While hybridization of selected CWRs with the target crop can rely on established cellular biotechnological approaches to overcome pre- and postzygotic barriers, such as *in vitro* fertilization, embryo rescue, somatic hybridization, and others, recent advances in ‘omics’ and biotech technologies discussed in this review may provide substantial support for the implementation of other requirements.

Although the reduced sequencing costs and the development of more efficient bioinformatics tools have prompted the production and analysis of telomere-to-telomere and pan-genomes in relatives of many vegetable crops, the costs and the assembling methodological hurdles are still too high to largely extend such analyses to resources maintained in Gene Banks, especially for species with large genomes and/or polyploids. In addition, ‘omics’ disciplines other than genome-wide sequencing, particularly the digitalization of metabolic and plant phenotyping, lag. A further critical step is the integrated analysis of so many intrinsically different data, although new informatics and bioinformatics procedures based on artificial intelligence will give a push to future developments (Scossa *et al.*, 2021; Yan and Wang, 2023; Chao *et al.*, 2023; Farooq *et al.*, 2024).

New Genomic Techniques, in particular genome editing and associated techniques, can support functional studies and breeding efforts with genetic resources. Nevertheless, especially in breeding, the *in vitro* regeneration bottleneck limits the wide implementation by seed companies, which deal with different proprietary genetic backgrounds. In addition, tissue culture and *in vitro* regeneration can induce undesirable somaclonal variation. Therefore, the development of tissue culture-free *in planta* delivery and editing methods is of paramount importance (Islam and Kasfy, 2023; Hu and Liu, 2025).

Furthermore, new genetic materials must be validated under “real” agronomic conditions using multidisciplinary collaborations, especially when the effect of single gene modifications on improving complex traits needs to be confirmed (Khaipho-Burch *et al.*, 2023).

However, besides methodological and cost efficiency issues, several side challenges must also be overcome to make genomics and biotechnology of plant genetic resources an asset at different levels. One aspect concerns the role and functioning of Gene Banks (Cardi, 2018; Brink and van Hintum, 2020; Dempewolf *et al.*, 2023). Indeed, a huge number of genetic resources are maintained worldwide in a variety of collection types, characterized by variable size, legal mandate, sustainability over time, ability to adhere to international

standards for conservation and management of stored resources, and coordination with other (international) organizations. It can be expected that only major internationally recognized Gene Banks will be able not only to properly maintain and curate stored biological materials, but also to implement the new tools discussed in this review and manage the derived datasets produced internally and in collaboration with research centers, facilitating the flow of materials and data with Gene Bank curators and scientists from various disciplines. To have seamless data exchange, however, the FAIR (Findable, Accessible, Interoperable and Reusable) principles and derived standards must always be adopted for the management of genotypic and phenotypic data, as well as a universal system to uniquely identify and track physical genetic resources (Halewood *et al.*, 2018; Mascher *et al.*, 2019, 2024).

A second important aspect includes the regulatory issues related to a) exchange of genetic resources and Access and Benefit-Sharing (ABS), b) biosafety of new breeding technologies, and c) intellectual property and plant variety rights (Eriksson *et al.*, 2020). The exchange and use of genetic resources, including VGR stored in Gene Banks, are presently regulated by three international agreements: the Convention on Biological Diversity (CBD), which entered into force in 1993, the International Treaty on Plant Genetic Resources for Food and Agriculture (ITPGRFA), in 2004, and the Nagoya Protocol (NP), in 2014. Each has been ratified by hundreds of countries worldwide, but not all countries have ratified all agreements, and there are differences in interpretation, making practical implementation sometimes difficult (Brink and van Hintum, 2020; Marden *et al.*, 2023). The CBD applies to all genetic resources and encompasses the conservation and sustainable use of biological diversity as well as the sharing of benefits associated with their use. The derived Nagoya Protocol is also general in scope in terms of types of genetic resources and uses, but focuses on the benefit-sharing objective of the CBD, extending it to traditional knowledge related to genetic resources. Both the CBD and the NP are based on bilateral agreements with GR-supplying countries and provide, albeit with some differences, for prior informed consent (PIC) from competent authorities and mutually agreed terms (MAT) on the use of GR. The ITPGRFA (the “Treaty”) focuses on genetic resources for food and agriculture, with a subset of the objectives of the CBD. It is important to note that the Treaty provides for a multilateral system regulating access to GR for their conservation and use for agricultural research, training, and breeding, as well as simplified benefit-sharing procedures. Consequently, the exchange of resources is based on a Standard Material Transfer Agreement (SMTA), which refers to the species included in Annex 1. The extension of its use to all species for food and agricultural use, however, has been advocated to avoid the complexity of the bilateral system foreseen by the Nagoya Protocol (Brink and van Hintum, 2020). Only a few vegetable crops are present in Annex 1, while important ones, as, for instance, tomato, pepper, Cucurbitaceae, and many others, are missing (Marden *et al.*, 2023). Although all three mentioned agreements refer to physical genetic resources (although NP, as said, includes GR “traditional knowledge”), the rapid recent development of genomics, molecular biology and biotechnology has made the issue of Digital Sequence Information (DSI) pressing (Halewood *et al.*, 2018; Mascher *et al.*, 2019; Aubry *et al.*, 2022; Scholz *et al.*, 2022; Marden *et al.*, 2023; Sett *et al.*, 2024). Indeed, albeit it is not clear whether this term include all digitized information derived by genetic resources (not only DNA sequences but also “omics” data in general, including phenotypic ones), the availability of such information as well as that of scientific and technological knowledge has opened a heated debate on how DSI should be considered within the framework of the existing rules. Undoubtedly, detailed information about genotypic and phenotypic data, coupled with current DNA synthesis and editing technologies, could allow, at least in some cases, to breed new traits without moving and directly using any physical resource (seeds and plants exchanged for hybridization purposes). Regarding the benefit-sharing system, several options are being discussed to

balance the concerns of countries providing the original genetic resources with the needs for best practices in the management and improvement of genetic resources held in collections, which rely largely on open access to sequences and other datasets held in public repositories.

Several countries in the world have recently approved a new regulation for plants produced with New Genomic Techniques, with a general orientation to exclude them from the GMO legislation when they do not contain any transgenic sequence (Sprink and Wilhelm, 2024). The EU Commission has also proposed a Regulation that is under discussion. According to this proposal, NGT plants will be classified into two categories, NGT1 and NGT2, mainly depending on the extent of the modification and the possibility of obtaining the latter also with conventional breeding methods. NGT1 will be considered equivalent to conventionally bred plants in conventional agriculture, but as a GMO in organic agriculture (EU Commission, 2023). This is unfortunate considering the potential of New Genomic Techniques to address the challenges of organic agriculture, where few alternative methods can be used against biotic stresses.

New plant varieties can be generally protected according to the 1991 Act of the UPOV (International Union for the Protection of New Varieties of Plants) Convention. In the EU, Plant Variety Rights (PVR), also known as Plant Breeders' Rights (PBR), are issued by CPVO (Community Plant Variety Office) and cover products of conventional breeding as well as those of biotechnology, including NGT. The EU Biotechnology Directive 98/44/EC covers the legal protection of biotechnological inventions in Europe. However, it is still a matter of debate which system will be adopted to protect plants produced by NGT in Europe (Ricroch, 2024).

ACKNOWLEDGEMENTS

This study was carried out within the Agritech National Research Center and received funding from the European Union Next-GenerationEU (PIANO NAZIONALE DI RIPRESA E RESILIENZA (PNRR) – MISSIONE 4 COMPONENTE 2, INVESTIMENTO 1.4 – D.D. 1032 17/06/2022, CN00000022). The manuscript reflects only the authors' views and opinions, neither the European Union nor the European Commission can be considered responsible for them.

REFERENCES

1. Alonge, M., Wang, X., Benoit, M., Soyk, S., Pereira, L., Zhang, L., Suresh, H., Ramakrishnan, S., Maumus, F., Ciren, D., et al. (2020). Major Impacts of Widespread Structural Variation on Gene Expression and Crop Improvement in Tomato. *Cell* 182, 145–161. <https://doi.org/10.1016/j.cell.2020.05.021>.
2. Arais, J.L., Kefauver, S.C., Vergara-Díaz, O., Gracia-Romero, A., Rezzouk, F.Z., Segarra, J., Buchailot, M.L., Chang-Espino, M., Vatter, T., Sanchez-Bragado, R., et al. (2022). Crop phenotyping in a context of global change: What to measure and how to do it. *J Integr Plant Biol* 64, 592–618. <https://doi.org/10.1111/jipb.13191>.
3. Aubry, S., Frison, C., Medaglia, J.C., Frison, E., Jaspars, M., Rabone, M., Sirakaya, A., Saxena, D., and van Zimmeren, E. (2022). Bringing access and benefit sharing into the digital age. *Plants People Planet* 4, 5–12. <https://doi.org/10.1002/ppp3.10186>.
4. Bao, H., Ding, Y., Yang, F., Zhang, J., Xie, J., Zhao, C., Du, K., Zeng, Y., Zhao, K., Li, Z., et al. (2022). Gene silencing, knockout and over-expression of a transcription factor ABORTED MICROSPORES (SIAMS) strongly affects pollen viability in tomato (*Solanum lycopersicum*). *BMC Genomics* 23 (Suppl 1), 346. <https://doi.org/10.1186/s12864-022-08549-x>.
5. Barrantes, W., Fernández-del-Carmen, A., López-Casado, G., González-Sánchez, M.Á., Fernández-Muñoz, R., Granell, A., and Monforte, A.J. (2014). Highly efficient genomics-assisted development of a library of introgression lines of *Solanum pimpinellifolium*. *Molecular Breeding* 34, 1817–1831. <https://doi.org/10.1007/s11032-014-0141-0>.

6. Bohra, A., Kilian, B., Sivasankar, S., Caccamo, M., Mba, C., McCouch, S.R., and Varshney, R.K. (2022). Reap the crop wild relatives for breeding future crops. *Trends Biotechnol* 40, 412–431. <https://doi.org/10.1016/j.tibtech.2021.08.009>.
7. Bozan, I., Achakkagari, S.R., Anglin, N.L., Ellis, D., Tai, H.H., and Strömvik, M. V. (2023). Pangenome analyses reveal impact of transposable elements and ploidy on the evolution of potato species. *Proc Natl Acad Sci U S A* 120, e2211117120. <https://doi.org/10.1073/pnas.2211117120>.
8. Brink, M., and van Hintum, T. (2020). Genebank Operation in the Arena of Access and Benefit-Sharing Policies. *Front Plant Sci* 10, 1712. <https://doi.org/10.3389/fpls.2019.01712>.
9. Bvindi, C., Lee, S., Tang, L., Mickelbart, M. V., Li, Y., and Mengiste, T. (2022). Improved pathogen and stress tolerance in tomato mutants of SET domain histone 3 lysine methyltransferases. *New Phytologist* 235, 1957–1976. <https://doi.org/10.1111/nph.18277>.
10. Cai, X., Chang, L., Zhang, T., Chen, H., Zhang, L., Lin, R., Liang, J., Wu, J., Freeling, M., and Wang, X. (2021). Impacts of allopolyploidization and structural variation on intraspecific diversification in *Brassica rapa*. *Genome Biol* 22, 166. <https://doi.org/10.1186/s13059-021-02383-2>.
11. Capdeville, N., Schindele, P., and Puchta, H. (2023). Getting better all the time — recent progress in the development of CRISPR/Cas-based tools for plant genome engineering. *Curr Opin Biotechnol* 79, 102854. <https://doi.org/10.1016/j.copbio.2022.102854>.
12. Cardi, T. (2016). Cisgenesis and genome editing: Combining concepts and efforts for a smarter use of genetic resources in crop breeding. *Plant Breeding* 135, 139–147. <https://doi.org/10.1111/pbr.12345>.
13. Cardi, T. (2018). Opportunità per l'agricoltura dalle varietà locali e antiche. *Terra è Vita* 17, 54–57.
14. Cardi, T., and Stewart, N.C. (2016). Progress of targeted genome modification approaches in higher plants. *Plant Cell Rep* 35, 1401–1416. <https://doi.org/10.1007/s00299-016-1975-1>.
15. Cardi, T., Murovec, J., Bakhsh, A., Boniecka, J., Bruegmann, T., Bull, S.E., Eeckhaut, T., Fladung, M., Galovic, V., Linkiewicz, A., et al. (2023). CRISPR/Cas-mediated plant genome editing: outstanding challenges a decade after implementation. *Trends Plant Sci* 28, 1144–1165. <https://doi.org/10.1016/j.tplants.2023.05.012>.
16. Chao, H., Zhang, S., Hu, Y., Ni, Q., Xin, S., Zhao, L., Ivanisenko, V.A., Orlov, Y.L., and Chen, M. (2023). Integrating omics databases for enhanced crop breeding. *J Integr Bioinform* 20, 20230012. <https://doi.org/10.1515/jib-2023-0012>.
17. Chen, Y., Qiu, S., Zhou, H., Gao, W., Cui, L., Qiu, Z., Dong, C., and Wang, X. (2023). Construction of a Tomato (*Solanum lycopersicum* L.) Introgression Line Population and Mapping of Major Agronomic Quantitative Trait Loci. *Horticulturae* 9, 823. <https://doi.org/10.3390/horticulturae9070823>.
18. Chetelat, R., and Meglic, V. (2000). Molecular mapping of chromosome segments introgressed from *Solanum lycopersicoides* into cultivated tomato (*Lycopersicon esculentum*). *Theoretical Applied Genetics* 100, 232–241.
19. Chetelat, R.T., Qin, X., Tan, M., Burkart-Waco, D., Moritama, Y., Huo, X., Wills, T., and Pertuzé, R. (2019). Introgression lines of *Solanum sitiens*, a wild nightshade of the Atacama Desert, in the genome of cultivated tomato. *Plant Journal* 100, 836–850. <https://doi.org/10.1111/tpj.14460>.
20. Choudhary, R., Ahmad, F., Kaya, C., Upadhyay, S.K., Muneer, S., Kumar, V., Meena, M., Liu, H., Upadhyaya, H., and Seth, C.S. (2025). Decrypting proteomics, transcriptomics, genomics, and integrated omics for augmenting the abiotic, biotic, and climate change stress resilience in plants. *J Plant Physiol* 305, 154430. <https://doi.org/10.1016/j.jplph.2025.154430>.
21. Coppens, F., Wuyts, N., Inzé, D., and Dhondt, S. (2017). Unlocking the potential of plant phenotyping data through integration and data-driven approaches. *Curr Opin Syst Biol* 4, 58–63. <https://doi.org/10.1016/j.coisb.2017.07.002>.
22. Corak, K.E., Genger, R.K., Simon, P.W., and Dawson, J.C. (2023). Comparison of genotypic and phenotypic selection of breeding parents in a carrot (*Daucus carota*) germplasm collection. *Crop Sci* 63, 1998–2011. <https://doi.org/10.1002/csc2.20951>.
23. Crossa, J., Jarquín, D., Franco, J., Pérez-Rodríguez, P., Burgueño, J., Saint-Pierre, C., Vikram, P., Sansaloni, C., Petrolí, C., Akdemir, D., et al. (2016). Genomic prediction of gene bank wheat landraces. *G3: Genes, Genomes, Genetics* 6, 1819–1834. <https://doi.org/10.1534/g3.116.029637>.
24. Cuccurullo, A., Francesca, S., Granell, A., Rambla, J., Yoneyama, K., D'Agostino, N., Festa, G., Cardi, T., Rigano, M., and Nicolai, A. (2023). Exploring the multifaceted nature of Dwarf27 (D27) genes in tomato. In *Proceedings of the LXVI SIGA Annual Congress*, p. 2.45.
25. D'Agostino, N., and Tripodi, P. (2017). NGS-based genotyping, high-throughput phenotyping and genome-wide association studies laid the foundations for next-generation breeding in horticultural crops. *Diversity (Basel)* 9, 38. <https://doi.org/10.3390/d9030038>.
26. Dempewolf, H., Krishnan, S., and Guarino, L. (2023). Our shared global responsibility: Safeguarding crop diversity for future generations. *Proc Natl Acad Sci U S A* 120, e2205768119. <https://doi.org/10.1073/pnas.2205768119>.
27. Ebert, A.W. (2020). The role of vegetable genetic resources in nutrition security and vegetable breeding. *Plants* 9, 1–20. <https://doi.org/10.3390/plants9060736>.

28. Epstein, R., Sajai, N., Zelkowski, M., Zhou, A., Robbins, K.R., and Pawlowski, W.P. (2023). Exploring impact of recombination landscapes on breeding outcomes. *Proc Natl Acad Sci U S A* *120*, e2205785119. <https://doi.org/10.1073/pnas.2205785119>.
29. Eriksson, D., Ortiz, R., Visser, R.G.F., Vives-Vallés, J.A., and Prieto, H. (2020). Editorial: Leeway to Operate With Plant Genetic Resources. *Front Plant Sci* *11*, 911. <https://doi.org/10.3389/fpls.2020.00911>.
30. Eshed, Y., Abu-Abied, M., Saranga, Y., and Zamir, D. (1992). *Lycopersicon esculentum* lines containing small overlapping introgressions from *L. pennellii*. *Theoretical Applied Genetics* *83*, 1027–1034.
31. Esposito, S., Cardi, T., Campanelli, G., Sestili, S., Díez, M.J., Soler, S., Prohens, J., and Tripodi, P. (2020). ddRAD sequencing-based genotyping for population structure analysis in cultivated tomato provides new insights into the genomic diversity of Mediterranean ‘da serbo’ type long shelf-life germplasm. *Hortic Res* *7*, 134. <https://doi.org/10.1038/s41438-020-00353-6>.
32. European Commission (2023). Proposal for a REGULATION OF THE EUROPEAN PARLIAMENT AND OF THE COUNCIL on plants obtained by certain new genomic techniques and their food and feed, and amending Regulation (EU) 2017/625 (Wiley-Blackwell Publishing Ltd).
33. Farooq, M.A., Gao, S., Hassan, M.A., Huang, Z., Rasheed, A., Hearne, S., Prasanna, B., Li, X., and Li, H. (2024). Artificial intelligence in plant breeding. *Trends in Genetics* *40*, 891–908. <https://doi.org/10.1016/j.tig.2024.07.001>.
34. Fernie, A.R., and Yan, J. (2019). De Novo Domestication: An Alternative Route toward New Crops for the Future. *Mol Plant* *12*, 615–631. <https://doi.org/10.1016/j.molp.2019.03.016>.
35. Fricano, A., Battaglia R., Cardi, T., and Cattivelli, L. (2016). L'applicazione delle moderne tecnologie genomiche al miglioramento genetico. *Dal Seme* *2*, 43–50.
36. Gao, C. (2021). Genome engineering for crop improvement and future agriculture. *Cell* *184*, 1621–1635. <https://doi.org/10.1016/j.cell.2021.01.005>.
37. Gao, L., Gonda, I., Sun, H., Ma, Q., Bao, K., Tieman, D.M., Burzynski-Chang, E.A., Fish, T.L., Stromberg, K.A., Sacks, G.L., et al. (2019). The tomato pan-genome uncovers new genes and a rare allele regulating fruit flavor. *Nat Genet* *51*, 1044–1051. <https://doi.org/10.1038/s41588-019-0410-2>.
38. Garg, V., Bohra, A., Mascher, M., Spannagl, M., Xu, X., Bevan, M.W., Bennetzen, J.L., and Varshney, R.K. (2024). Unlocking plant genetics with telomere-to-telomere genome assemblies. *Nat Genet* *56*, 1788–1799. <https://doi.org/10.1038/s41588-024-01830-7>.
39. Gasparini, K., Moreira, J. dos R., Peres, L.E.P., and Zsögön, A. (2021). De novo domestication of wild species to create crops with increased resilience and nutritional value. *Curr Opin Plant Biol* *60*, 102006. <https://doi.org/10.1016/j.pbi.2021.102006>.
40. Golicz, A.A., Bayer, P.E., Barker, G.C., Edger, P.P., Kim, H.R., Martinez, P.A., Chan, C.K.K., Severn-Ellis, A., McCombie, W.R., Parkin, I.A.P., et al. (2016). The pangenome of an agronomically important crop plant *Brassica oleracea*. *Nat Commun* *7*, 13390. <https://doi.org/10.1038/ncomms13390>.
41. Gramazio, P., Prohens, J., Plazas, M., Mangino, G., Herraiz, F.J., and Vilanova, S. (2017). Development and genetic characterization of advanced backcross materials and an introgression line population of *Solanum incanum* in a *S. Melongena* background. *Front Plant Sci* *8*, 1477. <https://doi.org/10.3389/fpls.2017.01477>.
42. Gu, A., Li, X., Wang, Z., Wang, Y., Xuan, S., Ma, W., Hong, Y., Zhao, Y., Chen, X., Luo, S., et al. (2025). An efficient allotriploid-mediated system of generating genomic introgression from *Brassica oleracea* to *B. rapa*. *Plant Biotechnol J* *23*, 1073–1075. <https://doi.org/10.1111/pbi.14564>.
43. Gu, R., Fan, S., Wei, S., Li, J., Zheng, S., and Liu, G. (2023). Developments on Core Collections of Plant Genetic Resources: Do We Know Enough? *Forests* *14*, 926. <https://doi.org/10.3390/f14050926>.
44. Guijarro-Real, C., Navarro, A., Esposito, S., Festa, G., Macellaro, R., Di Cesare, C., Fita, A., Rodríguez-Burruezo, A., Cardi, T., Prohens, J., et al. (2020). Large scale phenotyping and molecular analysis in a germplasm collection of rocket salad (*Eruca vesicaria*) reveal a differentiation of the gene pool by geographical origin. *Euphytica* *216*, 53. <https://doi.org/10.1007/s10681-020-02586-x>.
45. Guo, N., Wang, S., Wang, T., Duan, M., Zong, M., Miao, L., Han, S., Wang, G., Liu, X., Zhang, D., et al. (2024). A graph-based pan-genome of *Brassica oleracea* provides new insights into its domestication and morphotype diversification. *Plant Commun* *5*, 100791. <https://doi.org/10.1016/j.xplc.2023.100791>.
46. Halewood, M., Chiurugwi, T., Sackville Hamilton, R., Kurtz, B., Marden, E., Welch, E., Michiels, F., Mozafari, J., Sabran, M., Patron, N., et al. (2018). Plant genetic resources for food and agriculture: opportunities and challenges emerging from the science and information technology revolution. *New Phytologist* *217*, 1407–1419. <https://doi.org/10.1111/nph.14993>.
47. Hao, N., Han, D., Huang, K., Du, Y., Yang, J., Zhang, J., Wen, C., and Wu, T. (2020). Genome-based breeding approaches in major vegetable crops. *Theoretical and Applied Genetics* *133*, 1739–1752. <https://doi.org/10.1007/s00122-019-03477-z>.
48. Hoopes, G., Meng, X., Hamilton, J.P., Achakkagari, S.R., de Alves Freitas Guesdes, F., Bolger, M.E., Coombs, J.J., Esselink, D., Kaiser, N.R., Kodde, L., et al. (2022). Phased, chromosome-scale genome assemblies of tetraploid potato reveal a complex genome, transcriptome, and predicted proteome landscape underpinning genetic diversity. *Mol Plant* *15*, 520–536. <https://doi.org/10.1016/j.molp.2022.01.003>.

49. Hu, M., and Liu, D. (2025). Unlocking the potential of genome editing in agriculture with tissue culture-free techniques. *New Phytologist* 246, 1478–1484. <https://doi.org/10.1111/nph.70046>.
50. Hunziker, J., Nishida, K., Kondo, A., Ariizumi, T., and Ezura, H. (2022). Phenotypic Characterization of High Carotenoid Tomato Mutants Generated by the Target-AID Base-Editing Technology. *Front Plant Sci* 13, 848560. <https://doi.org/10.3389/fpls.2022.848560>.
51. Ishikawa, M., Yoshida, T., Matsuyama, M., Kouzai, Y., Kano, A., and Ishibashi, K. (2022). Tomato brown rugose fruit virus resistance generated by quadruple knockout of homologs of TOBAMOVIRUS MULTIPLICATION1 in tomato. *Plant Physiol* 189, 679–686. <https://doi.org/10.1093/plphys/kiac103>.
52. Islam, T., and Kasfy, S.H. (2023). CRISPR enables heritable genome editing in planta. *Trends in Genetics* 39, 646–648. <https://doi.org/10.1016/j.tig.2023.06.009>.
53. Jarquin, D., Specht, J., and Lorenz, A. (2016). Prospects of genomic prediction in the USDA Soybean Germplasm Collection: Historical data creates robust models for enhancing selection of accessions. *G3: Genes, Genomes, Genetics* 6, 2329–2341. <https://doi.org/10.1534/g3.116.031443>.
54. Jeuken, M.J.W., and Lindhout, P. (2004). The development of lettuce backcross inbred lines (BILs) for exploitation of the *Lactuca saligna* (wild lettuce) germplasm. *Theoretical and Applied Genetics* 109, 394–401. <https://doi.org/10.1007/s00122-004-1643-7>.
55. Jia, C., Wang, J., Guo, B., Yang, T., Yang, H., Wang, B., and Yu, Q. (2024). Editing and genome-wide analysis upstream open reading frames contributes to enhancing salt tolerance in tomato. *Plant Biotechnol J* 22, 3312–3325. <https://doi.org/10.1111/pbi.14450>.
56. Jo, K.R., Kim, C.J., Kim, S.J., Kim, T.Y., Bergervoet, M., Jongsma, M.A., Visser, R.G.F., Jacobsen, E., and Vossen, J.H. (2014). Development of late blight resistant potatoes by cisgene stacking. *BMC Biotechnol* 14, 50. <https://doi.org/10.1186/1472-6750-14-50>.
57. Khaipho-Burch, M., Cooper, M., Crossa, J., de Leon, N., Hollamd, J., Lewis, R., McCouch, S., Murray, S., Rabbi, I., Ronald, P., et al. (2023). Scale up trials to validate modified crops' benefits. *Nature* 621, 470–473. <https://doi.org/10.26434/chemrxiv-2022-ntd3r>.
58. Khan, A.W., Garg, V., Roorkiwal, M., Golicz, A.A., Edwards, D., and Varshney, R.K. (2020). Super-Pangenome by Integrating the Wild Side of a Species for Accelerated Crop Improvement. *Trends Plant Sci* 25, 148–158. <https://doi.org/10.1016/j.tplants.2019.10.012>.
59. Khazaei, H., Street, K., Bari, A., Mackay, M., and Stoddard, F.L. (2013). The FIGS (Focused Identification of Germplasm Strategy) Approach Identifies Traits Related to Drought Adaptation in *Vicia faba* Genetic Resources. *PLoS One* 8, e63107. <https://doi.org/10.1371/journal.pone.0063107>.
60. Kulwal, P.L. (2018). Trait mapping approaches through linkage mapping in plants. In *Plant Genetics and Molecular Biology. Advances in Biochemical Engineering/Biotechnology*, vol 164, R. Varshney, M. Pandey, and A. Chitkineni, eds. (Springer Science and Business Media Deutschland GmbH), pp. 53–82. https://doi.org/10.1007/10_2017_49.
61. Lee, J.H., Venkatesh, J., Jo, J., Jang, S., Kim, G.W., Kim, J.M., Han, K., Ro, N., Lee, H.Y., Kwon, J.K., et al. (2022). High-quality chromosome-scale genomes facilitate effective identification of large structural variations in hot and sweet peppers. *Hortic Res* 9, uhac210. <https://doi.org/10.1093/hr/uhac210>.
62. Li, B., Sun, C., Li, J., and Gao, C. (2024). Targeted genome-modification tools and their advanced applications in crop breeding. *Nat Rev Genet* 25, 603–622. <https://doi.org/10.1038/s41576-024-00720-2>.
63. Li, H., Wang, S., Chai, S., Yang, Z., Zhang, Q., Xin, H., Xu, Y., Lin, S., Chen, X., Yao, Z., et al. (2022). Graph-based pan-genome reveals structural and sequence variations related to agronomic traits and domestication in cucumber. *Nat Commun* 13, 682. <https://doi.org/10.1038/s41467-022-28362-0>.
64. Li, L., Zhang, Q., and Huang, D. (2014). A review of imaging techniques for plant phenotyping. *Sensors (Switzerland)* 14, 20078–20111. <https://doi.org/10.3390/s141120078>.
65. Li, N., He, Q., Wang, J., Wang, B., Zhao, J., Huang, S., Yang, T., Tang, Y., Yang, S., Aisimutuola, P., et al. (2023). Super-pangenome analyses highlight genomic diversity and structural variation across wild and cultivated tomato species. *Nat Genet* 55, 852–860. <https://doi.org/10.1038/s41588-023-01340-y>.
66. Li, X., Wang, Y., Cai, C., Ji, J., Han, F., Zhang, L., Chen, S., Zhang, L., Yang, Y., Tang, Q., et al. (2024). Large-scale gene expression alterations introduced by structural variation drive morphotype diversification in *Brassica oleracea*. *Nat Genet* 56, 517–529. <https://doi.org/10.1038/s41588-024-01655-4>.
67. Lin, K., Zhang, N., Severing, E.I., Nijveen, H., Cheng, F., Visser, R.G.F., Wang, X., de Ridder, D., and Bonnema, G. (2014). Beyond genomic variation - comparison and functional annotation of three *Brassica rapa* genomes: A turnip, a rapid cycling and a Chinese cabbage. *BMC Genomics* 15, 250. <https://doi.org/10.1186/1471-2164-15-250>.
68. Liu, F., Zhao, J., Sun, H., Xiong, C., Sun, X., Wang, X., Wang, Z., Jarret, R., Wang, J., Tang, B., et al. (2023). Genomes of cultivated and wild *Capsicum* species provide insights into pepper domestication and population differentiation. *Nat Commun* 14, 5487. <https://doi.org/10.1038/s41467-023-41251-4>.
69. Lucoli, A., Tavazza, R., Baima, S., Fatyol, K., Burgyan, J., and Tavazza, M. (2022). CRISPR-Cas9 Targeting of the eIF4E1 Gene Extends the Potato Virus Y Resistance Spectrum of the *Solanum tuberosum* L. cv. Désirée. *Front Microbiol* 13, 873930. <https://doi.org/10.3389/fmicb.2022.873930>.

70. Ma, S., Wang, M., Wu, J., Guo, W., Chen, Y., Li, G., Wang, Y., Shi, W., Xia, G., Fu, D., et al. (2021). WheatOmics: A platform combining multiple omics data to accelerate functional genomics studies in wheat. *Mol Plant* *14*, 1965–1968. <https://doi.org/10.1016/j.molp.2021.10.006>.
71. Marden, E., Hamilton, R.S., Halewood, M., and McCouch, S. (2023). International agreements and the plant genetics research community: A guide to practice. *Proceedings National Academy of Sciences USA* *120*, e2205773119. <https://doi.org/10.1073/pnas.2205773119/-/DCSupplemental>.
72. Mascher, M., Jayakodi, M., Shim, H., and Stein, N. (2024). Promises and challenges of crop translational genomics. *Nature* *636*, 585–593. <https://doi.org/10.1038/s41586-024-07713-5>.
73. Mascher, M., Schreiber, M., Scholz, U., Graner, A., Reif, J.C., and Stein, N. (2019). Genebank genomics bridges the gap between the conservation of crop diversity and plant breeding. *Nat Genet* *51*, 1076–1081. <https://doi.org/10.1038/s41588-019-0443-6>.
74. McLoughlin, F., Augustine, R.C., Marshall, R.S., Li, F., Kirkpatrick, L.D., Otegui, M.S., and Vierstra, R.D. (2018). Maize multi-omics reveal roles for autophagic recycling in proteome remodelling and lipid turnover. *Nat Plants* *4*, 1056–1070. <https://doi.org/10.1038/s41477-018-0299-2>.
75. Mieulet, D., Aubert, G., Bres, C., Klein, A., Droc, G., Vieille, E., Rond-Coissieux, C., Sanchez, M., Dalmais, M., Mauxion, J.P., et al. (2018). Unleashing meiotic crossovers in crops. *Nat Plants* *4*, 1010–1016. <https://doi.org/10.1038/s41477-018-0311-x>.
76. Miyatake, K., Shinmura, Y., Matsunaga, H., Fukuoka, H., and Saito, T. (2019). Construction of a core collection of eggplant (*Solanum melongena* L.) based on genome-wide snp and ssr genotypes. *Breed Sci* *69*, 498–502. <https://doi.org/10.1270/jsbbs.18202>.
77. Monforte, A.J., and Tanksley, S.D. (2000). Development of a set of near isogenic and backcross recombinant inbred lines containing most of the *Lycopersicon hirsutum* genome in a *L. esculentum* genetic background: A tool for gene mapping and gene discovery. *Genome* *43*, 803–813.
78. Moon, K.B., Park, S.J., Park, J.S., Lee, H.J., Shin, S.Y., Lee, S.M., Choi, G.J., Kim, S.G., Cho, H.S., Jeon, J.H., et al. (2022). Editing of StSR4 by Cas9-RNPs confers resistance to *Phytophthora infestans* in potato. *Front Plant Sci* *13*, 997888. <https://doi.org/10.3389/fpls.2022.997888>.
79. Nandi, S., Varotariya, K., Luhana, S., Kyada, A.D., Saha, A., Roy, N., Sharma, N., and Rambabu, D. (2024). GWAS for identification of genomic regions and candidate genes in vegetable crops. *Funct Integr Genomics* *24*, 203. <https://doi.org/10.1007/s10142-024-01477-x>.
80. Nonaka, S., Ito, M., and Ezura, H. (2023). Targeted modification of CmACO1 by CRISPR/Cas9 extends the shelf-life of *Cucumis melo* var. *reticulatus* melon. *Front Genome Ed* *5*, 1176125. <https://doi.org/10.3389/fgeed.2023.1176125>.
81. Noureen, A., Khan, M.Z., Amin, I., Zainab, T., and Mansoor, S. (2022). CRISPR/Cas9-Mediated Targeting of Susceptibility Factor eIF4E-Enhanced Resistance Against Potato Virus Y. *Front Genet* *13*, 922019. <https://doi.org/10.3389/fgene.2022.922019>.
82. Odong, T.L., Jansen, J., van Eeuwijk, F.A., and van Hintum, T.J.L. (2013). Quality of core collections for effective utilisation of genetic resources review, discussion and interpretation. *Theoretical and Applied Genetics* *126*, 289–305. <https://doi.org/10.1007/s00122-012-1971-y>.
83. Ou, L., Li, D., Lv, J., Chen, W., Zhang, Z., Li, X., Yang, B., Zhou, S., Yang, S., Li, W., et al. (2018). Pan-genome of cultivated pepper (*Capsicum*) and its use in gene presence–absence variation analyses. *New Phytologist* *220*, 360–363. <https://doi.org/10.1111/nph.15413>.
84. Palmgren, M.G., Edenbrandt, A.K., Vedel, S.E., Andersen, M.M., Landes, X., Østerberg, J.T., Falhof, J., Olsen, L.I., Christensen, S.B., Sandøe, P., et al. (2015). Are we ready for back-to-nature crop breeding? *Trends Plant Sci* *20*, 155–164. <https://doi.org/10.1016/j.tplants.2014.11.003>.
85. Palomba, E., Cremona, G., Paparo, R., Tripodi, P., Nocolia, A., De Paola, D., Iovene, M., Consiglio, M., and Termolino, P. (2024). Induction of chromosome rearrangements in the wild species “*Solanum pimpinellifolium*” using multi-target Crispr-Cas system. In LXVII SIGA Annual Congress, p. 6.40.
86. Pazhamala, L.T., Kudapa, H., Weckwerth, W., Millar, A.H., and Varshney, R.K. (2021). Systems biology for crop improvement. *Plant Genome* *14*, e20098. <https://doi.org/10.1002/tpg2.20098>.
87. Pechar, G.S., Donaire, L., Gosálvez, B., García-Almodovar, C., Sánchez-Pina, M.A., Truniger, V., and Aranda, M.A. (2022). Editing melon eIF4E associates with virus resistance and male sterility. *Plant Biotechnol J* *20*, 2006–2022. <https://doi.org/10.1111/pbi.13885>.
88. Peng, H., Wang, K., Chen, Z., Cao, Y., Gao, Q., Li, Y., Li, X., Lu, H., Du, H., Lu, M., et al. (2020). MBKbase for rice: An integrated omics knowledgebase for molecular breeding in rice. *Nucleic Acids Res* *48*, D1085–D1092. <https://doi.org/10.1093/nar/gkz921>.
89. Pereira, L., Santo Domingo, M., Argyris, J., Mayobre, C., Valverde, L., Martín-Hernández, A.M., Pujol, M., and Garcia-Mas, J. (2021). A novel introgression line collection to unravel the genetics of climacteric ripening and fruit quality in melon. *Sci Rep* *11*, 11364. <https://doi.org/10.1038/s41598-021-90783-6>.
90. Perpiñá, G., Esteras, C., Gibon, Y., Monforte, A.J., and Picó, B. (2016). A new genomic library of melon introgression lines in a cantaloupe genetic background for dissecting desirable agronomical traits. *BMC Plant Biol* *16*, 154. <https://doi.org/10.1186/s12870-016-0842-0>.

91. Phippen, W., Kresovich, S., Candelas, F., and McFerson, J. (1997). Molecular characterization can quantify and partition variation among genebank holdings: a case study with phenotypically similar accessions of *Brassica oleracea* var. capitata L. (cabbage) 'Golden Acre.' *Theoretical Applied Genetics* 94, 227–234.
92. Prestwich, B.D., Cardi, T., Bakhsh, A., Nicolia, A., and Bhati, K.K. (2023). Novel delivery methods for CRISPR-based plant genome editing. In *A Roadmap for Plant Genome Editing* (Springer Nature), pp. 41–67. https://doi.org/10.1007/978-3-031-46150-7_3.
93. Prohens, J., Gramazio, P., Plazas, M., Dempewolf, H., Kilian, B., Díez, M.J., Fita, A., Herraiz, F.J., Rodríguez-Burruezo, A., Soler, S., et al. (2017). Introgressomics: a new approach for using crop wild relatives in breeding for adaptation to climate change. *Euphytica* 213, 158. <https://doi.org/10.1007/s10681-017-1938-9>.
94. Puchta, H., Jiang, J., Wang, K., and Zhao, Y. (2022). Updates on gene editing and its applications. *Plant Physiol* 188, 1725–1730. <https://doi.org/10.1093/plphys/kiac032>.
95. Purugganan, M.D., and Jackson, S.A. (2021). Advancing crop genomics from lab to field. *Nat Genet* 53, 595–601. <https://doi.org/10.1038/s41588-021-00866-3>.
96. Rajasekharan, P.E. (2019). Vegetable Genetic Resources: Diversity, Distribution and Conservation. In *Conservation and Utilization of Horticultural Genetic Resources* (Springer Singapore), pp. 117–138. https://doi.org/10.1007/978-981-13-3669-0_5.
97. Raza, A., Razzaq, A., Mehmood, S.S., Hussain, M.A., Wei, S., He, H., Zaman, Q.U., Xuekun, Z., Yong, C., and Hasanuzzaman, M. (2021). Omics: The way forward to enhance abiotic stress tolerance in *Brassica napus* L. *GM Crops Food* 12, 251–281. <https://doi.org/10.1080/21645698.2021.19562031>.
98. Ricroch, A. (2024). CRISPR processes patents in green biotechnology: Collaborative licensing models. In *A Roadmap for Plant Genome Editing*, A. Ricroch, D. Eriksson, D. Miladinović, J. Sweet, K. Van Laere, and E. Woźniak-Gientka, eds. (Springer Nature), pp. 453–467. https://doi.org/10.1007/978-3-031-46150-7_27.
99. Schneider, K., Barreiro-Hurle, J., Vossen, J., Schouten, H.J., Kessel, G., Andreasson, E., Kieu, N.P., Strassemer, J., Hristov, J., and Rodriguez-Cerezo, E. (2023). Insights on cisgenic plants with durable disease resistance under the European Green Deal. *Trends Biotechnol* 41, 1027–1040. <https://doi.org/10.1016/j.tibtech.2023.02.005>.
100. Scholz, A.H., Freitag, J., Lyal, C.H.C., Sara, R., Cepeda, M.L., Cancio, I., Sett, S., Hufton, A.L., Abebaw, Y., Bansal, K., et al. (2022). Multilateral benefit-sharing from digital sequence information will support both science and biodiversity conservation. *Nat Commun* 13, 1086. <https://doi.org/10.1038/s41467-022-28594-0>.
101. Schreiber, M., Jayakodi, M., Stein, N., and Mascher, M. (2024). Plant pangenomes for crop improvement, biodiversity and evolution. *Nat Rev Genet* 25, 563–577. <https://doi.org/10.1038/s41576-024-00691-4>.
102. Scossa, F., Alseekh, S., and Fernie, A.R. (2021). Integrating multi-omics data for crop improvement. *J Plant Physiol* 257, 153352. <https://doi.org/10.1016/j.jplph.2020.153352>.
103. Sett, S., Kress, W.J., Halewood, M., Nicholson, D., Nuñez-Vega, G., Faggionato, D., Rouard, M., Jaspars, M., da Silva, M., Prat, C., et al. (2024). Harmonize rules for digital sequence information benefit-sharing across UN frameworks. *Nat Commun* 15, 8745. <https://doi.org/10.1038/s41467-024-52994-z>.
104. Sha, G., Sun, P., Kong, X., Han, X., Sun, Q., Fouillen, L., Zhao, J., Li, Y., Yang, L., Wang, Y., et al. (2023). Genome editing of a rice CDP-DAG synthase confers multipathogen resistance. *Nature* 618, 1017–1023. <https://doi.org/10.1038/s41586-023-06205-2>.
105. Simko, I., Jia, M., Venkatesh, J., Kang, B.C., Weng, Y., Barcaccia, G., Lanteri, S., Bhattarai, G., and Foolad, M.R. (2021). Genomics and Marker-Assisted Improvement of Vegetable Crops. *CRC Crit Rev Plant Sci* 40, 303–365. <https://doi.org/10.1080/07352689.2021.1941605>.
106. Sprink, T., and Wilhelm, R. (2024). Genome editing in biotech regulations worldwide. In *A Roadmap for Plant Genome Editing*, A. Ricroch, D. Eriksson, D. Miladinović, J. Sweet, K. Van Laere, and E. Woźniak-Gientka, eds. (Springer Nature), pp. 425–435. https://doi.org/10.1007/978-3-031-46150-7_25.
107. Stenberg, J.A., and Ortiz, R. (2021). Focused Identification of Germplasm Strategy (FIGS): polishing a rough diamond. *Curr Opin Insect Sci* 45, 1–6. <https://doi.org/10.1016/j.cois.2020.11.001>.
108. Sun, Y., Shang, L., Zhu, Q.H., Fan, L., and Guo, L. (2022). Twenty years of plant genome sequencing: achievements and challenges. *Trends Plant Sci* 27, 391–401. <https://doi.org/10.1016/j.tplants.2021.10.006>.
109. Szymański, J., Bocobza, S., Panda, S., Sonawane, P., Cárdenas, P.D., Lashbrooke, J., Kamble, A., Shahaf, N., Meir, S., Bovy, A., et al. (2020). Analysis of wild tomato introgression lines elucidates the genetic basis of transcriptome and metabolome variation underlying fruit traits and pathogen response. *Nat Genet* 52, 1111–1121. <https://doi.org/10.1038/s41588-020-0690-6>.
110. Tang, D., Jia, Y., Zhang, J., Li, H., Cheng, L., Wang, P., Bao, Z., Liu, Z., Feng, S., Zhu, X., et al. (2022). Genome evolution and diversity of wild and cultivated potatoes. *Nature* 606, 535–541. <https://doi.org/10.1038/s41586-022-04822-x>.
111. Tang, X., and Zhang, Y. (2023). Beyond knockouts: fine-tuning regulation of gene expression in plants with CRISPR-Cas-based promoter editing. *New Phytologist* 239, 868–874. <https://doi.org/10.1111/nph.19020>.
112. Tao, Y., Zhao, X., Mace, E., Henry, R., and Jordan, D. (2019). Exploring and Exploiting Pan-genomics for Crop Improvement. *Mol Plant* 12, 156–169. <https://doi.org/10.1016/j.molp.2018.12.016>.

113. Taranto, F., D'Agostino, N., Greco, B., Cardi, T., and Tripodi, P. (2016). Genome-wide SNP discovery and population structure analysis in pepper (*Capsicum annuum*) using genotyping by sequencing. *BMC Genomics* 17, 943. <https://doi.org/10.1186/s12864-016-3297-7>.
114. Teper-Bamnolker, P., Roitman, M., Katar, O., Peleg, N., Aruchamy, K., Suher, S., Doron-Faigenboim, A., Leibman, D., Omid, A., Belausov, E., et al. (2023). An alternative pathway to plant cold tolerance in the absence of vacuolar invertase activity. *Plant Journal* 113, 327–341. <https://doi.org/10.1111/tpj.16049>.
115. Tran, M.T., Son, G.H., Song, Y.J., Nguyen, N.T., Park, S., Thach, T.V., Kim, J., Sung, Y.W., Das, S., Pramanik, D., et al. (2023). CRISPR-Cas9-based precise engineering of SIHyPRP1 protein towards multi-stress tolerance in tomato. *Front Plant Sci* 14, 1186932. <https://doi.org/10.3389/fpls.2023.1186932>.
116. Tripodi, P. (2023). The Evolution of Molecular Genotyping in Plant Breeding. *Agronomy* 13, 2569. <https://doi.org/10.3390/agronomy13102569>.
117. Tripodi, P., Massa, D., Venezia, A., and Cardi, T. (2018). Sensing Technologies for Precision Phenotyping in Vegetable Crops: Current Status and Future Challenges. *Agronomy* 8, 57. <https://doi.org/10.3390/agronomy8040057>.
118. Tripodi, P., Soler, S., Campanelli, G., Díez, M.J., Esposito, S., Sestili, S., Figàs, M.R., Leteo, F., Casanova, C., Platani, C., et al. (2021). Genome wide association mapping for agronomic, fruit quality, and root architectural traits in tomato under organic farming conditions. *BMC Plant Biol* 21, 481. <https://doi.org/10.1186/s12870-021-03271-4>.
119. Uchida, K., Sawada, Y., Ochiai, K., Sato, M., Inaba, J., and Hirai, M.Y. (2020). Identification of a unique type of isoflavone O-Methyltransferase, GmIOMT1, Based on Multi-Omics analysis of soybean under biotic stress. *Plant Cell Physiol* 61, 1974–1985. <https://doi.org/10.1093/pcp/pcaa112>.
120. Van Hintum, T.J.L., and Kntipffer, H. (1995). Duplication within and between germplasm collections I. Identifying duplication on the basis of passport data. *Genet Resour Crop Evol* 42, 127–133.
121. van Workum, D.-J.M., Mehrem, S.L., Snoek, B.L., Alderkamp, M.C., Lapin, D., Mulder, F.F.M., Van den Ackerveken, G., de Ridder, D., Schranz, M.E., and Smit, S. (2024). *Lactuca* super-pangenome reduces bias towards reference genes in lettuce research. *BMC Plant Biol* 24, 1019. <https://doi.org/10.1186/s12870-024-05712-2>.
122. van Zonneveld, M., Volk, G.M., Dulloo, M.E., Kindt, R., Mayes, S., Quintero, M., Choudhury, D., Achigan-Dako, E.G., and Guarino, L. (2023). Safeguarding and Using Fruit and Vegetable Biodiversity. In *Science and Innovations for Food Systems Transformation* (Springer International Publishing), pp. 553–567. https://doi.org/10.1007/978-3-031-15703-5_30.
123. Varshney, R.K., Bohra, A., Yu, J., Graner, A., Zhang, Q., and Sorrells, M.E. (2021). Designing Future Crops: Genomics-Assisted Breeding Comes of Age. *Trends Plant Sci* 26, 631–649. <https://doi.org/10.1016/j.tplants.2021.03.010>.
124. Verlaan, M.G., Szinay, D., Hutton, S.F., De Jong, H., Kormelink, R., Visser, R.G.F., Scott, J.W., and Bai, Y. (2011). Chromosomal rearrangements between tomato and *Solanum chilense* hamper mapping and breeding of the TYLCV resistance gene Ty-1. *Plant Journal* 68, 1093–1103. <https://doi.org/10.1111/j.1365-3113X.2011.04762.x>.
125. Visakh, R.L., Anand, S., Reddy, S.B., Jha, U.C., Sah, R.P., and Beena, R. (2024). Precision Phenotyping in Crop Science: From Plant Traits to Gene Discovery for Climate-Smart Agriculture. *Plant Breeding* 0, 1–29. <https://doi.org/10.1111/pbr.13228>.
126. Wada, N., Osakabe, K., and Osakabe, Y. (2022). Genome editing in plants. *Gene and Genome Editing* 3–4, 100020. <https://doi.org/10.1016/j.ggedit.2022.100020>.
127. Wambugu, P.W., Ndjioudjop, M.-N., Rangan, P., and Henry, R.J. (2024). Application of Genomics in Supporting Efficient Conservation and Utilization of Plant Genetic Resources. In *Sustainable Utilization and Conservation of Plant Genetic Diversity*, J. M. , Al-Khayri, S. M. , Jain, and S. Penna, eds. (Springer), pp. 339–361. https://doi.org/10.1007/978-981-99-5245-8_10.
128. Yadav, A.K., Banerjee, S.K., Das, B., and Chaudhary, K. (2022). Editorial: Systems Biology and Omics Approaches for Understanding Complex Disease Biology. *Front Genet* 13, 896818. <https://doi.org/10.3389/fgene.2022.896818>.
129. Yan, J., and Wang, X. (2023). Machine learning bridges omics sciences and plant breeding. *Trends Plant Sci* 28, 199–210.
130. Yang, Y., Saand, M.A., Huang, L., Abdelaal, W.B., Zhang, J., Wu, Y., Li, J., Sirohi, M.H., and Wang, F. (2021). Applications of Multi-Omics Technologies for Crop Improvement. *Front Plant Sci* 12, 563953. <https://doi.org/10.3389/fpls.2021.563953>.
131. Yang, Y., Tilman, D., Jin, Z., Smith, P., Barrett, C.B., Zhu, Y.G., Burney, J., D'Odorico, P., Fantke, P., Fargione, J., et al. (2024). Climate change exacerbates the environmental impacts of agriculture. *Science* 385, eadn3747. <https://doi.org/10.1126/science.adn3747>.
132. Yu, X., Li, X., Guo, T., Zhu, C., Wu, Y., Mitchell, S.E., Roozeboom, K.L., Wang, D., Wang, M.L., Pederson, G.A., et al. (2016). Genomic prediction contributing to a promising global strategy to turbocharge gene banks. *Nat Plants* 2, 16150. <https://doi.org/10.1038/nplants.2016.150>.

133. Zenda, T., Liu, S., Dong, A., Li, J., Wang, Y., Liu, X., Wang, N., and Duan, H. (2021). Omics-Facilitated Crop Improvement for Climate Resilience and Superior Nutritive Value. *Front Plant Sci* 12, 774994. <https://doi.org/10.3389/fpls.2021.774994>.
134. Zewdie, Y., Tong, N., and Bosland, P. (2004). Establishing a core collection of Capsicum using a cluster analysis with enlightened selection of accessions. *Genet Resour Crop Evol* 51, 147–151.
135. Zhang, J., Lyu, H., Chen, J., Cao, X., Du, R., Ma, L., Wang, N., Zhu, Z., Rao, J., Wang, J., et al. (2024). Releasing a sugar brake generates sweeter tomato without yield penalty. *Nature* 635, 647–656. <https://doi.org/10.1038/s41586-024-08186-2>.
136. Zhang, R., Zhang, C., Yu, C., Dong, J., and Hu, J. (2022). Integration of multi-omics technologies for crop improvement: Status and prospects. *Frontiers in Bioinformatics* 2, 1027457. <https://doi.org/10.3389/fbinf.2022.1027457>.
137. Zhang, X., Liu, T., Wang, J., Wang, P., Qiu, Y., Zhao, W., Pang, S., Li, X., Wang, H., Song, J., et al. (2021). Pan-genome of Raphanus highlights genetic variation and introgression among domesticated, wild, and weedy radishes. *Mol Plant* 14, 2032–2055. <https://doi.org/10.1016/j.molp.2021.08.005>.
138. Zhang, Y., Zhao, M., Tan, J., Huang, M., Chu, X., Li, Y., Han, X., Fang, T., Tian, Y., Jarret, R., et al. (2024). Telomere-to-telomere Citrullus super-pangenome provides direction for watermelon breeding. *Nat Genet* 56, 1750–1761. <https://doi.org/10.1038/s41588-024-01823-6>.
139. Zhao, A., Li, Q., Meng, P., Liu, P., Wu, S., Lang, Z., Song, Y., and Macho, A.P. (2024). Reduced content of gamma-aminobutyric acid enhances resistance to bacterial wilt disease in tomato. *Plant Biotechnol J* 23, 792–806. <https://doi.org/10.1111/pbi.14539>.
140. Zhou, Y., Zhang, Z., Bao, Z., Li, H., Lyu, Y., Zan, Y., Wu, Y., Cheng, L., Fang, Y., Wu, K., et al. (2022). Graph pangenome captures missing heritability and empowers tomato breeding. *Nature* 606, 527–534. <https://doi.org/10.1038/s41586-022-04808-9>.

Molecular characterization and relationships among some fig (*Ficus carica* L.) genotypes from the collection of the Faculty of Horticulture, Bucharest, using simple sequence repeat (SSR) markers

E. Moisesescu¹, S. Teoman Duran^{2*}, Y. Insel³, B. Oler³, A. Peticilă¹ and F. Stănică¹

¹Faculty of Horticulture, University of Agronomic Sciences and Veterinary Medicine of Bucharest, Romania

²Vocational School of Karacabey, Department of Crop and Animal Production, Organic Agriculture Programme ², Faculty of Agriculture, Department of Horticulture ³, University of Bursa Uludag, Turkey

*Corresponding author email: sevinteoman@uludag.edu.tr

ABSTRACT

The development of molecular marker technology in the late 20th century has revolutionized plant breeding, particularly for minor species that previously lacked sufficient data. Accurate information on structure and genetic diversity is crucial to improve this activity. This study was conducted on fig accessions from various regions of Romania. The accessions are part of the fig germplasm collection at the Faculty of Horticulture, Bucharest. The genetic variability among 31 fig (*Ficus carica* L.) accessions was examined using 17 simple sequence repeat (SSR) markers previously developed for this crop. The primer pairs revealed a total of 54 alleles. The number of alleles per locus ranged from two to six, with an average of 3.60 alleles per locus. The mean expected (H_e) was 0.55, and the mean polymorphic information content (PIC) was 0.61. The genetic relationships are illustrated by hierarchical clustering. UPGMA cluster analysis grouped the fig accessions analyzed into two principal clusters. All of these suggest a molecular diversity among the studied fig accessions.

Keywords: Accessions, alleles, cluster, germplasm, microsatellites.

INTRODUCTION

Figs, the fruits of *Ficus carica* L. ($2n=2x=26$) from the *Moraceae* family, are among the oldest domesticated fruit species, before millet and wheat (Kislev *et al.*, 2006). This may be due to the easy propagation of the fig tree, which is generally done through cutting (Zohary and Hopf, 2000). It is believed that fig originated from the Middle Asia and the Mediterranean Basin (Condit, 1955). However, it is now also cultivated in other temperate countries (Sadder and Ateyyeh, 2006). The fig tree belongs to the genus *Ficus*, a large group that includes 600 to 1,900 species (Flaishman *et al.*, 2008).

The fig tree grows easily in warm and temperate climates, being a xerophilous plant (Minone *et al.*, 2011). It is tolerant to drought (Ammar *et al.*, 2022), does not require rich soils, and is resistant to pests (Jullien and Jullien, 2011).

Figs are consumed both as fresh and especially dried fruits (Duenas *et al.*, 2008), being very perishable fruits due to fungal attack after harvest (Dogan *et al.*, 2018). Fig trees are cultivated on 290,000 ha worldwide (FAOSTAT, 2019). Turkey is the world's main producer of figs (Veberic and Mikulic-Petkovsek, 2016).

The fruits of *Ficus* species are reported to represent an important source of essential medicinal and dietary nutrients, among which figs, the fruits of *Ficus carica*, are the most used (Shi et al., 2018). Consumption of figs has positive effects on human health (Khatib and Vaya, 2010). This is based on their content in amino acids (Saki et al., 2019), vitamins, fibers, mineral, carbohydrates (Hssaini et al., 2019 and Hssaini et al., 2020a), their antioxidant capacity (Arvaniti et al., 2019), their polyphenolic compounds such as flavones, quercetin, and rutin (Pourghayoumi et al., 2017), and other nutraceutical qualities (dos Anjos Cruz et al., 2022).

The first characterizations of this species relied on agronomic and morphological traits, following the descriptors developed by IPGRI and CIHEAM (2003).

Although numerous descriptors exist, a standardized method for fig characterization is still lacking. Generally, figs are characterized by region, and environmental conditions strongly influence the morphological and biometric traits, which makes it difficult to compare genotypes, especially if they are very similar. Morphological characterizations of some fig genotypes were made in Slovenia (Podgornik et al., 2010), Tunisia (Gaaliche et al., 2012), Iran (Khadivi et al., 2018), Algeria (Mahmoudi et al., 2018), Morocco (Hssaini et al., 2020b), and for male fig (caprifig) in Iran (Khadivi-Khub and Anjam, 2014; Mirheidari et al., 2020), and Tunisia (Essid et al., 2017).

The development of molecular marker technology in recent years has significantly improved precision in plant breeding (Nadeem et al., 2018). Examples of molecular markers include microsatellite or Simple Sequence Repeat (SSR) data, Restriction Fragment Length Polymorphism (RFLP) data, and Single Nucleotide Polymorphism (SNP) data. Due to their high degree of polymorphism, reproducibility, and codominance, SSR (microsatellite markers) are most commonly used to create a genetic fingerprint for most plant species (Gupta and Varshney, 2000). A good genetic characterization depends on the high quality of the extracted DNA (Armiteye, 2021). For fig, among the first research where microsatellite markers (SSR) were used, we find Khadari et al., 2001; Giraldo et al., 2005; Giraldo et al., 2008; Bandelj et al., 2007, but also more recent studies, Costa et al., 2017; Ferarra et al., 2017; Marcotuli et al., 2019; Ergul et al., 2021; Essid et al., 2021.

The main objective of this study was to characterize and assess the genetic diversity of fig genotypes from the collection of the Faculty of Horticulture, in Bucharest, using SSR markers to support the development of conservation strategies for the endangered genetic resources of this species.

MATERIALS AND METHODS

Plant material

This study was conducted on 31 fig accessions with diverse phenotypic traits from various Romanian regions (Table 1). All accessions are part of the fig germplasm collection of the Faculty of Horticulture, Bucharest. The plant material was propagated through hardwood cuttings.

Table 1. List of genotypes of collection and their origin area

Genotype	Location	Genotype	Location	Genotype	Location	Genotype	Location
4 i	Bucharest	241 cv	Lunca	106 m	Popești-Leordeni	184 cv	Viisoara
10 i	Mangalia	4 m	Bucharest	125 m	Moara Domneasă	185 cv	Tulcea
16 ia	Bucharest	4 ma	Bucharest	19 L	Bucharest	187 cv	Mangalia
100 i	Bucharest	4 mb	Bucharest	28 L	Brazi	189 cv	Tulcea
103 i	Săvârșin	11 m	Giurgiu	50 L	Săvârșin	266 cv	Constanța
109 i	Svinița	19 m	Bucharest	183 L	Alba-Iulia	267 cv	Craiova
161 i	Svinița	21 m	Bucharest	207 L	Croatia	275	Snagov
125 i	Moara Domneasă	63 m	Moara Domneasă	181 cv	Voiteg		

Genomic DNA extraction

Genomic DNA was extracted from 25 mg lyophilized and fine-powdered winter dormant buds following a cytrimethylammonium bromide CTAB-based method (Futterer *et al.*, 1995) with modifications. The concentrations of DNA were determined by using a Quibit fluorometer (Invitrogen, USA) and diluted to 50 ng/μL.

SSR analysis

A total of 17 SSR primer pairs, previously developed by Giraldo *et al.* (2005), were used for SSR analysis in this study (Table 2). Amplification reactions were performed in a final volume of 20 μL containing of 50 ng of template DNA, 1X reaction buffer, 1.0 U Taq DNA polymerase (Thermo Scientific, USA), 0.10 μM M13 sequence-tailed forward primer, 0.20 μM reverse primer, 0.20 μM M13 primer labeled with infrared dye at either 700 nm or 800 nm (Licor, USA) and 0.25 mM dNTPs. PCR reactions were conducted using a Veriti 96-well thermal cycler (Applied Biosystems, USA). PCR conditions were as follows: 2 min at 94°C; 6 cycles of 40s at 94°C, 1 min at 61°C (with the annealing temperature reduced 1°C after every cycle for touchdown PCR protocol), 1 min and 30s at 72°C; 28 cycles of 40s at 94°C, 1 min at 55 °C and 1 min and 30s at 72°C; 7 cycles of 40s at 94°C, 1 min at 54°C and 1 min and 30s at 72°C and final extension step of 10 min at 72°C. The PCR products were separated on 6.0% polyacrylamide gels at 30 W for 2 to 3 h using a Li-COR 4300 DNA analyzer (LI-COR).

Data analysis

A genetic similarity matrix of fig accessions was calculated using a Dice coefficient (Dice, 1945) and the NTSYSpc v2.21 program (Exeter Software, New York, USA), based on the presence (1) and absence (0) of the SSR primers. Genetic distance among accessions was determined using the unweighted pair group method with arithmetic mean (UPGMA). The method of Levene (1949) and the POPGEN32 software v.1.32 (Yeh *et al.*, 1997) were used to calculate expected heterozygosity (He) and observed heterozygosity (Ho). The polymorphism information content (PIC) values for SSR markers were calculated according to the method of Botstein *et al.* (1980). Population structure was analyzed using the Structure v.2.3.4 software, based on a Bayesian clustering model. (Pritchard *et al.*, 2000). The range of possible numbers of clusters (K) varied from 1 to 12, with 5 replications for each K value. Each independent run was carried out with a burn-in period of 100,000 steps followed by 100.000 Monte Carlo Markov Chain (MCMC) replications. Furthermore, the method defined by Evanno *et al.* (2005) was used to detect the most likely number of subpopulations (K).

Table 2. Genetic diversity parameters assessed for 17 SSR loci in 31 fig genotypes

Locus	Na	Ho	He	PIC
LMFC 12	monomorphic	-	-	-
LMFC 13	5	0.29	0.66	0.86
LMFC 14	3	0.35	0.54	0.61
LMFC 15	4	0.77	0.66	0.60
LMFC 17	3	0.68	0.63	0.57
LMFC 19	4	0.90	0.74	0.64
LMFC 21	3	0.34	0.40	0.54
LMFC 22	4	0.38	0.54	0.58
LMFC 25	3	0.48	0.37	0.55
LMFC 26	4	0.48	0.39	0.59
LMFC 27	2	0.37	0.50	0.57
LMFC 28	monomorphic	-	-	-
LMFC 30	6	0.83	0.79	0.75
LMFC 32	5	0.38	0.46	0.66
LMFC 35	3	0.74	0.55	0.66
LMFC 37	2	0.41	0.37	0.56
LMFC 40	3	0.74	0.70	0.54
Mean	3.60	0.54	0.55	0.61

Na – number of different alleles; Ho – observed heterozygosity; He – expected heterozygosity; PIC – polymorphism information content

RESULTS AND DISCUSSIONS

A set of 17 SSR markers was used to assess genetic distance among the 31 fig accessions. It was found that 15 SSRs were polymorphic; meanwhile, 2 of them were monomorphic (LMFC 12 and LMFC 28). LMFC 12 was also found monomorphic by Teoman *et al.* (2017), but polymorphic by Costa *et al.* (2017) with 6 alleles, Giraldo *et al.* (2005) with 3 alleles. LMFC marker was found to be polymorphic by Giraldo *et al.* (2005) with 4 alleles, Costa *et al.* (2017) with 6 alleles, Teoman *et al.* (2017) with 5 alleles, and Essid *et al.* (2021) with 5 alleles. 15 SSR markers amplified 54 polymorphic SSR alleles, and the number of polymorphic alleles per SSR marker ranged from 2 (LMFC-27 and LMFC-37) to 6 (LMFC-30), with an average of 3.60 (Table 2). A higher number of alleles for LMFC 30 was also reported by Ergul *et al.* (2021) with 9 alleles and Essid *et al.* (2021) with 6 alleles. The observed (H_o) values for each SSR marker ranged from 0.29 (LMFC-13) to 0.90 (LMFC-19) with an average of 0.54, while the expected (H_e) heterozygosity varied from 0.37 (LMFC-25 and LMFC-37) to 0.79 (LMFC-30) with an average of 0.55 (Table 2). Comparing both H_o and H_e values of the primer pairs, a heterozygote excess ($H_o > H_e$) was observed for LMFC-15, LMFC-17, LMFC-19, LMFC-25, LMFC-26, LMFC-30, LMFC-35, and LMFC-40, whereas a heterozygote deficiency ($H_o < H_e$) was observed for the remaining SSR markers.

The polymorphism information content (PIC) value ranged from 0.54 (LMFC-21 and LMFC-40) to 0.86 (LMFC-13), with a mean of 0.61. These results indicated that all loci were highly informative ($PIC > 0.50$), and the most powerful locus for discriminating fig genotypes was LMFC-13. $PIC = 0.86$ for LMFC-13 was higher than $PIC = 0.42$ found by Costa *et al.* (2017), but lower than $PIC = 0.90$ found by Teoman *et al.* (2017).

The number of gene pools indicated by the STRUCTURE analysis for these 31 fig genotypes was estimated to be $K = 2$ (Figure 1), with the highest ΔK value of 6.36. As shown in Figure 2, gene pool 1 contained 17 genotypes collected from the Southwestern part of the country (Svinița, Săvârșin) - 5, Bucharest - 4, Constanța - 3, Teleorman - 1, Alba - 1, Prahova - 1, Snagov - 1, Croatia - 1, while gene pool 2 consisted of 14 genotypes collected from Bucharest - 10, Tulcea - 3, Craiova - 1. It can be observed that the genotypes from the Bucharest area were present in both gene pools, although about 71% of genotypes in gene pool 2 originate from Bucharest. Even the genotypes from the Dobrogea area are found in both gene pools, but they are well separated. The genotypes from the southeastern part of Dobrogea (Constanța) are all in gene pool 1, while the genotypes from the northern part of Dobrogea (Tulcea) are in gene pool 2. Similarly, it was observed that 47 caprificus genotypes were placed in two gene pools based on STRUCTURE analysis (Teoman *et al.* 2017).

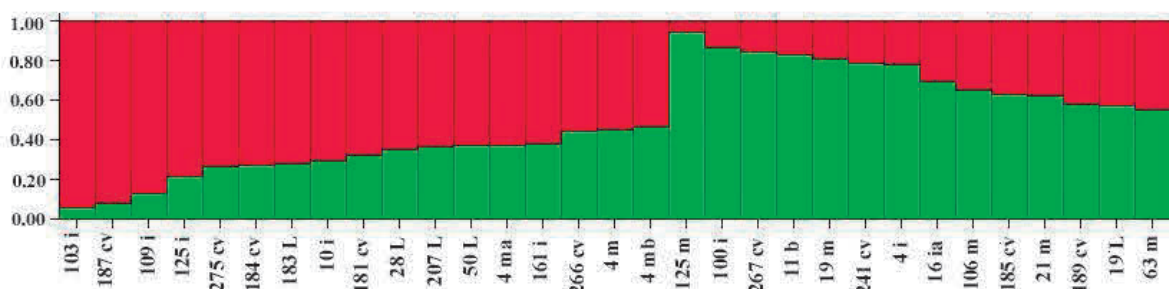


Figure 1. The STRUCTURE bar graph based on 15 SSR markers. Different colours represent different gene pools

According to the UPGMA dendrogram, the 31 fig genotypes were mainly divided into two distinct clusters (A and B), which correspond to the two gene pools identified by STRUCTURE analysis for $K = 2$ (Figure 2). Each cluster was further separated into two

subgroups: Cluster A into Group I and Group II, and Cluster B into Group III and Group IV. Genetic similarity among the fig genotypes ranged from 0.34 to 0.87, indicating high genetic variation in this study. The highest similarity was observed between the genotypes 185 cv and 187 cv (0.87), while the lowest similarity was recorded between 184 cv and 125 m genotypes (0.34).

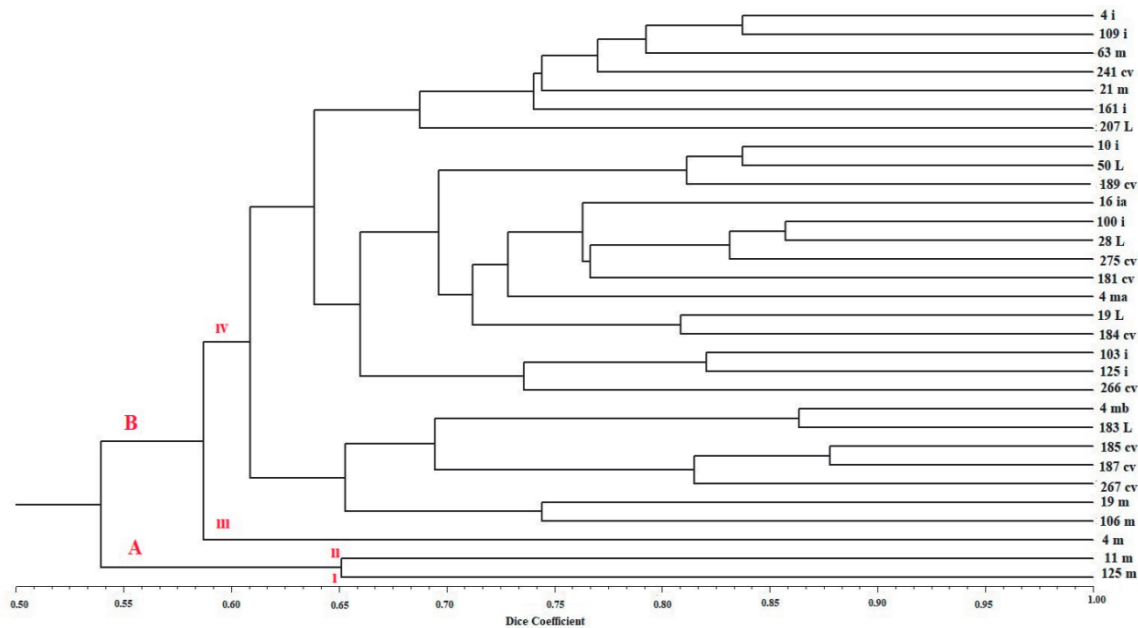


Figure 2. UPGMA dendrogram based on the Dice similarity of 15 SSR markers showing the genetic relationships among 31 fig genotypes.

CONCLUSIONS

The present research demonstrated that all studied fig genotypes from the collection of the Faculty of Horticulture in Bucharest, are genetically distinct. Additionally, there were no homonyms or synonyms among the genotypes in this study, indicating that all genotypes are unique. Genetically related genotypes may share a common genetic origin; however, they are influenced by external factors such as the environment in which they are cultivated. Furthermore, the high genetic variation between the fig genotypes will be valuable for developing national fig breeding programmes. The results of this study also highlight that SSR analysis is an efficient tool to identify fig genotypes.

REFERENCES

1. Ammar, A., Aissa, I.B., Gouiaa, M., Mars, M. 2022. Fig (*Ficus carica* L.) vulnerability to climate change: Combined effects of water stress and high temperature on ecophysiological behaviour of different cultivars. South African Journal of Botany 147 (2022) 482-492. <https://doi.org/10.1016/j.sajb.2022.02.014>
2. Amiteye, S. (2021). Basic concepts and methodologies of DNA marker systems in plant molecular breeding. Heliyon 7 (2021) e08093. <https://doi.org/10.1016/j.heliyon.2021.e08093>.
3. Arvaniti, O.S., Samaris, Y., Gatidou, G., Thomaidis, N. S., Stasinakis, A.S. (2019). Review on fresh and dried figs: Chemical analysis and occurrence of phytochemical compounds, antioxidant capacity and health effects. Food Research International 119 (2019) 244-267. <https://doi.org/10.1016/j.foodres.2019.01.055>
4. Botstein, D., White, R.L., Skolnick, M., Davis, R.W. (1980). Construction of a genetic-linkage map in man using restriction fragment length polymorphisms. Am J Hum Genet 32:314-331. <https://www.ncbi.nlm.nih.gov/pmc/articles/PMC1686077/pdf/ajhg00189-0020.pdf> on 13 May 2023

5. Bandelj, D., Javornik B., Jakse, J. (2007). Development of microsatellite markers in the common fig, *Ficus carica* L. *Molecular Ecology Notes* (2007) 7, 1311–1314. <https://doi.org/10.1111/j.1471-8286.2007.01866.x>.
6. Condit J., (1955). *Fig Varieties*. A Monograph. Hilgardia, A Journal of Agricultural Science Published by the California Agricultural Experiment Station, vol. 23, nr. 11
7. Costa, F., Marchese, A., Mafra, R., Di Vaio, C., Ferrara, G., Fretto, S., Quartararo, A., Marra, F.P., Mennone, C., Vitale, F., S. Reale, S., T. Caruso, T. (2017). Genetic diversity of fig (*Ficus carica* L.) genotypes grown in Southern Italy revealed by the use of SSR markers. *Acta Hort.* 1173. ISHS 2017. <https://doi.org/10.17660/ActaHortic.2017.1173.13>.
8. Dice LR (1945) Measures of the amount of ecologic association between species. *Ecology* 26:297–302. <http://doi.org/10.2307/1932409>.
9. dos Anjos Cruz J. M., Corrêa R. F., Lamarão C. V., Kinupp V. F., Sanches E. A., Campelo P. H., de Araújo Bezerra J. (2022). *Ficus* spp. fruits: Bioactive compounds and chemical, biological and pharmacological properties, *Food Research International*, Volume 152, 2022, 110928, <https://doi.org/10.1016/j.foodres.2021.110928>
10. Dogan, A., Cat, A., Catal, M., Erkan, M. (2018). First report of *Alternaria alternata* causing postharvest decay in fig (*Ficus carica* cv. Bursa Siyahi) fruit in Turkey, *Journal of Biotechnology*. 2018, 280, Supplement, S84 <https://doi.org/10.1016/j.jbiotec.2018.06.275>
11. Duenas, M., Perez-Alonso, J.J., Santos-Buelga, C., Teresa Escribano-Bailon, T. (2008). Anthocyanin composition in fig (*Ficus carica* L.) *Journal of Food Composition and Analysis* 21 (2008) 107–115. <http://doi.org/10.1016/j.jfca.2007.09.002>.
12. Ergül, A., Büyük, B.P., Hazrati, N., Yılmaz, F., Kazan, K., Arslan, N., Özmen, C.Y., Aydın, S.S., Melike Bakır, M., Tan, N., Kösoğlu, I., Çobanoğlu, F. (2021). Genetic characterisation and population structure analysis of Anatolian figs (*Ficus carica* L.) by SSR markers. *Folia Hort.* 33(1) (2021): 49–78 <http://doi.org/2478/fhort-2021-0005>.
13. Essid A., Aljane F., Ferchichi A. (2017). Morphological characterization and pollen evaluation of some Tunisian ex situ planted caprifig (*Ficus carica* L.) ecotypes. *South African Journal of Botany* 111 (2017) 134–143. <http://dx.doi.org/10.1016/j.sajb.2017.03.001>
14. Essid, A., Aljane, F., Neily, M.H., Ferchichi, A., Hormaza, J.J. (2021). Assessment of genetic diversity of thirty Tunisian fg (*Ficus carica* L.) accessions using pomological traits and SSR markers. *Molecular Biology Reports* (2021) 48:335–346 <https://doi.org/10.1007/s11033-020-06051-9>.
15. Evanno, G., Regnaut, S., Goudet, J. (2005) Detecting the number of clusters of individuals using the software STRUCTURE. *Asimulation Study Mol Ecol* 14:2611–2620. <http://doi.org/10.1111/j.1365-294X.2005.02553.x>
16. FAOSTAT (2019). Food and Agriculture Organization of the United Nations (FAO), FAOSTAT Crop Database. www.faostat.org.
17. Ferrara, G., Mazzeo, A., Gallotta, A., Pacucci, C., Matarrese, A. M. S., Tarantino, A., Incerti, O., Marcotuli, I., Nigro, D., Blanco, A., Gadaleta, A. (2017). Fruit-set and SSR markers of fig cultivars from Puglia region, Southeastern Italy. *Acta Horticulturae*, (1173), 39–44. <http://doi.org/10.17660/actahortic.2017.1173.7>
18. Flaishman, M. A., Rodov, V., Stover, E. (2008). The Fig: Botany, Horticulture, and Breeding. *Horticultural Reviews*, 113–196. <http://doi.org/10.1002/9780470380147.ch2>.
19. Futterer, J., Gisel, A., Iglesias, V., Kloti, A., Kost, B., Mittelsten-Scheid, O., Neuhaus, G., Schrott, M., Shillito, R., Spangenberg G., Wang, Z.Y. (1995). Standard molecular techniques for the analysis of transgenic plants. In: Potrykus I, Spangenberg G (Eds). *Gene transfer to plants*. New York, NY, USA: Springer-Verlag pp 215–263.
20. Gaaliche B. Olfa Saddoud,2 and Messaoud Mars 2012. Morphological and Pomological Diversity of Fig (*Ficus carica* L.) Cultivars in Northwest of Tunisia. *ISRN Agronomy*, 2012(), 1–9. doi:10.5402/2012/326461
21. Giraldo, E., Viruel, M.A., López-Corrales, M., Hormaza, J.I. (2005). Characterization and cross-species transferability of microsatellites in the common fig (*Ficus carica* L.), *The Journal of Horticultural Science and Biotechnology*, 80:2, 217–224. <http://dx.doi.org/10.1080/14620316.2005.11511920>.
22. Giraldo, E., López-Corrales, M., Hormaza, J.I. (2008). Optimization of the Management of an Ex-situ Germplasm Bank in Common Fig with SSRs. *J. AMER. SOC. HORT. SCI.* 133(1):69–77. 2008. <http://doi.org/10.21273/JASHS.133.1.69>.
23. Gupta, P.K. and Varshney, R.K. 2000. The development and use of microsatellite markers for genetic analysis and plant breeding with emphasis in bread wheat. *Euphytica* 113: 163–185.

24. Hssaini, L., Hanine, H., Razouk, R., Ennahli, S., Mekaoui, A., Ejilani, A., Charafi, J., 2019. Assessment of genetic diversity in Moroccan fig (*Ficus carica* L.) collection by combining morphological and physicochemical descriptors. *Genet Resour Crop Evol.* <https://doi.org/10.1007/s10722-019-00838-x> (0123456789)
25. Hssaini, L., Hanine, H., Charafi, J., Razouk, R., Elantari, Abderraouf, E. S., Hernandez, F., Ouaabou, R., (2020a). First report on fatty acids composition, total phenolics and antioxidant activity in seeds oil of four fig cultivars (*Ficus carica* L.) grown in Morocco. *OCL*, 27(), 8– <http://doi.org/10.1051/ocl/2020003>.
26. Hssaini, L., Charafi, J., Razouk, R., Hernández, F., Fauconnier, M-L., Ennahli, S., Hanine, H., (2020b): Assessment of Morphological Traits and Fruit Metabolites in Eleven Fig Varieties (*Ficus Carica* L.), *International Journal of Fruit Science*, <http://doi.org/10.1080/15538362.2019.1701615>.
27. Khatib, S and Vaya, J. (2010). Chapter 17. Fig, Carob, Pistachio, and Health. *Bioactive Foods in Promoting Health: Fruits and Vegetables*. 2010 Elsevier Inc.
28. Kislev, M.E., Hartmann, A., Bar-Yosef, O. (2006). Early Domesticated Fig in the Jordan Valley. *Science* 312, 1372 (2006); <http://doi.org/10.1126/science.1125910>.
29. Khadari, B., Hochu, I., Santoni, S., Kjellberg, F. (2001). Identification and characterization of microsatellite loci in the common fig (*Ficus carica* L.) and representative species of the genus *Ficus*. *Molecular Ecology Notes* (2001) 1, 191–193. <http://doi.org/10.1046/j.1471-8278.2001.00072.x>.
30. Khadivi A., Anjam R., Anjam K., 2018. Morphological and pomological characterization of edible fig (*Ficus carica* L.) to select the superior trees. *Scientia Horticulturae* 238 (2018) 66-74 <https://doi.org/10.1016/j.scienta.2018.04.031>
31. Khadivi-Khub, A. and Anjam, K., 2014. Characterization and evaluation of male fig (caprifig) accessions in Iran. *Plant Syst Evol.* <http://doi.org/10.1007/s00606-014-1038-4>.
32. IPGRI, CIHEAM. (2003). Descriptors for Figs. International Plant Genetic Resources Institute (IPGRI), Rome, Italy, and the International Centre for Advanced Mediterranean Agronomic Studies (CIHEAM), Paris, France.
33. Jullien E. et Jullien J., (2011). Soigner les fruitiers, reconnaître et traiter les maladies et ravageurs. Les Editions Eugen Ulmer, printed in Italy
34. Levene H (1949) On a matching problem arising in genetics. *Annal Math Stat* 20:91–94. DOI: 10.1214/aoms/1177730093
35. Mahmoudi, S., Khali, M., Benkhaled, A., Boucetta, I., Dahmani, Y., Attallah, Z. and Belbraouet, S., (2018). Fresh figs (*Ficus carica* L.): pomological characteristics, nutritional value, and phytochemical properties. *Eur. J. Hortic. Sci.* 83(2), 104–113 | ISSN 1611-4426 print, 1611-4434 online <https://doi.org/10.17660/eJHS.2018/83.2.6>. © ISHS 2018
36. Marcotuli, I., Mazzeo, A., Nigro, D., Giove, S.L., Giancaspro, A., Colasuonno, P., Prgomet, Z., Prgomet, I., Tarantino, A., Ferrara, G., Gadaleta, A. (2019). Analysis of genetic diversity of *Ficus carica* L. (*Moraceae*) collection using simple sequence repeat (SSR) markers. *Acta Sci. Pol. Hortorum Cultus*, 18(4) 2019, 93-109. DOI: 10.24326/asphc.2019.4.9
37. Minonne, F., Belloni, P., De Leonardis, V., (2011). Fichi di Puglia, storia, paesaggi, cucina, biodiversit e, conservazione del fico in Puglia. Editrice Coop. Ulliside
38. Mirheidari, F., Khadivi, A., Moradi, Y., Paryan, S. (2020). Phenotypic variability of naturally grown edible fig (*Ficus carica* L.) and caprifig (*Ficus carica* var. *caprificus* Risso) accessions. *Scientia Horticulturae*. Volume 267, 1 June 2020, 109320. <http://doi.org/10.1016/j.scienta.2020.109320>.
39. Nadeem, M.A., Nawaz, M.A., Shahid, M.Q., Doğan, Y., Comertpay, G., Yıldız, M., Hatipoğlu, R., Ahmad, F., Alsaleh, A., Labhane, N., Özkan, H., Chung, G., Baloch, F.S. (2018). DNA molecular markers in plant breeding: current status and recent advancements in genomic selection and genome editing. *Biotechnology & Biotechnological Equipment*, 32:2, 261-285, <https://doi.org/10.1080/13102818.2017.1400401>.
40. Podgornik, M., Vuk, I., Vrhovnik, I., Bandelj, D., Mavsar, D.B., (2010). A survey and morphological evaluation of fig (*Ficus carica* L.) genetic resources from Slovenia. *Scientia Horticulturae* 125 (2010) 380–389. <http://doi.org/10.1016/j.scienta.2010.04.030>.
41. Pourghayoumi, M., Bakhshi, D., Rahemi, M., Noroozisharaf, A., Jafari, M., Salehi, M., Chamane, R., Hernandez, F. (2017). Phytochemical Attributes of Some Dried Fig (*Ficus carica* L.) Fruit Cultivars Grown in Iran. *Agric. conspec. sci.* Vol. 81 (2016) No. 3.
42. Pritchard, J.K., Stephens, M., Rosenberg, N.A., Donnelly, P. (2000). Association mapping in structured populations. *American Journal of Human Genetics* 67:170-181. <http://doi.org/10.1086/302959>

43. Sadler, M.T. and Ateyyeh, A.F. (2006). Molecular assessment of polymorphism among local Jordanian genotypes of the common fig (*Ficus carica* L.). *Scientia Horticulturae* 107 (2006) 347–351. <http://doi.org/10.1016/j.scienta.2005.11.006>.
44. Saki, M., ValizadehKaji, B., Abbasifar, A., Shahrjerdi, I. (2019). Effect of chitosan coating combined with thymol essential oil on physicochemical and qualitative properties of fresh fig (*Ficus carica* L.) fruit during cold storage. *Journal of Food Measurement and Characterization* <https://doi.org/10.1007/s11694-019-00030-w>.
45. Shi, Y., Mon, A. M., Fu, Y., Zhang, Y., Wang, C., Yang, X., Wang, Y. (2018) The genus *Ficus* (*Moraceae*) used in diet: Its plant diversity, distribution, traditional uses and ethnopharmacological importance, *Journal of Ethnopharmacology*, 226, 185-196, <https://doi.org/10.1016/j.jep.2018.07.027>
46. Teoman, S., Ipek M., Erturk U., Aktepe Tangu N., Durgut E., Barut E., Ercisli S., Ipek A. (2017). Assessment of Genetic Relationship among Male and Female Fig Genotypes Using Simple Sequence Repeat (SSR) Markers. *Not Bot Horti Agrobo*, 2017, 45(1):172-178. <http://doi.org/10.15835/nbha45110756>
47. Veberic, R and Mikulic-Petkovsek, M. (2016). Phytochemical Composition of Common Fig (*Ficus carica* L.) Cultivars. *Nutritional Composition of Fruit Cultivars* <http://dx.doi.org/10.1016/B978-0-12-408117-8.00011-8>.
48. Yeh, F.C., Yang, R.C., Boyle, T.B.J., Ye, Z.H., Mao, J.X. (1997) POPGENE the user-friendly shareware for population genetic analysis. Molecular Biology and Biotechnology Centre, Alberta. Retrieved 13 May 2023 from <https://sites.ualberta.ca/~fyeh/popgene.html>
49. Zohary, D. and Hopf, M. (2000). *Domestication of plants in the old world*. 3rd ed. Oxford Univ. Press, New York

Effect of alternative carbohydrates on micropropagation in plant species with special reference to *Vanilla planifolia* Jacks.

C. Córdova-Nieto^{1,2}, L.G. Iglesias-Andreu^{1*}, J.C. Noa-Carrazana¹, A. Alonso-López², G. Hernández -Zárate², J.C. López-Collado² and C. Garcias-Morales¹

¹Instituto de Biotecnología y Ecología Aplicada (INBIOTECA),
Universidad Veracruzana, Campus para la Cultura, las Artes y el Deporte.
Avenida de las Culturas Veracruzanas, México

²Colegio de Postgraduados, Campus Veracruz. Carretera Xalapa-Veracruz, México

*Corresponding author: liglesias@uv.mx

ABSTRACT

One of the challenges posed by cultivating certain agricultural species, such as *Vanilla planifolia* Jacks., is obtaining high-quality plantlets to meet the high global demand for vanillin. However, despite the progress achieved, this crop is severely damaged by fungal infections such as *Fusarium oxysporum* f. sp. *vanillae* (Fov). Through *in vitro* selection, the RH₃₅₀ genotype, which shows a certain level of resistance to this pathogen, is currently available. Therefore, in this study, it was proposed to review the role of different concentrations and sources of carbohydrates in the micropropagation of various plant species. According to the literature, sucrose is the most commonly used carbohydrate, probably because it contributes to greater shoot proliferation and shoot growth. Considering that vegetative propagation techniques require a precise propagation protocol to obtain plants faithful to the promising genotype of interest, it was proposed to develop the present literature review to evaluate the advances obtained in this area between 2000 and 2025. The impact of different carbon concentration and sources is the primary focus of the micropropagation process of the valuable genotype RH₃₅₀ which was obtained through *in vitro* selection, against Fov filtrates for future field evaluation by vanilla producers.

Keywords: carbon sources, orchid, plant tissue culture, *Fusarium oxysporum*, *in vitro*.

INTRODUCTION

In vitro plant culture has proven to be an essential tool, especially useful in clonal propagation and germplasm conservation (Rodríguez-Deméneghi, 2023). However, as plant cells and tissues develop in a controlled artificial environment, they present impairments in the optimal functioning of photosynthesis (Xiao *et al.*, 2011). This situation limits the ability of plants to generate their own assimilable carbon (George *et al.*, 2008), which adversely affects the micropropagation process itself (Fotopoulos and Sotiropoulos, 2004).

To compensate for this deficiency, carbohydrate sources are typically added to the culture medium. In addition to sucrose, which is the most commonly used disaccharide due to its low cost and availability, other carbohydrate sources that are translocated and metabolized in plants are used for this purpose (Hema and Murthy, 2007). These include hexose monosaccharides (glucose, fructose, galactose, and mannose), pentoses (arabinose, ribose, xylose), disaccharides (maltose, lactose, cellobiose, trehalose), and some trisaccharide

(raffinose) (George *et al.*, 2008), as well as some sugar alcohols, also known as polyols, among which are sorbitol, glycerol, as well as mannitol, etc. In addition to their energy function, these exogenous carbon sources are important signaling molecules that interfere with osmotic regulation, morphogenetic responses, biochemical synthesis, and cell differentiation (Lemoine *et al.*, 2013; Ikeuchi *et al.*, 2013), regeneration, and developmental control (Bhojwani and Razdan, 1986). They also participate in central metabolic pathways such as the Krebs cycle and glycolysis. Because each species may have different needs for these carbohydrates, it is imperative to assess their impact on each species and at each stage of cultivation (Thomson and Thorpe, 1987).

These requirements have not yet been defined and optimized in micropropagation systems due to the lack of understanding surrounding certain aspects of carbon utilization and metabolism in cell and tissue cultures. Despite their importance in cell growth, maintenance, and differentiation *in vitro* (Romano *et al.*, 1995). In recent years, particular emphasis has been placed on evaluating the effects of different carbon sources and concentrations, both alone and in combination with other nutrients and growth regulators. This is done in order to maximize seedling quality and propagation rates and to optimize micropropagation work. However, despite the voluminous information available on the subject, there is still no precise analysis of the impact of different carbohydrate sources on micropropagation work, especially in the propagation of such valuable species as *Vanilla planifolia* Jacks. Thus, the goal of the current literature review was to evaluate the advancements in this field from 2000 to 2025. This could support efforts to enhance the micropropagation protocol of the RH₃₅₀ genotype, which was acquired through *in vitro* selection against the main fungal pathogen that poses a serious threat to this crop, *Fusarium oxysporum* f. *vanillae* (Fov).

Carbon sources commonly used in vitro culture

The most popular carbon source for plant micropropagation is sucrose, particularly for orchid species, which have little to no *in vitro* photosynthetic capacity. Several studies have demonstrated that the source and concentration of carbohydrates in the culture have a significant impact on the growth and spread of shoots, which is a crucial component of successful plant regeneration *in vitro*. The use of a concentration of 30 gL⁻¹ sucrose has proven to be the most effective concentration for stimulating the growth of some plant species. According to various authors, this sucrose dose caused a high rate of shoot multiplication in *Zingiber officinale* Roscoe, *Dendrobium nobile* Lindl., and *Cymbopogon flexuosus* Nees ex Steud. W. Watson (Nayak *et al.*, 1997; Rout *et al.*, 2001; Ravi-Kumar *et al.*, 2011).

Similarly, *Cattleya cernua* Lindl. Van den Berg benefited from the application of such a concentration of sucrose, whereas *Cattleya tigrina* A. Rich. benefited from the effective stimulation of protocorms conversion into seedlings (Hisayuki *et al.*, 2021; Silva, 2022). The addition of 30 gL⁻¹ sucrose to *Metroxylon sagu* Rottb. can result in improvements in height growth, leaf number, and shoot fresh weight (Sumaryono *et al.*, 2012). It was found that this carbon source helps to encourage shoot elongation in *Quercus suber* L., demonstrating its effectiveness as the primary carbon source in micropropagation (Romano *et al.*, 1995). Similarly, the use of 30 gL⁻¹ sucrose in *Solanum tuberosum* L. has been shown to promote a high proliferation rate and significant starch accumulation (Ahmad *et al.*, 2021).

However, it has been observed that the optimal sucrose concentration can vary significantly between orchid species and even between different stages of development *in vitro*. Several studies have indicated that different species respond better to lower sucrose concentrations (Martins *et al.*, 2015; Koene *et al.*, 2019). The advantages of using lower concentrations of sucrose for better shoot growth outcomes have been underlined in this context. When Nayak *et al.* (1997) added a concentration of 20 gL⁻¹ sucrose to *D. nobile* they were able to increase shoot length.

The optimal results for *Phalaenopsis* mass propagation were found when a concentration of 15 gL⁻¹ sucrose was combined with a four-hour immersion frequency in temporary immersion systems (like RITA® bioreactors). However, other orchids might react better to media like MS that contain 20–30 gL⁻¹ sucrose (Murashige and Skoog, 1962).

Numerous studies, including those on *Cattleya maxima* Lindl. suggest that sucrose concentration may not significantly affect germination *in vitro* because high germination percentages have been obtained even in the absence of sucrose or with low concentrations of sucrose. Higher sucrose concentrations between 40 and 60 gL⁻¹ are effective in other studies (Sasamori *et al.*, 2021). However, it has been proposed that utilizing a higher sucrose concentration (90 gL⁻¹) is not beneficial for plant growth (George *et al.*, 2008).

Accordingly, it has been recommended that high sucrose concentrations in the growing medium may also harm plants by impeding their ability to absorb nutrients and water (Koene *et al.*, 2019). At higher sucrose concentrations in the culture media, Yildiz *et al.* (2007) observed that three sugar beet lines exhibited a marked decrease in shoot regeneration in addition to an increase in phenolic concentration and necrosis.

These outcomes are also in line with those of Kadota *et al.* (2001), who discovered that high sucrose concentrations were harmful to pears (*Pyrus communis* L.). This could be because a higher carbohydrate concentration causes a decrease in osmotic potential. During micropropagation in temporary immersion systems, high sucrose concentrations are known to induce osmotic stress in orchid tissues, which can occasionally inhibit orchid growth or even raise the risk of certain physiological disorders such as vitrification. Because sugars provide carbon to bacteria and fungi, high concentrations of carbohydrates can also change the structure of the photosynthetic apparatus, prevent the synthesis of chlorophyll and lowering the photosynthetic capacity of the tissues (Texeira Da Silva, 2004). This is particularly low *in vitro* (Rolland *et al.*, 2006). One aspect of interest that has been observed is the fact that sucrose is not always the best carbohydrate (Blanc *et al.*, 1999).

In terms of morphogenic development, it was discovered that using a concentration of 19.82 gL⁻¹ of glucose and fructose produced shoots with superior growth compared to those obtained with sucrose in *Phalaenopsis gigantea* J.J.Sm. and *Cattleya tigrine* A. Rich (Silva *et al.*, 2022). Since fructose and glucose both exhibit a shoot multiplication rate that is at least as high as sucrose, they may be helpful substitutes in *in vitro* culture protocols and have been utilized successfully in the genus *Dendrobium* (Khusairy *et al.*, 2022; Hernández, 2024).

Other recent studies have focused on trehalose, a non-reducing disaccharide involved in critical metabolic pathways and the synthesis of advanced glycation products. In orchids, there is evidence that the ability to effectively utilize trehalose is linked to their mycorrhizal symbiotic relationship with fungi in nature, making it a particularly good carbon source for orchid protocorms and plantlets (Ponert *et al.*, 2021). When compared to sucrose, the application of 30 gL⁻¹ trehalose greatly increased the formation of shoots and roots in species such as *Phalaenopsis* and *Dendrobium officinale* Kimura & Migo (Lee *et al.*, 2015; Nhut *et al.*, 2022).

It has been observed in certain studies, such as those conducted on *Fagus* species, that both sucrose and glucose are appropriate for increasing shoot length; however, in certain clones of this species, glucose demonstrated an exceptional level of effectiveness (Cuenca and Vieitez, 2000). Similarly, it has been shown that early stages of *V. planifolia* development, specifically protocorm formation and seed germination, are significantly impacted by the addition of 20 gL⁻¹ of glucose to the culture medium (Gayatri and Kavyashree, 2005).

Other studies have highlighted that not only glucose but also fructose is involved in the induction of axillary buds of several species of the *Prunus* genus when concentrations of 16 gL⁻¹ are used (Sumaryono *et al.*, 2012; Cheong and An, 2015). These researchers discovered that while the overall vigor of the seedlings produced was marginally lower than that seen with this concentration of sucrose, the use of this concentration of glucose and fructose resulted in sufficient shoot elongation. This also led to an increase in fresh weight. According

to Al-Khayri (2011), *Phoenix dactylifera* shoot length increased moderately when 20 gL⁻¹ fructose was added to the culture medium. A wide range of carbohydrates, including glucose, fructose, and oligosaccharides, are appropriate for the growth and development of *Phalaenopsis* shoots. This is according to research by Hew and Mah (1989) on the asymbiotic mechanism for the quick uptake of the monosaccharide fructose by differentiated and undifferentiated tissues of *Dendrobium* (Arditti, 1990). However, when the effects of sucrose, glucose, and maltose on *Rubus chamaemorus* L. were compared, it was found that both glucose and maltose outperformed sucrose in terms of the efficiency of shoot multiplication (Nayak *et al.*, 1997). Recent studies on *Doritaenopsis* orchids suggest that maltose may be more effective than sucrose in promoting protocorm-like bodies (PLBs) and subsequent seedling development, while sorbitol may affect seedling regeneration (Rahman *et al.*, 2005).

Maltose, a byproduct of the hydrolytic breakdown of starch, is another source of carbohydrates that has drawn special attention from orchids. It is involved in the metabolism of carbohydrates in these species, particularly in CAM plants (Crassulacean Acid Metabolism). One of the reasons maltose performs better than sucrose is thought to be that it has the lowest rate of extracellular hydrolysis, which inhibits the release of phenolic compounds into the culture medium (Kumar *et al.*, 2015).

The use of maltose produced more vigorous shoots in *D. nobile* than those obtained with similar concentrations of sucrose, which was particularly noteworthy (Nayak *et al.*, 1997). Maltose has been less successful in promoting shoot growth and proliferation in other contexts, such as the smaller-sized shoots grown on apple rootstocks, where it is the least effective carbon source (Bahmani *et al.*, 2009). Carrion-Pereira *et al.* (2019) discovered that increasing plant regeneration occurs when sucrose is substituted for maltose in *Urochloa brizantha* Hochst. ex. A. Rich. *in vitro* culture media.

In *Stevia rebaudiana* Bertoni, fructose was found to be effective in maintaining a constant number of shoots, followed by sucrose, maltose, and glucose (Preeth *et al.*, 2011). However, for the *in vitro* propagation of the Marsol chestnut hybrid cultivar, Akyüz (2025) found that the best results were obtained when 30 gL⁻¹ of sucrose was added to the culture medium. Glucose produced results that were better than maltose but not as good as sucrose.

Acclimatization of seedlings to the *ex vitro* environment is a significant challenge in *in vitro* culture. Optimizing the supply of carbohydrates in the later stages of *in vitro* culture can influence the ability of seedlings to develop a strong photosynthetic capacity and improve their survival after transplanting. As a result, it has been noted that an adequate supply of carbohydrates at this stage improves the starch and sucrose reserves in the leaves of the micropropagated plants. These act as organs for energy storage and provide the acclimatization stage for the development of new leaves that are appropriate for the *ex-vitro* environment. Thus, improving acclimatization performance (Fuentes *et al.*, 2005). Similarly, it has been noted that adding 40 gL⁻¹ of sucrose to *Cattleya loddigesii* Lindl. plants during their acclimatization phase are ineffective.

This could be because the high concentration of sugar causes excessive osmotic stress, which lowers metabolic activity *in vitro* (Jo *et al.*, 2009) and subsequently impacts *ex vitro* establishment. Nonetheless, it has been found that the photosynthetic apparatus operates most effectively when the amount of sucrose in the culture medium is decreased. Secondary oxidative stress and slower vine plant growth during acclimatization can result from a higher concentration of sucrose in the culture medium, which alters the synthesis of pigments, particularly carotenoids (Sundryeva *et al.*, 2020).

Impact of various carbon concentrations and sources on vanilla

V. planifolia is an orchid of immense economic importance, recognized worldwide for its aromatic pods, from which vanillin is extracted, a flavoring agent highly valued in the food,

beverage, cosmetics, and pharmaceutical industries. Natural vanilla extract is ranked as the second most expensive spice globally (Cardone *et al.*, 2020).

Despite its high value, traditional vanilla propagation, primarily through stem cuttings, is a labor-intensive, time-consuming, and inefficient process. This results in low multiplication rates and insufficient availability of quality planting material. This limitation directly contributes to the high cost and scarcity of natural vanilla on the market. Global demand for vanillin is projected to exceed 59,000 tons by 2025; however, natural vanilla barely meets 1% of this annual demand. This disparity creates considerable economic pressure to find more efficient production methods. In addition to propagation limitations, conventionally cultivated vanilla is susceptible to the effects of climate change, pests, and diseases.

The reliance on asexual propagation also affects the levels of genetic variation, which reduces the species' ability to adapt and resist different biotic and abiotic factors. Searching for propagation alternatives is a current priority.

Micropropagation represents a vital solution to overcome the challenges of conventional vanilla propagation, allowing for the mass production of disease-free seedlings and facilitating the genetic improvement of this highly valued orchid. However, research on how various elements, such as carbon source and concentration, can enhance mass micropropagation techniques for this valuable species is still ongoing.

To date, 30 gL⁻¹ of sucrose has been added to the culture medium commonly used for this crop's shoot multiplication (Oliveira *et al.*, 2013; Banerjee and Roy, 2023). Other authors, however, have confirmed that utilizing a lower sucrose concentration (20 gL⁻¹) can result in improvements in shoot and root proliferation and length (Samir, 2005; Oliveira, 2013).

More recent studies carried out on *V. planifolia* (Córdova-Nieto *et al.*, 2025, non-published data) have also shown significant differences between the carbohydrate sources and concentrations evaluated (sucrose, glucose, galactose, maltose, and fructose) in terms of shoot length. The use of 15 and 30 gL⁻¹ of maltose and 30 gL⁻¹ of glucose, as well as a similar concentration of fructose, were those that showed the most promising results.

Based on these authors' analysis of the effect on photosynthetic pigment content, the optimal concentrations of maltose for chlorophyll A content were 15 and 30 gL⁻¹, while the lowest concentration (15 gL⁻¹) was found for chlorophyll B content.

A concentration of 15 gL⁻¹ of sucrose and maltose yielded the highest concentration of carotenoids. Figure 1 shows the beneficial effect of some carbohydrate sources and concentrations that were most suitable for this species.

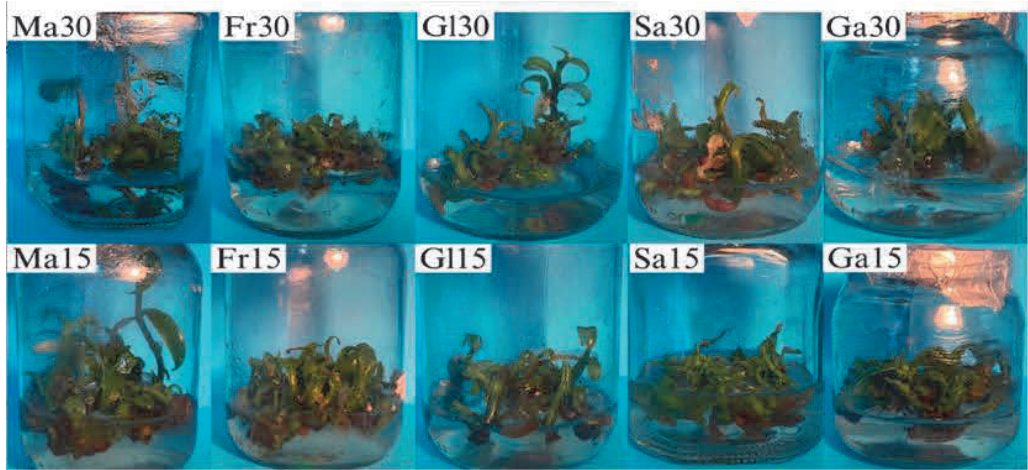


Figure 1. Effect of two concentrations (15 and 30 gL⁻¹) and five carbohydrate sources (*maltose, Ma; fructose, Fr; glucose, Gl; sucrose, Sa; galactose, Ga*) on the characteristics of *in vitro* *V. planifolia* RH₃₅₀ seedlings.

The findings indicated that maltose outperformed fructose in terms of shoot growth and production, while fructose outperformed sucrose and other carbohydrates in terms of shoot proliferation. When compared to the other carbohydrates assessed, galactose performed the worst in terms of shoot growth and proliferation. Finally, studies conducted on *V. planifolia* RH₃₅₀ and other species of the same genus confirm that carbohydrates are essential for establishing an effective micropropagation system. Finding their source and dosage will not only support environmentally conscious, technologically sophisticated, and sustainable production. It may also open up new avenues for study in the fields of plant physiology, biotechnology, and metabolomics when applied to species like *V. planifolia*. These species have significant ecological and commercial value.

CONCLUSIONS

The findings of the review emphasize the significance of carbohydrates for the *in vitro* culture of plants, particularly orchid species, whose photosynthetic efficiency is generally lower *in vitro* than *in vivo*. Therefore, selecting and having an adequate supply of a suitable carbon source is one of the most important factors affecting the success of micropropagation attempts. Even though 30 gL⁻¹ of sucrose has been shown to produce the best results in a variety of plant species, it hasn't always been the best concentration or carbon source to produce higher-quality shoots and faster rates of proliferation. It is crucial to carefully consider the species and cultivation stage because the choice of carbon source can have a direct impact on the outcomes of *in vitro* propagation processes.

REFERENCES

1. Ahmad, M., Khan, M.A. and Anis, M. (2021). Gibberellic acid and thidiazuron promote micropropagation of an endangered woody tree *Pterocarpus marsupium* Roxb. using *in vitro* seedlings. *Plant Cell, Tiss Organ Cult*, 145(2):345–356. <https://doi.org/10.1007/s11240-020-01969-1>
2. Arditti, J.E.R. (1990). Carbohydrate physiology of orchid seedlings. iii. hydrolysis of maltooligosaccharides by *Phalaenopsis* (Orchidaceae) seedlings. *Amer. J. Bot*, 77(2):188-195. <https://doi.org/10.1002/j.1537-2197.1990.tb13545.x>
3. Al-Khayri, J.M. (2011). Somatic embryogenesis of date palm (*Phoenix dactylifera* L.) improved by coconut water. In: S.M. Jain, J.M., Al-Khayri, and D.V. Johnson (Eds.), *Date Palm Biotechnology*. (Pp. 179–190). Springer. <https://doi.org/10.1007/978-94-007-1318-5>
4. Akyüz, B. (2025). Effect of different carbon sources and concentrations on *in vitro* propagation of chestnut. *Plant Cell Tiss Organ Cult*. 160: 25. <https://doi.org/10.1007/s11240-024-02960-w>
5. Bahmani, R., Karam, i O. and Gholam, i M. (2009). Effect of different carbohydrates on *in vitro* proliferation of apple rootstock MM.106. *Plant Cell, Tiss Organ Cult*, 98(2):147–151.
6. Banerjee A. and Roy S.C. (2023). Micropropagation of *Vanilla planifolia* Andrews with modification of cytokinin. *Plant Cell Biotechnology and Molecular Biology*, 24(17–18):147–153. <https://www.researchgate.net/publication/338465072>
7. Bhojwani, S. S. and Razdan, M. K. (1986). *Plant tissue culture: Theory and practice a Revised Edition*. Elsevier, Science Ltd. 756 p.
8. Blanc, G., Michaux-Ferrière N., Teisson, C., Lardet, L. and Carron, M.P. (1999). Effects of carbohydrate addition on the induction of somatic embryogenesis in *Hevea brasiliensis*. *Plant Cell, Tiss Organ Cult*, 59(2): 103–112. <https://doi.org/10.1023/A:1006437731011>
9. Cardone, L., Castronuovo, D., Perniola, M., Cicco, N. and Candido V. (2020). Saffron (*Crocus sativus* L.), the king of spices: An overview. *Sc Horticolt*, 272: 109560. <https://doi.org/10.1016/j.scienta.2020.109560>
10. Carrion-Pereira, A.V., Takamori, L.M., Yaguinuma, D.H., Mendonça de Oliveira, A. and Ferreira-Ribas, A. (2019). La maltosa en los medios de cultivo mejora la regeneración *in vitro* de plantas de *Urochloa brizantha* cv. 'Marandu'. *Bioteecnología Vegetal*, 19(3): 205–213. Retrieved from: https://scielo.sld.cu/scielo.php?pid=S2074-86472019000300205&script=sci_arttext
12. Cheong, E.J. and An, C. (2015). Effect of carbohydrates on *in vitro* Shoot Growth of various *Prunus* Species. *Korean Journal of Plant Research*, 28(3): 357–362. <https://doi.org/10.7732/kjpr.2015.28.3.357>
13. Cuenca, B., Ballester, A. and Vieitez, A. M. (2000). *In vitro* adventitious bud regeneration from internode segments of beech. *Plant Cell, Tiss Organ Cult*, 60(3): 213–220. <https://doi.org/10.1023/A:1006428717309>

14. Fotopoulos, S. and Sotiropoulos, T.E. (2004). *In vitro* propagation of the peach rootstock: the effect of different carbon sources and types of sealing material on rooting. *Biologia Plantarum*, 48 (4): 629-631.
15. Fuentes, G., Talabera, C., Oropeza, C., Desjardins, Y. and Santamaria, J.M. (2005). Exogenous sucrose can decrease *in vitro* photosynthesis but improve field survival and growth of coconut (*Cocos nucifera* L.) *in vitro* plantlets. *In vitro Cellular & Developmental Biology-Plant*, 41: 69-76. <https://doi.org/10.1079/IVP2004597>
16. Gayatri, M.C. and Kavyashree, R. (2005). *In vitro* seed germination studies in *Vanilla planifolia*. *Journal of Horticultural Sciences*, 1(1): 69-72.
17. George, E.F., Hall, M.A. and De Klerk, G.-J. (2008). *Plant Propagation by Tissue Culture* (3rd ed.). Volume 1. 502p. Springer. <https://doi.org/10.1007/978-1-4020-5005-3>
18. Hew, C.S. and Mah, T.C. (1989). Sugar uptake and invertase activity in *Dendrobium* tissues. *New Phytologist*, 111(2): 167-171. <https://doi.org/10.1111/j.1469-8137.1989.tb00678.x>
19. Hema, B.P. and Murthy, H.N. (2007). Effect of sugars on Niger embryogenesis and plant regeneration in anther culture. *Biologia Plantarum*, 51(4): 773-776. <https://doi.org/10.1007/s10535-007-0158-x>
20. Hernández, A. (2024). *In vitro* production of *Phalaenopsis* orchids. *Journal of Orchid Propagation*, 12(2):88-96. Retrieved from: <https://www.researchgate.net/publication/381752779>
21. Hisayuki, M., Sasamori, E D.J. and Droste, A. (2021). Optimal conditions for *in vitro* culture of *Cattleya cernua*, a small orchid native of Atlantic Forest and Cerrado. *Rodriguésia*, 72:3-12. <https://doi.org/10.1590/2175-7860202172059>
22. Ikeuchi, M., Sugimoto, K. and Iwase, A. (2013). Plant callus: mechanisms of induction and repression. *The Plant Cell*, 25(9):3159-3173. <https://doi.org/10.1105/tpc.113.116053>
23. Jo E.A., Tewari R. K., Hahn E.J. and Paek K.Y. (2009). *In vitro* sucrose concentration affects growth and acclimatization of *Alocasia amazonica* plantlets. *Plant Cell Tiss Organ Cult*, 96(3): 307-315. <https://doi.org/10.1007/s11240-008-9488-4>
24. Kadota, M., Imizu, K. and Hirano, T. (2001). Double-phase *in vitro* culture using sorbitol increases shoot proliferation and reduces hyperhydricity in Japanese pear. *Sci Hortic*, 89(3): 207-215. [https://doi.org/10.1016/S0304-4238\(00\)00234-X](https://doi.org/10.1016/S0304-4238(00)00234-X).
25. Khusairy, M. (2022). New insights into tissue culture plant-regeneration mechanisms. *Frontiers in Plant Science*, 13: 926752. <https://doi.org/10.3389/fpls.2022.926752>.
25. Koene, F.M., Amano, É. and Ribas, L.L.F. (2019). Asymbiotic seed germination and *in vitro* seedling development of *Acianthera prolifera* (Orchidaceae). *South African Journal of Botany*, (121): 83-91 <https://doi.org/10.1016/j.sajb.2019.06.005>
26. Khoiratty S., Kodja, H. and Verpoorte, R. (2018). *Vanilla flavor production methods: A review*. *Industrial Crops and Products*, 125: 433-442. <https://doi.org/10.1016/j.indcrop.2018.09.028>
27. Kumar, P.G., Sivakumar, S., Govindarajan, S., Sadasivam, V., Manickam, V., Mogilicherla, K., Kumar-Thiruppathi S.K. and Narayanasamy, J. (2015). Evaluation of different carbon sources for high frequency callus culture with reduced phenolic secretion in cotton (*Gossypium hirsutum* L.) cv. SVPR-2. *Biotechnology Reports*, (7):72-80. <https://doi.org/10.1016/j.btre.2015.06.003>
28. Lee, S.Y., Lee, J.H. and Kim, Y.W. (2015). An efficient method for *in vitro* plant regeneration and micropropagation of *Aloe saponaria*. *Journal of Plant Biotechnology*, 42(3):234-240. <https://doi.org/10.13005/bbra/2164>
29. Lemoine, R., La Camera, S., Atanassova, R., Dédaldéchamp, F., Allario, Th., Pourtau, N., Bonnemain, J.-L., Laloi, M., Coutos-Thévenot, P., Maurousset, L., Faucher, M., Girousse, C., Lemonnier, P., Parrilla, J. and Durand, M. (2013). Source-to-sink transport of sugar and regulation by environmental factors. *Front. Plant Sci*, 4:272. <https://doi.org/10.3389/fpls.2013.00272>
30. Martins, J.P.R., Verdoot, V., Pasqual, M. and De Proft, M. (2015). Impacts of photoautotrophic and photomixotrophic conditions on *in vitro* propagated *Billbergia zebrina* (Bromeliaceae). *Plant Cell Tiss Organ Cult*, 122 (1):53-62. <https://doi.org/10.1007/s11240-015-0820-5>
31. Murashige T. and Skoog F. (1962). A revised medium for rapid growth and bio-assays with tobacco tissue cultures. *Physiologia Plantarum*, 15(3): 473-497. <https://doi.org/10.1111/j.1399-3054.1962.tb08052.x>
32. Nayak, N.R., Patnaik, S. and Debata, B.K. (1997). *In vitro* propagation of *Dendrobium nobile* from axillary bud segments. *Plant Growth Regulation*, 22(1): 65-68.
33. Nhut, D.T., Duc, H.H. and Hoang, N.H. (2022). Efficient transgenic plantlet regeneration from hairy roots via somatic embryogenesis and hardening plantlets of *Panax vietnamensis* by iron nanoparticles-supplied culture. *Plant Cell Tiss Organ Cult*, 151: 335-345. <https://doi.org/10.1007/s11240-022-02355-9>
34. Oliveira, M.L., Matsuoka, K. and Umehara, T. (2013). A new procedure for *in vitro* propagation of vanilla (*Vanilla planifolia*) using a double-phase culture system. *Sci Hort.*, 160: 395-400. <https://doi.org/10.1016/j.scienta.2013.06.039>
35. Ponert, J., Šoch, J., Vosolsobe, S., Čiháková, K. and Lipavská, H. (2021) Integrative Study Supports the Role of Trehalose in Carbon Transfer From Fungi to Mycotrophic Orchid. *Front. Plant Sci.*, 12:793876. <https://doi.org/10.3389/fpls.2021.793876>

36. Preeth, D., Sridhar, T.M. and Naidu, C.V. (2011). Carbohydrate concentration influences on *in vitro* plant regeneration in *Stevia rebaudiana*. Journal of Phytotherapy, 3(5): 61-64. <https://doi.org/10.1016/j.scienta.2013.06.039>
37. Rahman, M.H., Islam, R. and Hossain-Islam, M.S. (2010). Role of sucrose, glucose and maltose on conventional potato micropropagation. Journal of Agricultural Technology, 6(4): 733-739. <https://thaiscience.info/journals/Article/IJAT/10842594.pdf>
38. Ravi-Kumar, K., Sudarsanam, G. and Reddy M.P. (2011). *In vitro* micropropagation of *Zingiber officinale* Rosc. through high frequency shoot proliferation. International Journal of Plant Sciences, 6(1): 85-89.
39. Romano, A., Noronha, C. and Martins-Loução, M.A. (1995). Influence of carbon source on the *in vitro* rooting of cork oak (*Quercus suber* L.). Plant Cell, Tiss Organ Cult, 40(2): 159-167.
40. Rodríguez-Deméneghi, M.V., Aguilar-Rivera N., Gheno-Heredia, Y.A. and Armas-Silva, A.A. (2023). Cultivo de vainilla en México: Tipología, características, producción, prospectiva agroindustrial e innovaciones biotecnológicas como estrategia de sustentabilidad. Scientia Agropecuaria, 14(1):93-109.<https://doi.org/10.17268/sci.agropecu.2023.009>
41. Rolland, F., Baena-González E. and Sheen, J. (2006). Sugar sensing and signaling in plants: conserved and novel mechanisms. Annu Rev Plant Biol, 57:675-709. <https://doi.org/10.1146/annurev.arplant.57.032905.105441>.
42. Rout, G.R., Samantaray, S. and Das, P. (2001). *In vitro* manipulation and propagation of medicinal plants. Biotechnology Advances, 19(2): 91-120. [https://doi.org/10.1016/S0734-9750\(99\)00026-9](https://doi.org/10.1016/S0734-9750(99)00026-9)
43. Sasamori, M.H., Júnior, D.E. and Droste, A. (2021). Optimal conditions for *in vitro* culture of *Cattleya cernua*, a small orchid native of Atlantic Forest and Cerrado. Rodriguesia, 72: 2-12. <https://doi.org/10.1590/2175-7860202172059>
44. Samir, C. D. (2005). Effects of carbon source and concentration on development of lingonberry (*Vaccinium vitis-idaea* L.) shoots cultivated *in vitro* from nodal explants. In Vitro Cellular and Developmental Biology-Plant, 41(2): 145-150. <https://doi.org/10.1079/IVP2004590>
45. Silva, M.A. and Khusairy, M.A. (2022). New insights into tissue culture plant-regeneration mechanisms. Frontiers in Plant Science, 13: 926752. <https://doi.org/10.3389/fpls.2022.926752>
46. Sumaryono, S. and Kasi, P.D. (2012). Effect of carbohydrate source on growth and performance of *in vitro* sago palm (*Metroxylon sagu* Rottb.) plantlets. HAYATI Journal of Biosciences, 19(3): 123-128. <https://doi.org/10.4308/hjb.19.2.88>
47. Sundyeva M., Rebrov A., and Mishko A. (2020). Influence of sucrose concentration in the culture medium on the condition of the photosynthetic apparatus of grapes cultured *in vitro*. BIO Web Conf, 25, 7p. <https://doi.org/10.1051/bioconf/20202504003>
48. Teixeira Da Silva, J. A. (2004). Evaluation of carbon sources as positive or negative factors in chrysanthemum thin cell layer explant culture. Bragantia, 63(2):166-177.
49. Thomson, M. and Thorpe, T.A. (1987). Metabolic and non-metabolic roles of carbohydrates. In: Bong J.M., Durzan, D.J. (eds) Cell and tissue culture in forestry. Martinus Nijhoff Publisher, Dordrecht. (Pp. 89-112). https://doi.org/10.1007/978-94-017-0994-1_6
50. Yildiz, M., Onde, S. and Ozgen, M. (2007). Sucrose effects on phenolic concentration and plant regeneration from sugar beet leaf and petiole explants. Journal Sugarbeet Research, 44: 1-16. <http://doi.org/10.5274/jsbr.44.1.1>
51. Xiao, Y., Niu G. and Kozai, T. (2011). Development and application of photoautotropic micropropagation systems. Plant Cell Tiss Organ Cult, 105 (2): 149-158. <http://doi.org/10.1007/s11240-010-9863-9>

Results of highlighting genes associated with ethylene production in apple genotypes cultivated in Romania

A.F. Iancu^{1,3}, M. Militaru² and S. Cosmulescu³

¹Doctoral School of Plant and Animal Resources Engineering, Faculty of Horticulture, University of Craiova, Craiova, Romania

²Research Institute for Fruit Growing, Pitesti, Romania

³Department of Horticulture and Food Science, Faculty of Horticulture, University of Craiova, Romania

Corresponding author email: adinafloricica@yahoo.com

ABSTRACT

Molecular screening using SSLP and CAPS techniques enabled the genotyping of 40 apple cultivars developed at the main national research centres in Romania. All markers proved to be informative, successfully amplifying the target fragments previously reported in multiple studies. Genotyping with SSLP and CAPS techniques revealed a moderate but statistically significant level of genetic diversity and a high degree of allelic polymorphism. SSLP-based genotyping detected all expected allelotypes in the cultivars, whereas the CAPS technique identified all three expected allelotypes only when digested with the TaqI enzyme; digestion with BstNI did not yield all anticipated allelotypes. Pearson correlation analysis between allelotypes identified through SSLP and firmness loss after 30 days of storage showed no significant correlation for the bb/aa allelotype, which is associated with efficient ethylene limitation. However, a weak negative correlation was observed for the bb/ab allelotype, corresponding to a moderate ethylene production rate. The estimated inbreeding coefficient indicated a moderate reduction in observed heterozygosity for the MdACS1 marker, while a moderate heterozygote excess ($H_o > H_e$) was recorded for MdACO1 and MdACS3a markers. The study highlights a significant level of genetic diversity and provides valuable information for marker-assisted selection aimed at developing cultivars with superior postharvest quality.

Keywords: Firmness, CAPS technique, SSLP technique, Md-ACS1, MdACO1, MdACS3a, Apple species.

INTRODUCTION

Fruit ripening is a complex physiological process regulated by the so-called System II of ripening and mediated through mechanisms involving the plant hormone ethylene. In the context of apple breeding, understanding the genetic mechanisms that regulate ethylene concentration is essential, as producing high-quality fruits and extending storage life are major objectives. To date, seven genes encoding 1-aminocyclopropane-1-carboxylate synthase (ACS) have been identified in apple: MdACS1 (U89156), MdACS3a (AB243060), MdACS3b (AB243061), MdACS3c (AB243062), MdACS4, MdACSSA (AB034992), and MdACSSB (AB034993), among which only MdACS1 and MdACS3a are expressed in fruit tissues (Li *et al.*, 2013). Fruit firmness is physiologically influenced by ethylene concentration, and previous studies in apple have shown that ethylene biosynthesis is regulated by MdACS1, MdACO1, and MdACS3a. The ACS enzyme is encoded by MdACS1 and

MdACS3a, with MdACS1 playing a limiting role in the ethylene production pathway, while MdACS3a appears to mediate the transition from System I (basal ethylene production) to System II (the climacteric ethylene surge). Yue *et al.* (2020) reported that the transcription factor MdERF2, a member of the ERF family, restricts the accumulation of ACC (1-aminocyclopropane-1-carboxylic acid), thereby delaying the shift from System I to System II. Consequently, reduced expression of MdERF2 leads to upregulation of MdACS3a and MdACS1, accelerating fruit ripening and promoting firmness loss (Li *et al.*, 2017). MdACO1 encodes an ACC oxidase responsible for converting ACC into ethylene, and the molecular marker developed by Costa *et al.* (2005), also used in the present study, amplifies two DNA fragments of 525 bp and 587 bp a result confirmed by other studies (Shamshin *et al.*, 2018; Lyzhin, 2020). Expression of the System II gene MdACS1 correlates with ethylene production at the onset of fruit maturity: the MdACS1-2 allele (655 bp) is associated with low ethylene production and extended storage life, whereas MdACS1-1 (489 bp) corresponds to higher ethylene levels and shorter storage potential (Costa *et al.*, 2005; Sunako *et al.*, 1999). Heterozygous genotypes MdACS1-1/2 exhibit intermediate ethylene production compared with MdACS1-1/1 (higher) and MdACS1-2/2 (lower) (Wu *et al.*, 2021). MdACO1 acts independently of MdACS1 and has a less pronounced effect on storage capacity (Costa *et al.*, 2005; Oraguzie *et al.*, 2004). Allelic variation in the System I gene MdACS3a, expressed during fruit development (Costa *et al.*, 2005; Dougherty, 2019; Lundmark, 2019; Shamshin *et al.*, 2018), also plays a key role in ripening regulation. A G289V point mutation renders the ACC synthase enzyme non-functional (Wang *et al.*, 2009). Dougherty (2019) found that the G/T allelotype produces more ethylene than G/G, but less than MdACS1-1/1. Using the CAPS₈₇₀ marker, the T/T genotype showed a one-day delay in the ethylene peak compared with C/C and C/T, although ethylene levels and firmness loss identified through CAPS₈₆₆ and CAPS₈₇₀ markers were not consistently correlated. Overall, allelic effects of MdACS3a seem to limit the ethylene peak, whereas firmness loss is likely influenced by factors other than ethylene concentration. The aim of this study is to identify and highlight the genes associated with ethylene production in apple genotypes cultivated in Romania, in order to better understand the genetic variation underlying fruit ripening processes and storage capacity.

MATERIALS AND METHODS

Biological material. The biological material studied consists of 40 cultivar of apple, cultivar from the collections of the Research Institute for Fruit Growing Pitești - Maracineni ('Colonade', 'Colmar', 'Nicol', 'Rebra', 'Remus', 'Romus 3', 'Romus 4', 'Romus 5', 'Rustic', 'Doina', 'Alex', 'Aura', 'Bistrițean', 'Dany', 'Jonaprim', 'Salva', 'Starkprim', 'Precoce de Ardeal', 'Auriu de Cluj', 'Productiv de Cluj', 'Estival') și Research Station for Fruit Growing Voinești ('Cezar', 'Ciprian', 'Dacian', 'Delicios de Voinești', 'Discoprim', 'Frumos de Voinești', 'Generos', 'Inedit', 'Iris', 'Irisem', 'Luca', 'Pomona', 'Real', 'Redix', 'Remar', 'Revidar', 'Valery', 'Voinea', 'Voinicel').

PCR-SSLP and PCR-CAPS amplification. The PCR reaction mixture was optimised to a final volume of 15 µl, of which 11.5 µl was constituted of MyTaq™ Red Mix. For each primer pair (forward and reverse), 0.1 µl was added at a concentration of 0.1 mM, resulting in a final primer concentration of 0.666 µM per primer. DNA concentration was maintained within the range of 75–100 ng/µl, and 0.3 µl of ultrapure water was added to achieve the final reaction volume. Reaction conditions were identical across all primer pairs and optimised as follows: initial denaturation at 95 °C for 3 min; 35 cycles of 94 °C for 1 min, 58 °C for 1 min, and 72 °C for 2.5 min; followed by a final extension at 72 °C for 10 min.

Enzymatic restriction. Amplicons generated through amplification with the dCAPS (ACS3a) primer were digested with two restriction enzymes, BstNI and TaqI. The digestion reaction

was optimised to a final volume of 15 µl, comprising 10 µl of DNA, 2 µl of 10X Buffer R, 2 µl of BstNI or TaqI, and 1 µl of ultrapure water. The mixtures were incubated for 1 h at 37 °C for BstNI and at 65 °C for TaqI using a FastGene analyser.

Statistical analysis. The Pearson correlation coefficient was calculated using Minitab v.18, and the interpretation of the resulting values followed the criteria proposed by Colton (1974) (Table 1). The inbreeding coefficient was estimated according to Sewall Wright's concept (Wright, 1965), which defines it as the proportional reduction in observed heterozygosity relative to the level expected under Hardy-Weinberg equilibrium. This coefficient is expressed mathematically as $F_{is} = 1 - H_o/H_e$, where H_o represents the observed heterozygosity and $H_e = 2pq$ denotes the expected heterozygosity, with p and q being the allele frequencies in the population, and $p + q = 1$. Allelic polymorphism was evaluated using the Polymorphic Information Content (PIC) index, calculated using the formula $2f(1 - f)$, as described by Roldán-Ruiz *et al.* (2000) and Soengas *et al.* (2006). Genetic diversity was further quantified using the Shannon and Simpson indices, based on the formulas reported by Shannon (1948) and Simpson (1960). Thus, the Shannon index was computed as $\sum(n/N * \ln(n/N))$, and the Simpson index as $(\sum n(n - 1)) / (N(N - 1))$, where n denotes the number of alleles at a given locus and N the total number of alleles.

Table 1. Colton's rules (enounced in 1974)

Range of values	Level of differentiation
$-0,25 < r < 0,25$	Without correlation
$-0,5 < r < -0,25$ sau $0,25 < r < 0,5$	Poor correlation
$-0,75 < r < -0,5$ sau $0,5 < r < 0,75$	Moderate correlation
$r < -0,75$ sau $r > 0,75$	Strong correlation

RESULTS AND DISCUSSIONS

Genotyping of the cultivars using the MdACS1 marker identified 13 cultivars homozygous for the Md-ACS1-1 allele (489 bp), 8 cultivars ('Cezar', 'Discoprim', 'Luca', 'Productiv de Cluj', 'Romus 5', 'Salva', 'Remar', and 'Valery') homozygous for the Md-ACS1-2 allele (655 bp) and 19 heterozygous cultivars corresponding to the ab allelotype (Md-ACS1-1/2) (Table 2). Analysis of the Md-ACO1 marker revealed an additional amplification fragment (512 bp) alongside the previously known fragments (525 bp and 587 bp), as reported by Costa (2005). Our results corroborate those of Shamshin *et al.* (2018). So, 19 cultivars were identified as homozygous for the Md-ACO1-1 allele (525 bp), and 20 cultivars were heterozygous, corresponding to the ab allele (Md-ACO1-1/2). The homozygous Md-ACO1-2 state (bb allelotype, 587 bp) was not observed in any of the cultivars analysed. Additionally, the genetic profile of the 'Generos' cultivar did not conform to the observed allelotypic patterns, presenting a rare atypical profile that may indicate a potential mutation at this locus. (Table 2). In the case of the MdACS3a marker, digestion with BstNI resulted in 21 heterozygous cultivars corresponding to the G/T allelotype (MdACS3a/MdACS3a-G289V) and 19 homozygous cultivars corresponding to the G/G allelotype (MdACS3a/MdACS3a).

No cultivars were identified for the T/T allelotype (MdACS3a-G289V/G289V) among the total cultivars analysed (Fig. 1), and digestion with Taq1 enzyme, resulted in 23 heterozygous cultivars corresponding to the C/T allelotype (MdACS3a/Mdacs3a), 13 homozygous cultivars associated with the T/T allelotype (Mdacs3a/Mdacs3a), and 4 homozygous cultivars corresponding to the C/C allelotype (MdACS3a/MdACS3a) (Figure 2).

Table 2. The result of genotyping and highlighting alleotypes*

Nr. crt.	Cultivar	Highlighted gene				Genotyping	FD
		Md-ACS1	Md-ACO1				
		Alleles of ethylene biosynthesis genes				Allelotype	loss of firmness
		Md-ACS1-1 489 bp	Md-ACS1-2 655 bp	Md- ACO1-1 525 bp	Md-ACO1- 2 587 bp	Md-ACS1 / Md-ACO1	Kgf/m ²
1	Alex	+	+	+	+	ab/ab	0,132
2	Ciprian	+	+	+	+	ab/ab	0,181
3	Dany	+	+	+	+	ab/ab	0,585
4	Delicios de Voinești	+	+	+	+	ab/ab	0,15
5	Frumos de Voinești	+	+	+	+	ab/ab	0,843
6	Irisem	+	+	+	+	ab/ab	1,17
7	Nicol	+	+	+	+	ab/ab	0,280
8	Redix	+	+	+	+	ab/ab	0,089
9	Rebra	+	+	+	+	ab/ab	0,122
10	Romus 4	+	+	+	+	ab/ab	0,324
11	Voinea	+	+	+	+	ab/ab	0,17
12	Voinicel	+	+	+	+	ab/ab	0,374
13	Colmar	+	-	+	-	aa/aa	0,11
14	Colonade	+	-	+	-	aa/aa	0,12
15	Dacian	+	-	+	-	aa/aa	0,43
16	Estival	+	-	+	-	aa/aa	0,428
17	Real	+	-	+	-	aa/aa	0,236
18	Remus	+	-	+	-	aa/aa	0,193
19	Romus3	+	-	+	-	aa/aa	0,324
20	Starkprim	+	-	+	-	aa/aa	0,504
21	Discoprim	-	+	+	-	bb/aa	0,377
22	Luca	-	+	+	-	bb/aa	0,888
23	Productiv de Cluj	-	+	+	-	bb/aa	0,275
24	Salva	-	+	+	-	bb/aa	0,560
25	Valery	-	+	+	-	bb/aa	0,21
26	Auriu de Cluj	+	-	+	+	aa/ab	0,367
27	Bistrițean	+	-	+	+	aa/ab	1,162
28	Doina	+	-	+	+	aa/ab	0,59
29	Jonaprim	+	-	+	+	aa/ab	0,39
30	Pomona	+	-	+	+	aa/ab	0,189
31	Aura	+	+	+	-	ab/aa	0,86
32	Iris	+	+	+	-	ab/aa	0,25
33	Inedit	+	+	+	-	ab/aa	0,300
34	Revidar	+	+	+	-	ab/aa	0,410
35	Precoce de Ardeal	+	+	+	-	ab/aa	0,60
36	Rustic	+	+	+	-	ab/aa	0,214
37	Remar	-	+	+	+	bb/ab	0,216
38	Romus5	-	+	+	+	bb/ab	0,213
39	Cezar	-	+	+	+	bb/ab	0,030
40	Generos	+	+	-	-	ab/0	0,435

* (bb/aa)= effective limitation of ethylene production; (bb/ab)= high ethylene level than bb/aa; (ab/aa)= high ethylene level than bb/ab; (ab/ab)= high ethylene level than ab/aa; (aa/aa)= high ethylene level than ab/ab; (aa/ab)= nivel de etilenă mai ridicat decât alelotipul aa/aa;

This distribution highlights the genetic diversity at the MdACS3a locus and suggests that heterozygous and homozygous allelotypes could be valuable for breeding programs targeting ethylene regulation and improved postharvest firmness.

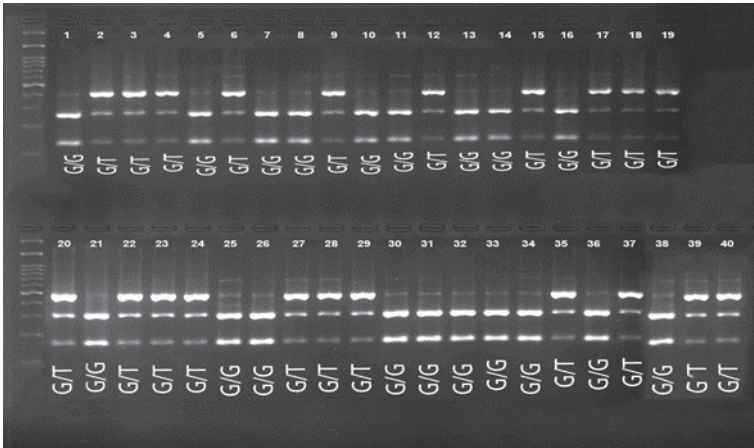


Figure 1. Electrophoretic profile for ACS3a marker with BstNI enzyme restriction.
1. 'Estival', 2. 'Rebra', 3. 'Bistrițean', 4. 'Aura', 5. 'Romus 3', 6. 'Dany', 7. 'Romus 5', 8. 'Productiv de Cluj', 9. 'Luca', 10. 'Ciprian', 11. 'Irisem', 12. 'Slava', 13. 'Jonaprim', 14. 'Rustic', 15. 'Precoce de Ardeal', 16. 'Iris', 17. 'Starkprim', 18. 'Auriu de Cluj', 19. 'Generos', 20. 'Colonade', 21. 'Nicol', 22. 'Colmar', 23. 'Delicios de Voinești', 24. 'Romus 4', 25. 'Remus', 26. Redix", 27. 'Alex', 28. 'Doina', 29. 'Voinicel', 30. 'Inedit', 31. 'Dacian', 32. 'Voinea', 33. 'Valery', 34. 'Real', 35. 'Discoprim', 36. 'Cezar', 37. 'Frumos de Voinești', 38. 'Pomona', 39. 'Revidar', 40. 'Remar'

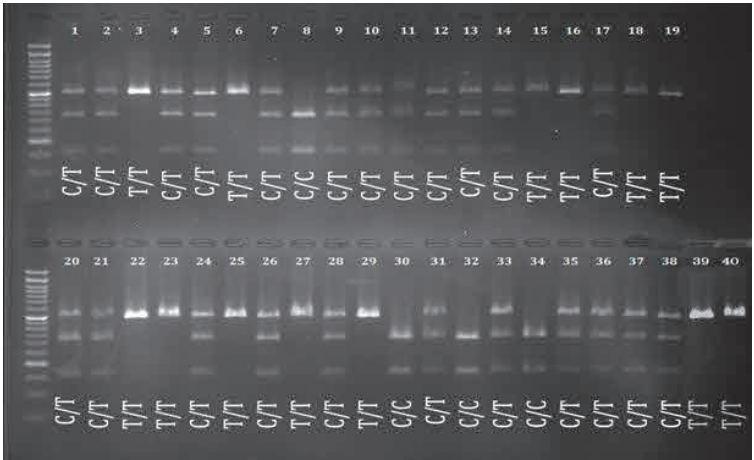


Figure 2. Electrophoretic profile for ACS3a marker with TaqI enzyme restriction.
1. 'Estival', 2. 'Rebra', 3. 'Bistrițean', 4. 'Aura', 5. 'Romus 3', 6. 'Dany', 7. 'Romus 5', 8. 'Productiv de Cluj', 9. 'Luca', 10. 'Ciprian', 11. 'Irisem', 12. 'Slava', 13. 'Jonaprim', 14. 'Rustic', 15. 'Precoce de Ardeal', 16. 'Iris', 17. 'Starkprim', 18. 'Auriu de Cluj', 19. 'Generos', 20. 'Colonade', 21. 'Nicol', 22. 'Colmar', 23. 'Delicios de Voinești', 24. 'Romus 4', 25. 'Remus', 26. Redix", 27. 'Alex', 28. 'Doina', 29. 'Voinicel', 30. 'Inedit', 31. 'Dacian', 32. 'Voinea', 33. 'Valery', 34. 'Real', 35. 'Discoprim', 36. 'Cezar', 37. 'Frumos de Voinești', 38. 'Pomona', 39. 'Revidar', 40. 'Remar'.

The inbreeding coefficient revealed a moderate reduction in observed heterozygosity for the MdACS1 marker and a moderate heterozygote excess ($H_o > H_e$) for the MdACO1 and MdACS3a markers (Table 3).

Table 3. Genetic diversity and inbreeding parameters for each marker

Marker	q	p	He	Ho	Fis
MdACS1	0,4375	0,5625	0,4921	0,475	0,034
MdACO1	0,2564	0,7435	0,3812	0,5128	-0,3452
MdACS3a (BstNI)	0,2625	0,7375	0,3871	0,525	-0,3562
MdACS3a (TaqII)	0,6125	0,3875	0,4746	0,575	-0,2115

Allelic polymorphism displayed a high information content (0.34125), surpassing the moderately informative threshold, while genetic diversity showed a moderately informative value (Table 4).

Table 4. Statistical analysis of allelic polymorphism and genetic diversity

PIC value>0,25 (moderately informative)	Indicele Shannon H > 3 (high genetic diversity)	Indicele Simpson (D) D=0 (infinite diversity) D=1 (lack of diversity)	Simpson diversity index (1-D), value € [0,1]
0,34125	2,0884191	0,1279803	0,8720197

Correlation analysis conducted using Minitab 18, in accordance with Colton (1974) criteria, between allelotypes identified through the SSLP technique and pulp firmness loss after 30 days of storage indicated a non-significant Pearson correlation coefficient (Table 5). Nevertheless, a weak negative correlation was observed for the *bb/ab* allelotype, suggesting that the presence of this allelotype contributes to reduced firmness loss. The homozygous *bb* state, corresponding to the *MdACS1* gene is associated with effective limitation of ethylene production, whereas the heterozygous *ab* state, corresponding to the *MdACO1* gene, is linked to an intermediate ethylene production level.

Table 5. Pearson correlation analysis between fruit firmness loss and identified allelotypes after 30 days of storage

Allelotip	bb/aa	bb/ab	ab/aa	aa/aa	aa/ab	ab/ab	ab/0
Pearson Index	0,110	-0,239	0,087	-0,163	0,217	-0,034	0,031
P-Value	0,500	0,138	0,595	0,314	0,179	0,835	0,851

Experimentally determined firmness values after 30 days of storage for the *bb/ab* allelotype showed that only three cultivars ('Discoprim', 'Productiv de Cluj', and 'Valery') exhibited good firmness retention, although they did not show the lowest overall firmness loss. As expected, the *bb/aa* allelotype should have conferred a more efficient reduction in ethylene production, compared to the *bb/ab* allelotype.

Nevertheless, genetic determinism alone does not fully account for the association between allelotype and firmness retention, suggesting that additional factors—climatic, agronomic, physiological, chemical, regulatory protein-related, and other genetic influences—also contribute to this trait.

Previous studies have shown that fruit firmness is a physico-structural characteristic influenced by physiological processes (Mangas *et al.*, 1992; Costa *et al.*, 2016; Ornelas-Paz *et al.*, 2018; Gwanpua *et al.*, 2015; 2016; Eshtiaghi and Kuldiloke, 20).

Consequently, the genotyping results obtained through the CAPS technique proved useful for studying polymorphism and genetic diversity (inbreeding coefficient, PIC, Shannon, and Simpson indices).

CONCLUSIONS

Apple fruit storability is largely governed by genetic factors but is also modulated by physiological influences, as no significant correlation was observed between allelotypes of System I ripening genes (*MdACS1* and *MdACO1*) and postharvest firmness loss. Analysis using molecular markers associated with ethylene biosynthesis demonstrated a notable

level of genetic diversity among the evaluated apple cultivars, reflected in a moderate excess of heterozygotes and in the values of Shannon and Simpson indices. The identification of allelotypes linked to firmness retention provides a solid foundation for marker-assisted selection, supporting the development of cultivars with enhanced postharvest quality.

ACKNOWLEDGEMENTS

The study presented in this paper is part of the doctoral thesis of A.F. Iancu and was carried out with the financial support of the University of Craiova, the Faculty of Engineering of Animal and Vegetal Resources and the Research Institute for Fruit Growing, Pitesti-Maracineni.

REFERENCES

- Colton, T. (1974). Statistics in Medicine. Little Brown and Company, Boston, 164-8.
- Costa, F., Stella, S., Van, De Weg, W.E., Guerra, W. Cecchine, M., Dallavia, J., Koller, B., Sansavini, S. (2005). Role of the genes Md-ACO1 and Md-ACS1 in ethylene production and shelf life of apple (*Malus domestica* Borkh). Euphytica 141:181-190.
- Costa, F., Cappellin, L., Longhi, S., Guerra, W., Magnago, P., Salvi S. (2016). Genetic and physiological factors affecting apple fruit firmness. Frontiers in Plant Science, 7, 35.
- Dougherty, L.E. (2019). Uncovering genes controlling apple tree architecture and fruit quality. PhD Thesis, Cornell University, 282.
- Eshtiaghi, M.N. and Kuldiloke J. (2013). Impact of hight hydrostativ pressure on gel formation of low methoxilpectin. African Journal of Pharmacy and Pharmacology, 7(22): 1455-1463.
- Gwanpua, S. G., Verlinden, B. E., Hertog, M. L. A. T. M., Nicola, A. B. M., Hendrickx, M. and Geeraerd, A. H. (2016). Understanding the regulation of texture degradation during apple softening-a kinetic modelling approach. In VIII International Postharvest Symposium: Enhancing Supply Chain and Consumer Benefits-Ethical and Technological Issues 1194, pp. 1399-1406.
- Li, T., Tan, D., Yang, X., Wang, A. (2013). Exploring the apple genome reveals six ACC synthase genes expressed during fruit ripening. Scientia Horticulturae 157:119-123.
- Li, T., Xu, Y., Zhang, L., Ji Y., Tan, D., Yuan, H., Wang, A. (2017). The jasmonate-activated transcription factor MdMYC2 regulates ethylene response factor and ethylene biosynthetic genes to promote ethylene biosynthesis during apple fruit ripening. The Plant Cell 29(6): 1316-1334.
- Lundmark, J. (2019). Genotyping ethylene production genes Md-ACS1 and Md-ACO1 for markerassisted selection in apple. First cycle, G2E. Alnarp: SLU, Dept. of Plant Breeding.
- Lyzhin, A.S. (2020). Molecular and genetic analysis of hybrid apple seedlings on genes MD-ACS1 and MD-ACO1 of ethylene biosynthesis. Scientific Works of North Caucasian Federal Scientific 30: 9-14. DOI:10.30679/2587-9847-2020-30-9-14.
- Mangas, J. J., Martínez, R., Garrido, J. (1992). Textural changes during fruit ripening. Journal of Food Science, 57(6): 1412-1415.
- Oraguzie, N.C., Iwanami, H., Soejima, J., Harada, T., Hall, A. (2004). Inheritance of the Md-ACS1 gene and its relationship to fruit softening in apple (*Malus domestica* Borkh.). Theoretical and Applied Genetics 108(8):1526-1533.
- Ornelas-Paz, J. J., Yahia E. M., González-Aguilar, G. A. (2018). Fruit firmness and quality during ripening. Postharvest Biology and Technology, 145: 49-60.
- Roldán-Ruiz, I., Dendauw, J., Van, Bockstaele, E., Depicke, A., De Loose M. (2000). AFLP markers reveal high polymorphic rates in ryegrasses (*Lolium* spp.). Molecular Breeding 6: 125-134.
- Shamshin I.N., Shlyavas A.V., Trifonova A.A., Boris K.V., Kudryavtsev A.M. (2018). Ethylene and expansin biosynthesis related genes polymorphism in local apple (*Malus domestica* Borkh.) cultivars from VIR Collection of plant genetic resources. Vavilov Journal of Genetics and Breeding 22(6):660-666.
- Shannon, C. E. (1948). A mathematical theory of communication. Bell System Technical Journal, 27, 379-423; 623-656.
- Simpson, E. H. (1960). Notes on the measurement of diversity. Nature 183: 688-688.
- Soengas, P., Ordás B., Malvar, R. A., Revilla, P. (2006). Isozyme variation and genetic relationships among Spanish populations of *Brassica rapa* L. Euphytica 150: 269-279.
- Sunako, T., Sakuraba, W., Senda, M., Akada, S., Ishikawa, R., Niizeki M., Harada, T. (1999). An allele of the ripening-specific 1-aminocyclopropane-1-carboxylic acid synthase gene (ACS1) in apple fruit with a long storage life. Plant Physiology 119(4):1297-1304.

20. Wang, A., Yamakake, J., Kudo, H., Wakasa, Y., Hatsuyama, Y., Ygarashi, M., Kasai ,A., Li T., Harada, T. (2009). Null mutation of the MdACS3 gene, coding for a ripening-specific 1-aminocyclopropane-1-carboxylate synthase, leads to long shelf life in apple fruit. *Plan Physiology* 151: 391-399.
21. Wright, S. (1965). The interpretation of population structure by F-statistics with special regard to systems of mating. *Evolution* 19: 395–420.
22. Wu, B., Shen, F., Chen, C. J., Liu, L., Wang, X., Zheng, W. Y., Zhan, X. Z. (2021). Natural variations in a pectin acetyltransferase gene, MdPAE10, contribute to prolonged apple fruit shelf life. *The Plant Genome* 14(1): e20084.
21. Yue, P., Lu, Q., Liu Z., Lv,, T., Li X., Bu H., Wang, A. (2020). Auxin-activated MdARF5 induces the expression of ethylene biosynthetic genes to initiate apple fruit ripening. *New phytologist* 226(6): 1781-1795.

Grapevine viruses – a global challenge and biotechnological solutions to obtain healthy material

E. C.Buciumeanu, I. C. Guță* and D.I. Sumedrea

National Research and Development Institute for Biotechnology
in Horticulture, Stefanesti - Arges, Romania

*Corresponding author email: gutaionelacatalina@yahoo.com

ABSTRACT

Grapevine (*Vitis* spp.) is one of the most important horticultural species, but also one of the most vulnerable to viral infections. To date, 102 viruses are known to infect grapevines. Of these, some viruses cause significant economic damage worldwide, affecting the quantity and quality production, plant growth and development, and the vineyards long-lasting. Other viruses have a broad spectrum, affecting several crop plants and represent scientific curiosities for the grapevine, both because they are rarely encountered and the damages induced are negligible. This paper summarizes the main knowledge regarding the diversity of viral agents affecting grapevine and the associated symptomatology characteristics. The economic effects of viral diseases are analyzed, highlighting their influence on propagation material and grape production. Once virus-infected, grapevine plants remain infected for their entire life. Prevention of infection includes the production of certified virus-free planting material and virus testing. Thus, the propagation of healthy material, originating from authorized sources, tested and certified as virus-free, is essential for sustainable viticulture. Also, virus-elimination methods are presented (meristem/shoot tip cultures, thermotherapy, *in vitro* chemotherapy, electrotherapy, cryotherapy, and combined methods), which allow the regeneration of healthy, virus-free plants. The integration of these technologies into virus-free germplasm selection and conservation programs represents a priority direction for a modern viticulture, ensuring the maintenance of grapevine health, the protection of genetic diversity and the sustainability of grapevine production.

Keywords: *Vitis*, GFLV, GLRaVs, GFkV, economic effects, virus-elimination

INTRODUCTION

Grapevine (*Vitis* spp.) is one of the most important horticultural plants in the world due to its economic and social impact (www.oiv.int).

The grapevine market is considered to be one of the most dynamic and fastest-growing agricultural markets in recent years.

Like most vegetatively propagated plants, grapevines are exposed to attacks by many pests and pathogens, among which intracellular infectious agents (viruses, viroids, phytoplasmas) play a major role, causing important losses, shortening the productive life of vineyards and endangering the survival of affected plants (Martelli and Boudon-Padieu, 2006; Moutinho-Pereira *et al.*, 2012; Cuozzo *et al.*, 2018).

Therefore, timely monitoring of factors that may have a negative effect on plant growth and product quality, primarily the control of the causal agents of grapevine diseases, is of critical importance for grape production (Martelli and Boudon-Padieu, 2006; Zherdev *et al.*, 2018).

Due to their latent mode of manifestation and transmission through vectors or vegetative propagation material, grapevine viruses constitute a major challenge for viticulture, with long-lasting economic and genetic effects.

In recent decades, the research has focused on understanding the mechanisms of infection, assessing the physiological impact of viral diseases, and developing efficient technologies to obtain virus-free plants. Thus, the present synthesis aims to analyze the main viral infections of grapevine, their consequences on the biological and economic performance of the crop, as well as the advances made in the field of virus-elimination methods and healthy germplasm conservation.

Viral infections in grapevine

To date, 102 viruses are known to infect grapevine, making it the plant with the highest number of known viral entities. These viruses have single-stranded or double-stranded RNA or DNA genomes and are classified into 44 genera and 21 families or taxonomically unclassified, identified in various *Vitis* germplasm collections worldwide (Fuchs, 2023; 2025). Of these, 31 viruses that cause significant economic damage worldwide are associated with four major disease complexes: leafroll (5 viruses), rugose wood complex (6 viruses), infectious degeneration and decline (12 and 4 viruses, respectively) and grapevine fleck (4 viruses) (Martelli, 2017; 2018; Fuchs, 2020). The viruses associated with these diseases are transmitted by mealybugs, soft scale insects or nematodes (Maliogka *et al.*, 2015); for the fleck virus (GFkV) that causes fleck disease (marbrure), the vector is unknown (Bovey and Martelli, 1992; Fuchs, 2020). The application of biological and chemical phytosanitary treatments is recommended for the control of virus vectors (Maliogka *et al.*, 2015).

New viral species, such as *Grapevine red blotch virus* (GRBV) and *Grapevine Pinot gris virus* (GPGV), as well as new strains of known viruses, have been identified and detected using high-throughput sequencing (HTS) technologies. These viruses have a substantial negative impact on production by reducing grapevine growth, thereby affecting the quality and quantity of production (Fuchs, 2020). High-Throughput Sequencing (HTS), known as Next Generation Sequencing (NGS), facilitates the detection of known viruses, the discovery of new viruses, and viruses associated with a disease of unknown etiology.

The diseases caused by the presence of *Grapevine fanleaf virus* (GFLV), *Arabidopsis mosaic virus* (ArMV), *Grapevine leafroll-associated virus-1* and *-3* (GLRaV-1, GLRaV-3), *Grapevine fleck virus* (GFkV), *Grapevine virus A* (GVA) in single infections or in combinations are the most aggressive for grapevine. Other viruses have a broad spectrum, affecting several important crop plants (*Italian artichoke latent virus* – AILV, *Alfalfa mosaic virus* – AMV, *Bean wilt virus* – BBWV, *Potato virus X* – PVX); for grapevine, these represent scientific curiosities, both because they are rarely found and because the damages induced are negligible (Martelli, 1999).

All these viruses are spread through infected propagation material and lead to a considerable decrease in fruit quality and yield, causing significant economic losses to producers and nurseries. In addition, over the years, after planting in the field, even healthy grapevines can become infected with numerous pathogens in the absence of rigorous management involving early diagnosis of infections and vector control.

Diagnostic methods such as visual inspection (Bell *et al.*, 2017), biological indexing (Krake *et al.*, 1999; Engel *et al.*, 2010; Hao *et al.*, 2021), enzyme-linked immunosorbent assay (ELISA), molecular methods (Stewart and Nassuth, 2001; Pacifico *et al.*, 2011), electron microscopy (Corbetta *et al.*, 1984; Russo, 2005), and multispectral (Mahlein, 2016) or hyperspectral (Nguyen *et al.*, 2021) imaging methods are used to monitor and detect viruses. Of these,

ELISA is the most used virological diagnostic method (Clark and Adams, 1977) to check the grapevines' sanitary status.

According to the technical annex to Directive 68/93/EEC (Council Directive of 9 April 1968 on the marketing of material for the vegetative propagation of the grapevine), *Vitis* propagating material other than seeds must be free from ArMV, GFLV, GLRaV-1, GLRaV-3; rootstocks of *Vitis* spp. and their hybrids, except *V. vinifera* L. must be free from ArMV, GFLV, GLRaV-1, GLRaV-3, and GFkV (<https://eur-lex.europa.eu/legal-content/RO/TXT/PDF/?uri=CELEX:01968L0193-20050714&from=LV>).

The grapevine viruses tested in Romania comply with the legislation in force (Annex 5 to Order 1267/2005 for the approval of the Rules and technical norms regarding the production for commercialization, control, quality certification and commercialization of vegetative propagation material of grapevine) harmonized with EU legislation.

THE INFLUENCE OF VIRAL INFECTION ON GRAPEVINE

Grapevine, as a woody species, is a model plant for the study of plant-virus interactions both under controlled and field conditions (Perrone *et al.*, 2017), but evaluating the effects of a viral disease is not easy to do because a plant can be infected with multiple viruses at the same time. Viral infection produces physiological and structural changes, affecting: photosynthesis, gas exchange, chlorophyll content, carbon translocation, phloem transport, cellular metabolism (Bertamini *et al.*, 2005; Martínez-Lüscher *et al.*, 2019; Kappagantu *et al.*, 2020). Also, the losses caused by viral infection depend on the age of the plant, environmental and growing conditions, grapevine cultivars and rootstocks (Golino *et al.*, 2003; Mannini, 2003; Pesqueira *et al.*, 2012). In addition, it should not be forgotten that plants grown in the field are simultaneously subjected to different abiotic and biotic stress factors (Suzuki *et al.*, 2014).

The impact of viral diseases is amplified by the fact that grapevine is a vegetatively propagated woody species, and many grapevine cultivars have been distributed as vegetative cuttings throughout the world with reduced attention to the presence of possible intracellular pathogens (Naidu *et al.*, 2015). Of all the factors that could influence the success of grafting, none is more important than the sanitary condition of the biological material, since the virus can multiply and spread throughout the plant, both from the scion and the rootstock. Furthermore, it is possible for the scion to be infected with one virus and the rootstock with another, resulting in a grafted grapevine containing two viruses, causing more severe symptoms and synergistic effects on plant health, grape production, and quality (Rayapati, 2017).

Plants infected with GFLV (genus *Nepovirus*, family *Secoviridae*) often exhibit abnormal growth, such as vine debilitation and shortened or deformed internodes, in addition to yellowing or deformed leaves and clarification of veins. The presence of this virus decreases production profits by reducing plant longevity, scion-rootstock compatibility, and the ability of the vine to multiply (Samaali *et al.*, 2019; Poojari *et al.*, 2020). Infected vines may show reduced resistance to adverse climatic factors, such as drought or frost. Fanleaf degeneration or decline is one of the most severe grapevine viral disease complexes worldwide. Also, fanleaf is one of the oldest known viral diseases in *V. vinifera*, the symptoms reported in Europe since 1841. The disease caused by GFLV infection affects grapevines in all temperate regions where *V. vinifera* and hybrid rootstocks are present (Oliver and Fuchs, 2011).

GFLV infection can cause variable losses, with potential for total loss of production, depending on the virulence of the virus strain and varietal susceptibility. Bunches are reduced in size and number and have irregular ripening. Infected berries have uneven size, many are small and seedless, and some fail to ripen. Fruit quality is affected due to reduced sugar content and titratable acidity. Rooting ability of rootstocks and grafting are both

substantially reduced in GFLV-infected material. Also, the productive life of vineyards infected with GFLV is significantly reduced. Poor vine health predisposes the vines to winter damage and death in colder climates (<https://grapes.extension.org/grapevine-fanleaf-degeneration-disease/>).

Grapevine leafroll-associated viruses (GLRaVs) are among the most important pathogens affecting grapevines and have a considerable economic impact (Atallah *et al.*, 2012; Fuchs *et al.*, 2021; Ricketts *et al.*, 2015). These virus species are diverse and belong to the family *Closteroviridae*, with six species and numerous strains grouped into three genera: the genus *Closterovirus* – GLRaV-2, the genus *Ampelovirus* – GLRaV-1, GLRaV-3, GLRaV-4, GLRaV-13, and the genus *Velarvirus* – GLRaV-7 (Fuchs, 2020; Naidu *et al.*, 2015). Grapevines are often infected with several of these viruses simultaneously (Prosser *et al.*, 2007; Al Rwahnih *et al.*, 2009). Given the global impact and distribution of leafroll disease, efforts have been undertaken to manage the spread of GLRaVs, characterize their effects, and understand the interaction between grapevine and GLRaVs. Leafroll, one of the most important viral diseases affecting grapevines worldwide, causes yield losses of up to 14–40% and delays fruit ripening by 3 weeks to 1 month (Rayapati *et al.*, 2014). Some GLRaV-3 isolates (LR101, LR127 and PA94.148) affect grapevine yield performance and berry composition, but for most parameters, the effect is variable between isolates, and in the case of cut wood weight and berry composition, it also depends on the rootstock (Rowhani *et al.*, 2015). Leafroll negatively affects rooting ability, graft attachment, plant vigor, photosynthesis, as well as the modulation of host genes involved in a cultivar of biological functions (Vondras *et al.*, 2021). A strain of GLRaV-2, constantly associated with Kober 5BB incompatibility in Europe, appears to be involved in the decline of young vines in California and has been detected in affected vines in Chile and Argentina. Other molecular variants of GLRaV-2 have been reported in New Zealand, Chile and Australia in association with decline conditions in young vines. Based on the differential responses of a panel of 18 rootstocks, up to five different graft-transmitted incompatibility-inducing agents have been detected in California (Martelli and Gallitelli, 2008).

In grapevine cultivars infected with GVA (genus *Vitivirus*, family *Betaflexiviridae*), a reduction in shoot number and growth, reduced sugar accumulation in berries, and vine senescence have been observed. Grape production can be reduced by 20-30% (Martelli and Boudon-Padieu, 2006; Minafra *et al.*, 2017). The Shiraz cultivar is very sensitive to GVA infections; symptoms associated with infection include delayed shoot growth, decreased sugar accumulation in berries, and, in some cases, plant death (Habili *et al.*, 2016).

The combination of GLRaV-1 and GVA infecting the scion caused incomplete healing of the grafting point and a decrease in the growth of the scions when Freedom and 101-14 rootstocks were used, while St. George (*V. rupestris*) and AXR#1 (*V. vinifera* × *V. rupestris*) rootstocks showed tolerance to viral infection under the same conditions (Golino *et al.*, 2015; Cui *et al.*, 2019). These observations suggested the existence of a virus-rootstock interaction that determines the destruction of the scions under the influence of viral infection. Furthermore, a synergistic effect between GLRaV and vitiviruses could increase the virulence on grapevine grafts, indicating an additional complexity underlying the virus-rootstock interaction. The genetic background determines the viral tolerance of the rootstock in the grafting system (Rowhani *et al.*, 2017).

Numerous observations and experiments highlighted the effects of the GFkV (genus *Maculavirus*, family *Tymoviridae*) presence, alone or in combination with other viruses and viral diseases. Many grapevine cultivars and rootstocks infected only with GFkV are asymptomatic, although the infection may be associated with graft incompatibility (<https://www.wineaustralia.com/getmedia/b1e5038d-cc30-48d8-b3c2-25844af8e795/201107-Grapevine-fleck-and-associated-viruses?ext=.pdf>). The presence of

GFkV reduces rhizogenesis and graft attachment; the effects depend on the virulence of the

viral isolate. GFkV alone or in the presence of leafroll induces a reduction in graft percentage and affects plant vigor in nurseries. Infection with GFkV associated with vein necrosis and vein mosaic reduced the amount of cutting wood on 420A and Kober 5BB rootstocks by 52% and 37%, respectively, but did not affect growth on Teleki 5A. Simultaneous infection with GFkV and leafroll produces ajinashika disease in the Japanese grape cultivar 'Koshu', characterized by a reduction in the amount of sugar in the fruit (Walter and Martelli, 1996). Following field experiments, it is considered that, in general, self-rooted grapevines are more tolerant to viral infections (Garau *et al.*, 1997).

GPGV, a member of the genus *Trichovirus* (family *Betaflexiviridae*), was first identified in the 'Pinot gris' cultivar in Trentino vineyards of Italy by next-generation sequencing (NGS) techniques (Giampetruzzi *et al.*, 2012), although symptoms of the new disease called *Grapevine Leaf Mottling and Deformation* (GLMD) (Martelli, 2014) have been reported since 2003.

Although GPGV-infected grapevines are often asymptomatic, they may sometimes exhibit leaf deformation, chlorotic spots, dwarfing, and shorter internodes that typically develop early in the growing season, similar to symptoms caused by mite bites, but also to those produced by nepoviruses (Hily *et al.*, 2020). Symptomatic plants infected with GPGV showed reduced vigor and significant yield losses (up to 96%), depending on the severity of symptoms (Malossini *et al.*, 2014; Messmer *et al.*, 2024).

The vector of GPGV is the eriophyid mite *Colomerus vitis* (Pagenstecher) (Malagnini *et al.*, 2016). The virus has been reported in the last few years in major grapevine-growing regions of the world (Saldarelli *et al.*, 2017). Since GPGV is transmitted by grafting, it is mainly spread through propagation material (Saldarelli *et al.*, 2015). GPGV is currently considered a major grapevine pathogen in Europe (Cieniewicz *et al.*, 2020). Although this virus is not regulated, it is recommended that all grapevine propagation material (potted plants, cuttings, graft buds) be tested for the presence/absence of GPGV (<https://www.dpi.nsw.gov.au/agriculture/horticulture/grapes/publications/grapevine-pinot-gris-virus>).

Healthy mother plants are important because they are used by nurseries to produce propagating material and distribute it worldwide, and strict regulatory measures are necessary to protect agricultural systems from the viruses spread. In general, germplasm selection (thanks to diagnostic tools and procedures developed in plant virology) leads to the identification of healthy cultivars that can be included in the vegetative production systems. However, in some cases, the identification of healthy plants among selected local cultivars with limited distribution may be difficult or impossible, making the virus-elimination procedures necessary to recover the useful healthy plants (Panattoni *et al.*, 2013).

The influence of viral infection on the grapevine is presented in Table 1.

Table 1. The impact of virus diseases in grapevine

Disease/ Virus	Symptoms	Economic losses	Observations	References
Fanleaf/GFLV	Leaf deformation; fan-shaped leaves, short internodes, zig-zag growth, bushy appearance, reduced plant growth, reduced number of clusters, berries are small-sized and ripen irregularly, and compromised propagation material.	Production reduction of up to 80% in severely affected areas.	One of the most damaging viral diseases; seriously affects the longevity of the plantation and the wine quality.	Walter and Martelli, 1996. Malossini <i>et al.</i> , 2003. Martelli and Boudon-Padieu, 2006.

Disease/ Virus	Symptoms	Economic losses	Observations	References
Leafroll/ GLRaV-1, -2, -3	Downward of leaf edges, reddening or yellowing (in red and white cultivars, respectively), green veins, uneven ripening, delayed ripening of grapes, and decreased production.	Production reduction by 20–60%.	Globally widespread; major effects on grape ripening and sugar content, lower quality wine.	Martelli and Boudon-Padieu, 2006. Martelli, 2014. Naidu <i>et al.</i> , 2014.
Rugose wood/ (viral complex)	Bark abnormalities, cracks, swellings, zones of necrosis on the trunk, weakening of the plant, and gradual reduction of production.	Information on production losses specific to this viral complex is limited.	Affects sap circulation; cumulative effects over time.	Martelli and Boudon-Padieu, 2006. Cuozzo <i>et al.</i> , 2018.
Grapevine mosaic/ArMV	Yellow spots, mosaic, reduced growth.	It ranges from slight decrease to severe losses (30–70%).	Plantation compromise; difficult to control.	Martelli and Boudon-Padieu, 2006.
Marbrure/ GFkV	Clearing of upper order veins, deformed, thin, and wrinkled leaves, light mosaic (on the rootstock <i>V. ruperstris</i>)	0% in <i>vinifera</i> cultivars; over 20% in the sensitive rootstocks.	Without symptoms in most commercial cultivars (<i>V. vinifera</i>), but dangerous in nurseries.	Walter, 1988. Angelini <i>et al.</i> , 2001. Martelli and Boudon-Padieu, 2006.
Grapevine leaf mottling and deformation/ GPGV	Chlorosis, mosaic, leaf deformation, growth reduction, plant decline; symptoms are present and easier to identify in spring, when new shoots and leaves begin to grow.	Yield losses of up to 80% have been reported in some cases, especially in susceptible cultivars.	Production losses vary depending on the severity of symptoms.	Giampetruzzi <i>et al.</i> , 2012. Maliogka <i>et al.</i> , 2015. Saldarelli <i>et al.</i> , 2015. Bertazzon <i>et al.</i> , 2017. Messmer <i>et al.</i> , 2024.

VIRUS-ELIMINATION METHODS

Strategies to promote virus-free propagation material in culture, essential for maintaining the health, productivity and sustainability of vineyards, include the application of viral elimination methods, starting with meristem culture and thermotherapy, to alternative methods such as *in vitro* chemotherapy, electrotherapy, cryotherapy and their combinations.

Meristem /shoot tip culture

Meristem culture is the oldest method used for the eradication of plant viruses (Faccioli and Marani, 1998). Due to the uneven distribution of viruses in the plant, it is assumed that the meristem is free of viruses (Quak, 1987). The mechanism of virus eradication by meristematic culture has not been fully elucidated, but various hypotheses are accepted, such as: the absence of plasmodesmata at the meristematic cell level, competition between the synthesis of nucleoproteins necessary for cell division and viral replication or the intervention of inhibitory substances.

For Mellor and Stace-Smith (1977), viral replication requires the presence of enzymes normally available in cells close to the meristematic dome. The excision of the meristem causes a trauma that renders these enzymes temporarily unavailable and therefore viral replication is interrupted, the viral RNA is degraded and it is integrated into the cell. Thus, the smaller the explant is, the more the chance of getting healthy plants increases.

Despite the successes achieved by this method, there are some problems that prevent the use and recommendation of this technique (Altmayer, 1989; Hatjinikolakis and Roubelakis – Angelakis, 1993).

One of the major problems is the difficulty of isolating the meristem of the smallest possible size (0.1 - 0.3 mm) followed by the multiple requirements necessary to ensure the viability of the explant and the triggering of regeneration processes: the appearance of adventive buds at the base of the pseudo-petiole of the phylloid formation, the formation of shoot primordia from the adventive buds, the multiplication itself.

Most of the time, apical meristems are free of viruses, but some viruses are known to invade the meristematic region of the growth tips, in which case healthy plants cannot be regenerated, requiring the combination of several virus-elimination methods (Manu and Manju, 2018).

Increasing the size of the explant cultured on aseptic medium determined the maintenance of viability, with satisfactory results regarding viral elimination (Saygaç and Önder, 2021).

Thermotherapy

The thermotherapy is based on the reduction of the virus multiplication rate under the influence of high temperatures, 35-45°C. Heat-treated plants can produce virus-free tissues and the lack of vascular elements in the meristem makes it difficult for viral particles to be transported to the meristematic dome. These tissues, if taken and cultured *in vitro*, can generate new plants identical to the mother plant.

The combination of tissue culture with thermotherapy led to the elimination of viruses from various grapevine species (Stellmach, 1980; Barlass *et al.*, 1982; Goussard *et al.*, 1991; Savino *et al.*, 1990, Buciumeanu and Vişoiu, 1996).

The results of research conducted by Hatzinikolakis and Roubelakis – Angelakis (1993) demonstrated that high percentages of healthy plants can also be obtained through *in vitro* thermotherapy of explants formed from a fragment of a node.

In vitro thermotherapy of small explants (apices of 5 mm) led to their necrosis after 8 weeks (Leonhardt *et al.*, 1998). Thus, for the elimination of viruses by *in vitro* thermotherapy, the authors recommended rooted microshoots; 2 months after thermotherapy, almost all clones induced in the experiment were virus-free.

Rooted grapevine plantlets grown on hormone-free medium were subjected at temperatures of 34°C, from 34 to 115 days in growth chambers. At the end of the thermotherapy, approximately 2 mm apices and nearby 2-3 axillary buds were excised and cultured on multiplication medium. *Grapevine rupestris stem pitting-associated virus* (GRSPaV) elimination in a proportion of 9.1% was achieved by this method. The percentage is low compared to other recovery techniques for the same virus: 28.9% by meristem culture, 23.6% by *in vivo* thermotherapy, 100% by somatic embryogenesis (Gribaudo *et al.*, 2006).

In vitro thermotherapy applied to some rootstock genotypes infected with ArMV, GLRaV-3 and GFLV provided satisfactory results (Křižan, 2008).

Elimination of GFLV in grapevine rootstocks was achieved by *in vivo* and *in vitro* thermotherapy in parallel, at 37°C for 45 days. *In vivo* thermotherapy gave better results although it is more laborious (Křižan, 2009).

In vivo thermotherapy is a method that requires large, energy-consuming equipment, the time required to regenerate new plants is long (only thermotherapy is applied up to 120 days) and the tolerance of genotypes to high temperatures is different. Some of the shortcomings of this method have been overcome by applying the thermic treatment to *in vitro* grown plantlets.

***In vitro* chemotherapy**

Initially used against human viruses, the action of viricides has also been transferred to the plant world. In order to block viral multiplication in cultivated plants, viricides have been either inoculated/injected (de Fazio *et al.*, 1978), sprayed on plants (Cheplick and Agrios, 1983), or introduced into the culture medium in the micropropagation process (Bittner *et al.*, 1989).

Antivirals have been used in the *in vitro* culture of plants as therapeutic supplements in integrated control programs targeting plant viral diseases. The most frequently studied compounds are antimetabolites, substances able to block the viral nucleic acid synthesis. These compounds, natural or synthetic, have antiviral effects, but none of them has a satisfactory selective action that would allow their use in specific prophylaxis and large-scale therapy of viral plant diseases (Nascimento *et al.*, 2003).

Among the antivirals used to eliminate various types of viruses in horticultural plants, ribavirin was the most commonly used, followed by 5-azacytidine (AZA), acycloguanosine, azidothymidine, 2,4-dioxohexahydro-1,2,5-triazine (DHT), dihydroxypropyladenine (DHPA), oseltamivir, mycophenolic acid (6-(4-hydroxy-6-methoxy-7-methyl-3-oxo-1,3-dihydroisobenzofuran-5-yl)-4-methyl-hex-enoic acid) (MPA), selenazole (2-β-D-ribofuranosylselenazole-4-carboxamide) (SE), 3-deazauridine (DZD), cyanoguanidine, etc.

The streamline of the virus-elimination method by *in vitro* chemotherapy determined the correlation of treatment parameters (viricide concentration, treatment time) with the phytotoxic effect induced by the viricide on *in vitro* cultivated explants and the regeneration rate of new virus-free plants. Increasing the concentration of ribavirin in the culture medium determined a dwarfing effect on the plantlets and a decrease of the multiplication rate compared to oseltamivir (Guță *et al.*, 2009a,b).

The concentration of 30 mg/L ribavirin did not lead to the regeneration of any GLRaV-1+3-free plants in the Ranâi Magaraci genotype, while the same concentration of oseltamivir determined a viral elimination rate of 71.42% after 30 days of treatment (Guță *et al.*, 2010). Testing of various concentrations of ribavirin and oseltamivir highlighted the lack of a correlation between the viricide concentration, the treatment time, and the viral elimination rate (Guță and Buciumeanu, 2011).

The need to improve the efficiency of virus-elimination methods led to the concomitant use of the viricides ribavirin and oseltamivir. GFKV was eliminated from both simple infections and viral complexes, the diagnosis being confirmed both by ELISA and RT-PCR (Guță *et al.*, 2014; 2017a). Similar results were not obtained in the case of GLRaV-1 and GFLV elimination, the rates being quite low, 13.3 and 6.8%, respectively (Guță *et al.*, 2017b). Increasing the viricides concentration to 60 mg/L ribavirin and 120 mg/L oseltamivir, used both individually and in combination, demonstrated once again the different behaviour of viral infections in grapevines to the application of virus-elimination methods. Thus, GFKV was completely removed with the viricide mixture only, while the best elimination rate for GLRaV-1 was recorded with oseltamivir (Guță *et al.*, 2017a).

In vitro chemotherapy, as a viral elimination method in grapevines, was also studied to maintain varietal identity in virus-free regenerated plants. RAPD analysis did not identify differences between plants subjected to chemotherapy treatments and healthy regenerated ones (Guță *et al.*, 2010).

The advantage of this method consists in the use of relatively large inocula (apices and nodal fragments), the success being overcoming the phytotoxic effect induced by the chemotherapeutic substance correlated with the regeneration of virus-free plants.

Electrotherapy

The use of electric current in the elimination of viral diseases in plants is known, although in-depth knowledge of these phenomena is not available. The description of the real causes of nucleoprotein inactivation will contribute to the optimization of future experiments aimed at streamlining the methods for the sanitation of different plant species. The energy dissipated in the form of heat depends on the specific treatment applied and on the properties of the plant tissues and the viral nucleoprotein.

Electrotherapy acts as a thermotherapy at the cellular level with controlled temperature at which the inactivation of the viral nucleoprotein occurs (Gonzales *et al.*, 2006).

Electrotherapy was effective in eliminating *Dasheen mosaic virus* (DMV) that affects 95% of *Xanthosoma sagittifolium* crops in Cuba (Igarza Castro *et al.*, 2001). Electrical treatment led to the regeneration of banana plants free of *Cucumber Mosaic Virus* (Helliot *et al.*, 2001).

The first studies on viral elimination in grapevines by electrotherapy reported a decrease in ELISA readings upon treatment of GFLV-infected plants with 1V/cm, with increasing treatment time (Burger, 1989).

The electric field applied to grapevine plants infected with GLRaV-3 led to high elimination rates (57.1-100%), without direct correlations between the exposure period and the efficiency of the treatment (Guță *et al.*, 2008 b,c).

The use of alternating electric current followed by *in vitro* culture was investigated as an alternative virus-elimination technique in grapevines with single or mixed infections. Electric current of 1000 and 10,000 kHz was applied to the ends of herbaceous grapevine cuttings; the treated axillary buds went through the micropropagation stages. ELISA results on regenerated and acclimatized plants showed encouraging results regarding the elimination of GLRaV-1 and -3 (12.5% elimination at 100 kHz and 4.5% at 10,000 kHz, respectively, after 10 min of treatment). The results were not reproducible in the case of GLRaV-1 for elimination from plants with mixed infections. Satisfactory results were not obtained regarding the elimination of GFLV and GFKV (Guță *et al.*, 2011; Guță and Buciumeanu, 2012). Electrotherapy in the horizontal electrophoresis tank led to satisfactory results for all viruses studied, GFKV being eliminated in simple infection and 50% in combination with GVA, GFLV and GLRaV-1, recording elimination rates of 50 and 30%, respectively (Guță *et al.*, 2019).

Cryotherapy

Cryotherapy is a relatively new method used to eliminate pathogens from infected plant tissue based on cryopreservation techniques.

The main methods employed in cryotherapy are encapsulation-dehydration, vitrification, encapsulation-vitrification and droplet vitrification, which are based on the immersion of preconditioned shoot tips in liquid nitrogen, followed by their recovery *in vitro* onto culture media for regeneration of healthy plantlets.

High frequency of virus-free plants among regenerants within a short time frame is the most desirable aspect of cryotherapy. Therefore, these advantages make the technique a promising tool for institutions mandated to the development of high-health planting materials with high genetic and agronomic potential for viticulture (Bettoni *et al.*, 2016).

The reported results show the dependence of the virus-elimination rate on the genotype, viral infection, and treatment method (Table 2).

Table 2. Efficiency of grapevine virus elimination by different methods

Virus/Viral complex	Species/cultivar	Efficiency (%)	References
Meristem culture			
GFLV	<i>V. vinifera</i> 'Merlot', 'Trebiana Toscana', and an unknown cultivar- JI	54	Barba <i>et al.</i> , 1992
	<i>V. vinifera</i> , 3 clones of the 'Refošk' cultivar	60	Koruza and Jelaska, 1993
	<i>V. vinifera</i> 'Thompson seedless'	85	Youssef <i>et al.</i> , 2009
	<i>V. vinifera</i> 'Flame seedless'	75	Fayek <i>et al.</i> , 2009
	<i>V. vinifera</i>	100	Miljanić <i>et al.</i> , 2022
GLRaV-1	<i>V. vinifera</i> 'Merlot', 'Trebiana Toscana', and an unknown cultivar - JI	73-93	Barba <i>et al.</i> , 1992
	<i>V. vinifera</i> , 3 clones of 'Refošk' cultivar	100	Koruza and Jelaska 1993
	<i>V. vinifera</i> 'Thompson seedless'	87.5	Youssef <i>et al.</i> , 2009
	<i>V. vinifera</i> 'Flame seedless'	82.5	Fayek <i>et al.</i> , 2009
GLRaV-3	<i>V. vinifera</i> 'Merlot', 'Trebiana Toscana' and an unknown cultivar- JI	73-93	Barba <i>et al.</i> , 1992
	<i>V. vinifera</i> 3 clones of 'Refošk' variety	100	Koruza and Jelaska, 1993
	<i>V. vinifera</i>	100	Miljanić <i>et al.</i> , 2022
GRSPaV	<i>V. vinifera</i> 'Albarola'	14.2	Gribaudo <i>et al.</i> , 2006
	<i>V. vinifera</i> 'Bosco'	72.7	
	<i>V. vinifera</i>	100	Miljanić <i>et al.</i> , 2022
GFkV	<i>V. vinifera</i>	100	Miljanić <i>et al.</i> , 2022
GFLV+GFkV	<i>V. vinifera</i>	60 – 84.2	Vişoiu and Teodorescu, 2000
GLRaV-1+3	<i>V. vinifera</i>	83 – 9	
GPGV+GFkV	<i>V. vinifera</i> 'Negru mare'	90.9	Guță <i>et al.</i> , 2025
	<i>V. vinifera</i> 'Sangiovese'	98.4	
Shoot tip culture			
GLRaV-3	<i>V. vinifera</i>	100	Bi <i>et al.</i> , 2018
	<i>V. vinifera</i> × <i>V. labrusca</i>	100	
	<i>V. pseudoreticulata</i>	100	
GPGV	<i>V. vinifera</i>	100	Rieger, 2016
GPGV+GFkV	<i>V. vinifera</i> 'Negru mare'	62.5	Guță <i>et al.</i> , 2025.
	<i>V. vinifera</i> 'Sangiovese'	100	
		70	
Thermotherapy			
GLRaV-1	<i>V. vinifera</i> 'Rauschling'	100	Staudt and Kassemeyer, 1994
GVA	<i>V. vinifera</i>	60	Panattoni <i>et al.</i> , 2007
	<i>V. berlandieri</i> × <i>V. riparia</i> , 'Kober 5BB'	70.2	
GPGV + GFkV	<i>V. vinifera</i> 'Negru mare'	56.5	Guță <i>et al.</i> , 2025.
	<i>V. vinifera</i> 'Sangiovese'	100	
GFkV	<i>V. champini</i>	57.1	Hu <i>et al.</i> , 2021
	<i>V. berlandieri</i> × <i>V. riparia</i> 'Kober 5BB'	0	Panattoni and Triolo, 2010
	<i>V. vinifera</i>	100	Miljanić <i>et al.</i> , 2022
GLRaV-3	<i>V. champini</i>	42.9	Hu <i>et al.</i> , 2020
	<i>V. berlandieri</i> × <i>V. riparia</i> 'Kober 5BB'	24.7	Panattoni and Triolo, 2010
	<i>V. vinifera</i>	100	Miljanić <i>et al.</i> , 2022
GRSPaV	<i>V. vinifera</i> 'Mantilaria'	39.62	Maliogka <i>et al.</i> , 2009
	<i>V. vinifera</i> 'Prevezaniko'	92.85	
	<i>V. vinifera</i>	100	Miljanić <i>et al.</i> , 2022
GFLV	<i>V. vinifera</i>	100	Miljanić <i>et al.</i> , 2022

Virus/Viral complex	Species/cultivar	Efficiency (%)	References
In vitro chemotherapy			
GFkV	<i>V. champini</i>	79,5	Hu <i>et al.</i> , 2021
	<i>V. vinifera</i>	100	Guță <i>et al.</i> , 2008a
	<i>V. vinifera</i>	100	Komínek <i>et al.</i> , 2016
	<i>V. vinifera</i> , 'Regina viilor'	100	Guță and Buciumeanu, 2021
GRSPaV	<i>V. champini</i>	13.6	Hu <i>et al.</i> , 2021
GLRaV-1 + 3	<i>V. vinifera</i>	78.6	Guță <i>et al.</i> , 2008a
GVA	<i>V. vinifera</i>	0	Guță <i>et al.</i> , 2008a
GLRaV-1+GVA	<i>V. vinifera</i> 'Servant'	11	Guță and Buciumeanu, 2010; 2011
GPGV	<i>V. vinifera</i>	66.7	Komínek <i>et al.</i> , 2016
		86.7	
		100	
GPGV+GFkV	<i>V. vinifera</i> , 'Negru mare'	100	Guță <i>et al.</i> , 2025
	<i>V. vinifera</i> , 'Sangiovese'	100	
GPGV+GFkV	<i>V. vinifera</i> 'Pinot Gris 14 Șt.'	86.9	Guță and Buciumeanu, 2021
	<i>V. vinifera</i> 'Merlot 146 Mn.'	75	
	<i>V. vinifera</i> 'Balaban alb'	100	
Electrotherapy			
GVA	<i>V. vinifera</i>	40	Bayati <i>et al.</i> , 2011
GLRaV-3	<i>V. vinifera</i>	57.1-100	Guță <i>et al.</i> , 2008b,c
GPGV+GFkV	<i>V. vinifera</i> , 'Sangiovese'	100	Guță <i>et al.</i> , 2025.
Cryotherapy			
GVA	<i>V. vinifera</i>	42	Bayati <i>et al.</i> , 2011
	<i>V. vinifera</i> 'Bruti'	97-100	Wang <i>et al.</i> , 2003
GLRaV-3	<i>V. vinifera</i> 'Cabernet Sauvignon'	100	Marković <i>et al.</i> , 2015
GFLV	<i>V. vinifera</i> 'Chardonnay'	77.8	Marković <i>et al.</i> , 2015
Combined methods: in vitro chemotherapy with thermotherapy			
GFkV	<i>V. champini</i>	100	Hu <i>et al.</i> , 2021
GLRaV-3	<i>V. champini</i>	42.9	Hu <i>et al.</i> , 2020
GRSPaV	<i>V. champini</i>	100	Hu <i>et al.</i> , 2021
Combined methods: cryotherapy with shoot tip culture			
GLRaV-3	<i>V. vinifera</i>	100	Bi <i>et al.</i> , 2018
	<i>V. vinifera</i> × <i>V. labrusca</i>	100	
	<i>V. pseudoreticulata</i>	100	

A study on the analytical hierarchy (Fig. 1) of the virus-elimination methods (meristem culture, intensely regenerative apex culture, *in vitro* thermotherapy, electrotherapy in uniform electric field, electrotherapy in horizontal electrophoresis tank), applied for elimination of GPGV+GFkV - led to the establishment of *in vitro* chemotherapy as the most efficient method in terms of the viral elimination rate, corroborated with the consumption of materials, energy and time required, until the regeneration of healthy plants (Guță *et al.*, 2025).

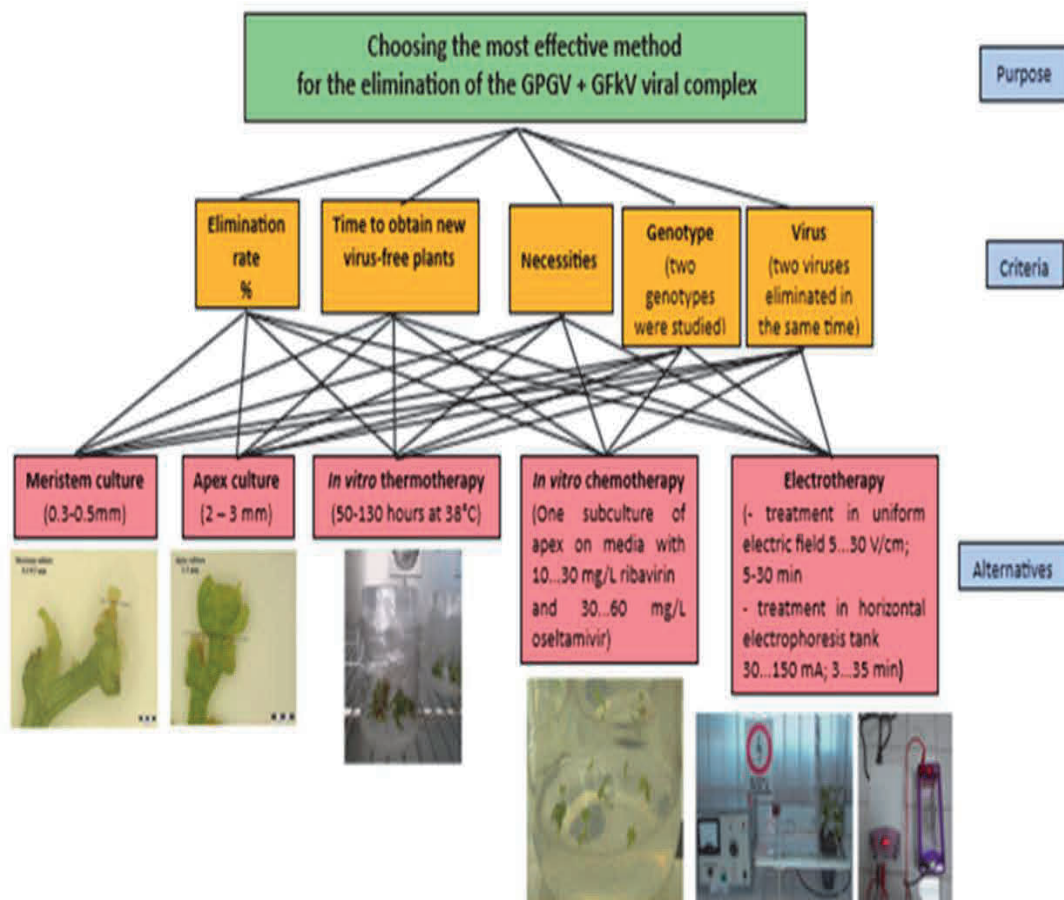


Figure 1. Analytic Hierarchy Process for GPGV + GFkVviruses elimination

CONCLUSIONS

Overall, grapevine viral infections constitute a complex problem, with major implications for the productivity, quality and sustainability of modern viticulture. The effects of these diseases, often cumulative and difficult to control, highlight the need for an integrated management that includes early diagnosis, the use of certified planting material, and the application of biotechnological methods of virus elimination. Progress registered in *in vitro* cultures, thermotherapy and chemotherapy techniques made it possible to obtain and preserve virus-free clones, essential for maintaining the genetic identity and economic value of cultivars. Therefore, the development and expansion of virus-free germplasm collections is not only a scientific necessity but also a fundamental condition for a sustainable viticulture capable of responding to current phytosanitary and economic challenges

ACKNOWLEDGEMENTS

The study is part of the research project “Maintaining the high biological value of the grapevine genotypes preserved in the germplasm collection”, No. 8399/2025-2027 which is supported jointly by Academy of Agricultural and Forestry Sciences “Gheorghe Ionescu-Sisesti, and Project “Procedure for elimination of *Grapevine Pinot gris virus* under conditions of economic efficiency in order to increase the biological value of grapevine propagation material”, No. 01.01, NUCLEU Programme 23.41/2023-2026 which is supported jointly by Romanian Ministry of Research, Innovation and Digitalization.

REFERENCES

1. Al Rwahnih, M., Daubert, S., Golino, D. and Rowhani, A. (2009). Deep sequencing analysis of RNAs from a grapevine showing Syrah decline symptoms reveals a multiple virus infection that includes a novel virus. *Virology* 387, 395–401.
2. Altmayer, B. (1989). Investigations on the elimination of *nepovirus* and *Grapevine leafroll* by shoot tip meristem culture of grape virus. *Phytoparasitica* 17, 72–73.
3. Angelini, E., Bertazzon, N. and Borgo, M. (2001). Sensitivity of grapevine rootstocks to GfKV infection. *Phytopathol. Mediterr.* 40 (Suppl.), 423–428.
4. Atallah, S.S., Gomez, M.I., Fuchs, M.F. and Martinson, T.E. (2012). Economic impact of *Grapevine leafroll* disease on *Vitis vinifera* cv. Cabernet Franc in Finger Lakes vineyards of New York. *Am. J. Enol. Vitic.* 63, 73–79.
5. Barba, M., Martino, L. and Cupidi, A. (1992). Il rasanamento della vite: tre tecniche a confronto (Grapevine sanitation: three methods compared). *Vignevini* 19(3), 33–36.
6. Barlass, M., Skene, K.G.M., Woodham, R.C. and Krake, L.R. (1982). Regeneration of virus free grapevines using *in vitro* apical culture. *Ann. Appl. Biol.* 101, 291–295.
7. Bayati, S., Shams-Bakhsh, M. and Moieni, A. (2011). Elimination of *Grapevine virus A* (GVA) by cryotherapy and electrotherapy. *J. Agric. Sci. Technol.* 13, 443–450.
8. Bell, V.A., Blouin, A.G., Cohen, D., Hedderley, D.I., Oosthuizen, T., Spreeth, N.A., Lester, P.J. and Pietersen, G. (2017). Visual symptom identification of *Grapevine leafroll associated virus 3* in red berry cultivars supports virus management by roguing. *J. Plant Pathol.* 99, 477–482.
9. Bertamini, M., Malossini, U., Muthuchelian, K. and Nedunchezian, N. (2005). Physiological response of field grown grapevine (*Vitis vinifera* L. cv. Marzemino) to *Grapevine leafroll-associated virus* (GLRaV-1). *Phytopathol.* 44(3), 256–265.
10. Bertazzon, N., Filippin, L., Forte, V. and Angelini, E. (2017). *Grapevine Pinot gris virus*: a threat for European viticulture. *Eur. J. Plant Pathol.* 149, 929–943. <https://doi.org/10.1007/s10658-017-1222-1>.
11. Bettoni, J.C., Costa, M.D., Gardin, J.P.P., Kretzschmar, A.A. and Pathirana, R. (2016). Cryotherapy: a new technique to obtain grapevine plants free of viruses. *Rev. Bras. Frutic.* 38:e- 833.
12. Bi, W.-L., Hao, X.-Y., Cui, Z.-H., Pathirana, R., Volk, G.M. and Wang, Q.-C. (2018). Shoot tip cryotherapy for efficient of *Grapevine leafroll-associated virus-3* from diseased grapevine *in vitro* plants. *Ann. Appl. Biol.* 173, 261–270.
13. Bittner, H., Schenk, G., Schuster, G. and Kluge, S. (1989). Elimination by chemotherapy of *Potato virus S* from potato plants grown *in vitro*. *J. Potatos Research* 32(2), 175–179.
14. Bovey, R. and Martelli, G.P. (1992) *Directory of Major Virus and Virus-Like Diseases of Grapevines*. MFCIC and ICVG, 111 pp.
15. Buciumeanu, E. and Vişoiu, E. (1996). Elimination of *Grapevine leafroll associated virus type III* by heat treatment and *in vitro* culture. In *Current problems and techniques in cellular and molecular biology* (Timișoara, Romania: Mirton Publishing House), pp. 626–631.
16. Burger, J.G. (1989). Electrotherapy: a possible method to eliminate *Grapevine fanleaf virus* from grapevine. Paper presented at: 9th ICVG (Kiryat Anavim, Israel, 1987).
17. Cheplick, S.M. and Agrios, G.N. (1983). Effect of injected antiviral compounds on *Apple mosaic*, *scar skin* and apple diseases of apple trees. *Plant Dis.* 67, 1130–1133.
18. Cieniewicz, E.J., Qiu, W., Saldarelli, P. and Fuchs, M. (2020). Believing Is Seeing: Lessons from Emerging Viruses in Grapevine. *J. Plant Pathol.* 102, 619–632.
19. Clark, M. and Adams, A. (1977). Characteristics of the microplate method of enzyme-linked immunosorbent assay for the detection of plant viruses. *J. Gen. Virol.* 34(3), 475–483.
20. Corbetta, M.K., Kasdorf, G.G.F., Engelbrecht, D.J. and Wiid, J. (1984). Detection of viral-like particles by electron microscopy of negatively stained extracts from grapevines. *S. Afr. J. Enol. Vitic.* 5(2), 43–49.
21. Cui, Z.H., Agüero, C.B., Wang, Q.C. and Walker, M.A. (2019). Validation of micrografting to identify incompatible interactions of rootstocks with virus-infected scions of Cabernet Franc. *Aust. J. Grape Wine Res.* 25, 268–275.
22. Cuozzo, D., Chitarra, W., Ferrandino, A., Gribaudo, I., Gambino, G. and Mannini, F. (2018). Field performances and grape quality of the same clone of ‘Nebbiolo’ (*Vitis vinifera* L.) when infected by GLRaV-1+GVA, GVA or healthy. Paper presented at: 19th ICVG (Santiago, Chile).
23. De Fazio, G., Caner, J. and Vicente, M. (1978). Inhibitory effect of Virazole (Ribavirin) on the replication of *Tomato White Necrosis – Virus* (VNBTV). *Arch. Virol.* 58, 153–156.
24. Engel, A.K. and Fries, P. (2010). Beta-band oscillations-signalling the status quo? *Curr. Opin. Neurobiol.* 20(2), 164–165.
25. Faccioli, V.C. and Marani, F. (1998). *Virus elimination by meristem tip culture and tip micrografting*. In *Plant virus disease control* (St. Paul: APS Press), pp. 346–380.

26. Fayek, M.A., Jomaa, A.H., A Shalaby, A.-B. and Al-Dhaher, M.-M. (2009). Meristem tip culture for *in vitro* eradication of *Grapevine leafroll-associated virus-1* (GLRaV-1) and *Grapevine fan leaf virus* (GFLV) from infected flame seedless grapevine plantlets. *Ini. Inv.*, 4:al, ISSN-e 1988-415X.
27. Fuchs, M. (2020). Grapevine viruses: a multitude of diverse species with simple but overall poorly adopted management solutions in the vineyard. *J. Plant Pathol.* 102, 643–653. <https://doi.org/10.1007/s42161-020-00579-2>.
28. Fuchs, M. (2023). Grapevine virology highlights: 2018-2023. Paper presented at: 20th ICVG (Thessaloniki, Greece).
29. Fuchs, M. (2025). Grapevine viruses: Did you say more than a hundred? *J. Plant Pathol.* 107, 217–227. <https://doi.org/10.1007/s42161-024-01819-5>.
30. Fuchs, M., Almeyda, C.V., Al Rwahnih, M., Atallah, S.S., Cieniewicz, E.J., Farrar, K., Foote, W.R., Golino, D.A., Gómez, M.I., Harper, S.J. *et al.* (2021). Economic studies reinforce efforts to safeguard specialty crops in the United States. *Plant Dis.* 105, 14–26.
31. Garau, R., Fiori, P.P., Prota, V.A., Tolu, G., Fiori, M. and Prota, U. (1997). Effect of virus infection on own-rooted clones of different wine grapes cultivars from Sardinia. Paper presented at: 12th ICVG (Lisbon, Portugal).
32. Giampetruzzi, A., Roumi, V., Roberto, R., Malossini, U., Yoshikawa, N., La Notte, P., Terlizzi, F., Credi, R., Saldarelli, P.A. (2012). New grapevine virus discovered by deep sequencing of virus-and viroid-derived small RNAs in cv. Pinot gris. *Virus Res.* 163(1): 262–8. <https://doi.org/10.1016/j.virusres.2011.10.010>.
33. Golino, D.A., Rowhani, A., Klaassen, V., Sim, S.T. and Al Rwahnih, M. (2015). *Grapevine leafroll-associated virus 1* effects on different grapevine rootstocks. Paper presented at: 18th ICVG (Ankara, Turkey).
34. Golino, D.A., Sim, S. and Rowhani, A. (2003). The role or rootstock genotype in the effects of single and mixed infections of grapevine viruses. Paper presented at: 14th ICVG (Locorotondo, Bari, Italy).
35. Gonzales, J.E., Sanchez, R. and Sanchez, A. (2006). Biophysical analysis of electric current mediated nucleoprotein inactivation process. Eprint arXiv:q-bio/0601014. Paper presented at: First International Workshop on Bioinformatics (Santa Clara, Cuba – Flanders).
36. Goussard, P.G., Wiid, J. and Kasdorf, G.G.F. (1991). The effect of *in vitro* somatic embryogenesis in eliminating fanleaf virus and leafroll associated viruses from grapevines. *S. Afr. J. Enol. Vitic.* 12, 77-81.
37. Gribaudo, I., Gambino, G., Cuzzo, D. and Mannini, F. (2006). Attempts to eliminate *Grapevine Rupestris Stem Pitting-Associated Virus* from grapevine clones. *J. Plant Pathol.* 88(3), 293-298.
38. Guță I.-C. and Buciumeanu, E.-C. (2010). Utilizarea chimioterapiei *in vitro* pentru eliminarea infecțiilor virale multiple la vița de vie. Paper presented at: A XXVIII-a sesiune științifică a SNBC (Constanța, Romania).
39. Guță I.-C. and Buciumeanu, E.-C. (2011). Grapevine chemotherapy for elimination of multiple virus infection. *Rom. Biotechnol. Lett.* 16(5), 6535-6539.
40. Guță, I.-C. and Buciumeanu, E.-C. (2012). Chemotherapy and electrotherapy: environmental friendly methods for virus elimination in grapevine. Paper presented at: 14th Serbian congress of fruit and grapevine producers with international participation (Vrnjacka Banja, Serbia).
41. Guță, I.-C. and Buciumeanu, E.-C. (2021). Quality improvement of the grapevine planting stock through virus elimination by *in vitro* chemotherapy. *RJH* 2(1), 17-22.
42. Guță, I.-C., Buciumeanu, E.-C., Gheorghe, R.N. and Teodorescu, Al. (2009a). The assessment of viricides phytotoxicity used in grapevine *in vitro* chemotherapy. *Bulletin UASVM, Horticulture* 66(1), 661.
43. Guță I.-C., Buciumeanu, E.-C., Gheorghe, R.N. and Teodorescu, AL. (2009b). Examination of phytotoxic effect of viricides on grapevine in controlled medium. *Scientifical Papers USAMVB, Serie B, Horticulture*, Vol. LIII, 559-563.
44. Guță I.-C., Buciumeanu, E.-C., Gheorghe, R.N. and Teodorescu, AL. (2010). Solutions to eliminate *Grapevine leafroll associated virus* serotype 1+3 from *V. vinifera* L. cv. Rânăi Magaraci. *Rom. Biotechnol. Lett.* 15(1)(Suppl.), 72-78.
45. Guță, I.-C., Buciumeanu, E.-C., Oprescu, B. and Tătaru, L. (2011). Effect of direct electric current on virus elimination in grapevine. Paper presented at: 4th International Symposium New Researches in Biotechnology Simp BTH 2011 (Bucharest, Romania).
46. Guță, I.-C., Buciumeanu, E.-C. and Sumedrea, D.I. (2025). Using the analytical hierarchy process to select the most appropriate method for grapevine Pinot gris virus and *Grapevine fleck virus* elimination. *Acta Hortic.* 1418, 151-158. <https://doi.org/10.17660/ActaHortic.2025.1418.19>.
47. Guță, I.-C., Buciumeanu, E.-C., Tătaru, L.D., Oprescu, B. and Topală, C.M. (2019). New approach of electrotherapy for grapevine virus elimination. *Acta Hortic.* 1242, 697-702. DOI: 10.17660/ActaHortic.2019.1242.103.
48. Guță, I.-C., Buciumeanu, E.-C., Tătaru, L.D. and Topală, C.M., (2017a). Regeneration of grapevine virus-free plants by *in vitro* chemotherapy. *Acta Hortic.* 1188, 319-322. DOI 10.17660/ActaHortic.2017.1188.42.
49. Guță, I.-C., Buciumeanu, E.-C., Topală, C.M. and Tătaru, L.D. (2017b). The use of *in vitro* chemotherapy in grapevine virus elimination. *Acta Hortic.* 1155, 425-430. DOI:10.17660/ActaHortic.2017.1155.63.
50. Guță, I.-C., Buciumeanu, E. and Vișoiu, E. (2008a). Evaluarea activității ribavirinei asupra proceselor de regenerare și devirozare *in vitro* la vița de vie. In Cachiță-Cosma D., Brezeanu A., Ardelean A. (Coord.), *Biotehnologii vegetale pentru secolul XXI*. Cluj-Napoca: Ed. Risoprint, 95-101.

51. Guță I.-C., Buciumeanu, E.-C. and Vișoiu, E. (2014). Elimination of *Grapevine fleck virus* by *in vitro* chemotherapy. *Not. Bot. Horti. Agrobo.* 42(1), 115-118.
52. Guță, I.-C., Buciumeanu, E.-C., Vișoiu, E., Teodorescu, Il. and Liță, I. (2008b). The possibility of grapevine virus free regeneration in the presence of electric field. Paper presented at: XXXVIII ESNA Annual Meeting (Krakow, Poland).
53. Guță, I.-C., Buciumeanu, E.-C., Vișoiu, E., Teodorescu, Il. and Liță, I. (2008c). The effect of electric field on *in vitro* regenerative processes and grapevine virus elimination *Lucrări științifice Seria B-LI-2008 Horticultură*, 482-486.
54. Habili, N., Wu, Q. and Pagay, V. (2016). Virus-associated Shiraz Disease may lead Shiraz to become an endangered variety in Australia. *Wine Vitic. J.* 31, 47-50.
55. Hatzinikolakis, H.K. and Roubelakis-Angelakis, K.A. (1993). A modified method for *in vitro* thermotherapy and meristem culture for production of virus-free grapevine plant material. Paper presented at: 11th ICVG (Montreux, Switzerland).
56. Hao, X.-Y., Jiao, B.-L., Wang, M.-R., Wang, Y.-L., Shang, B.-X., Wang, J.-Y., Wang, Q.-C. and Xu, Y. (2021). *In vitro* biological indexing of *Grapevine leafroll-associated virus 3* in red- and white-berried grapevines (*Vitis vinifera*). *Aust. J. Grape Wine Res.* 27, 483-490. <https://doi.org/10.1111/ajgw.12505>.
57. Helliot, B., Panis, B., Locicero, A., Reyniers, K., Muylle, H., Vandewalle, M., Michel, C., Swennen, R. and Lepoivre, P. (2001). Development of *in vitro* technique for the elimination of *cucumber mosaic virus* from *Musa*. *Acta Hortic. (ISHS)* 560, 535-538. DOI: 10.17660/ActaHortic.2001.560.109.
58. Hily, J.-M., Poulicard, N., Candresse, T., Vigne, E., Beuve, M., Renault, L., Velt, A., Spilmont, A.-S. and Lemaire, O. (2020). Datamining, Genetic Diversity Analyses, and Phylogeographic Reconstructions Redefine the Worldwide Evolutionary History of *Grapevine Pinot Gris Virus* and *Grapevine Berry Inner Necrosis Virus*. *Phytophymes J.* 4, 165-177.
59. Hu, G.-J., Dong, Y., Zhang, Z., Fan, X. and Ren, F. (2020). Efficiency of chemotherapy combined with thermotherapy for eliminating *Grapevine leafroll-associated virus 3* (GLRaV-3). *Sci. Hortic.* 271, 109462.
60. Hu, G.-J., Dong, Y.-F., Zhang, Z.-P., Fan, X.-D. and Ren, F. (2021). Elimination of *Grapevine fleck virus* and *Grapevine rupestris stem pitting-associated virus* from *Vitis vinifera* 87-1 by ribavirin combined with thermotherapy. *J. Integr. Agric.* 20, 2463- 2470.
61. Igarza Castro, J., Hernandez, P.R. and Cruz Castelanos, B. (2001). Electrotherapy as an alternative for elimination of DMV virus in tania. *Manejo integrado de plagas* 60, 57-60.
62. Leonhardt, W., Wowrosch, Ch., Auer, A. and Koop, B. (1998). Monitoring of virus diseases in Austrian grapevine varieties and virus elimination using *in vitro* thermotherapy. *Plant Cell, Tissue and Organ Culture* 52, 71-74.
63. Kappagant, M., Collum, T.D., Dardick, C. and Culver, J.N. (2020). Viral Hacks of the Plant Vasculature: The Role of Phloem Alterations in Systemic Virus Infection. *Ann. Rev. Virol.* 7(1), 351-370.
64. Komínek, P., Komínková, M. and Jandová, B. (2016). Effect of repeated Ribavirin treatment on grapevine viruses. *Acta virologica* 60, 400-403, doi: 10.4149/av_2016_04_400.
65. Koruza, B. and Jelaska, S. (1993). Influence of meristem culture and virus elimination on phenotypical modifications of grapevine (*Vitis vinifera* L., cv. Refošk) *Vitis* 22, 59-60.
66. Krake, L.R., Scott, N.S., Rezaian, M. and Taylor, R. (1999). *Graft-Transmitted Diseases of Grapevines* (Collingwood, Australia: CSIRO Publishing), pp. 137.
67. Kian, B., Moravcová, K., Ondrušiková, E., Idam, E., Holleínova, V. and Pidra, M. (2008). Thermotherapy of grapevines and apricots by reason of viruses and phytoplasma elimination. *Acta Hortic.* 781, 93-96.
68. Kian, B., Moravcová, K., Ondrušiková, E., Idam, E., Holleínová, V. and Bláhová, L. (2009). Elimination of grapevine fanleaf virus in grapevine by *in vivo* and *in vitro* thermotherapy. *Hort. Sci.* 36(3), 105-108.
69. Mahlein, A.K. (2016). Plant disease detection by imaging sensors - Parallels and specific demands for precision agriculture and plant phenotyping. *Plant Dis.* 100, 241-251.
70. Malagnini, V., de Lillo, E., Saldarelli, P., Beber, R., Duso, C., Raiola, A., Zanotelli, L., Valenzano, D., Giampetruzzi, A., Morelli, M. et al. (2016). Transmission of *Grapevine Pinot gris virus* by *Colomerus vitis* (Acari: *Eriophyidae*) to grapevine. *Arch. Virol.* 161(9), 2595-9.
71. Maliogka, V.I., Martelli, G.P., Fuchs, M. and Katis, N.I. (2015). Control of viruses infecting grapevine. *Adv. Virus Res.* 91, 175-227.
72. Maliogka, V.I., Skiada, F.G., Eleftheriou, E.P. and Katis, N.I. (2009). Elimination of a new ampelovirus (GLRaV-PR) and *Grapevine rupestris stem pitting-associated virus* (GRSPaV) from two *Vitis vinifera* cultivars combining *in vitro* thermo-therapy with shoot tip culture. *Sci. Hortic.* 123, 280-282.
73. Malossini, U., Bianchedi, P., Villegas, T.R., Varner, M., Gualandri, V. and Nicolini, G. (2014). An updating about the performances of Pinot Gris and Traminer vines affected by the GPGV trichovirus-related grapevine disease. Paper presented at: 37th World Congress of Vine and Wine (Mendoza, Argentina).
74. Malossini, U., Ciccotti, A.M., Bragagna, P., Vindimian, M.E., Moser, S., Versini, G., and Nicolini, G. (2003). Changes in agronomical and oenological performances of clones of the grapevine cv Gewürztraminer after GFLV elimination by heat therapy. Paper presented at: 14th ICVG (Locorotondo, Bari, Italy).
75. Mannini, F. (2003). Virus elimination in grapevine and crop performance. Paper presented at: 14th ICVG (Locorotondo, Bari, Italy).

76. Manu, V. and Manju, M. (2018). Elimination of viruses through thermotherapy and meristem culture in apple cultivar „Oregon Spur-II”. *Virus diseases* **29**(1), 75-82
77. Marković, Z., Preiner, D., Stupić, D., Andabaka, Ž., Šimon, S., Vončina, D., Maletić, E., Kontić, J.K., Chatelet, P. and Engelmann, F. (2015). Cryopreservation and cryotherapy of grapevine (*Vitis vinifera* L.). *Vitis* **54**, 247-251.
78. Martelli, G.P. (1999). Infectious diseases and certification of grapevines. In *Proceedings of the Mediterranean network on grapevine closteroviruses 1992-1997 and the viroses and virus-like diseases of the grapevine a bibliographic report, 1985-1997. Options méditerranéennes, Série A* Nr. 29 (Bari, Italy: CIHEAM), pp. 47-64.
79. Martelli, G.P. and Gallitelli, D. (2008). Emerging and Reemerging Virus Diseases of Plants. In *Encyclopedia of Virology*, 3rd edn. pp. 86-92. <https://doi.org/10.1016/B978-012374410-4.00705>. Academic Press.
80. Martelli G.P. (2014). Directory of virus and virus-like diseases of the grapevine and their agents. *J. Plant Pathol.* **96**, 1–136. <http://dx.doi.org/10.4454/JPP.V96I1SUP>.
81. Martelli, G.P. (2017). An Overview on Grapevine Viruses, Viroids, and the Diseases They Cause. In *Grapevine Viruses: Molecular Biology, Diagnostics and Management*. Springer, Cham. pp. 31-46. DOI: 10.1007/978-3-319-57706-7_2.
82. Martelli G.P. (2018). Where grapevine virology is heading to. Paper presented at: 19th ICVG, (Santiago, Chile).
83. Martelli, G.P. and Boudon-Padieu, E. (Eds.) (2006). Directory of infectious diseases of grapevines and viroses and virus-like diseases of the grapevine: bibliographic report 1998–2004. In *Directory of infectious diseases of grapevines and viroses and virus-like diseases of the grapevine: bibliographic report 1998–2004*. Vol. 55. Options méditerranéennes: Série B. Etudes et Recherches (Bari, Italy: CIHEAM), pp. 10-21
84. Martínez-Lüscher, J., Plank, C.M., Brillante, L., Cooper, M.L., Smith, R.J., Al-Rwahnih, M., Yu, R., Oberholster, A., Girardello, R. and Kurtural, S.K. (2019). *Grapevine Red Blotch Virus* May Reduce Carbon Translocation Leading to Impaired Grape Berry Ripening. *J. Agric. Food Chem.* **67**(9), 2437-2448.
85. Mellor, F.C. and Stace-Smith, R. (1977). Virus-free potatoes by tissue culture. In *Applied and fundamental aspects of plant cell, tissue, and organ culture*. Germany: Springer-Verlog Publisher. pp. 616-638.
86. Messmer, N., Bohnert, P., Askani, L., Schumacher, S., Voegelé, R.T. and Fuchs, R. (2024). *Grapevine Pinot gris virus* spreads in infected vineyards: latent infections have no direct impact on grape production. *Virol. J.* **21**(1), 178. doi: 10.1186/s12985-024-02453-4.
87. Miljanić, V., Rusjan, D., Škvarč, A., Chatelet, P. and Štajner, N. (2022). Elimination of eight viruses and two viroids from preclonal candidates of six grapevine varieties (*Vitis vinifera* L.) through *in vivo* thermotherapy and *in vitro* meristem tip micrografting. *Plants* **11**, 1064.
88. Minafra, A., Mawassi, M., Goszczynski, D. and Saldarelli, P. (2017). Grapevine Vitiviruses. In *Grapevine Viruses: Molecular Biology, Diagnostics and Management*. (Springer: Cham, Switzerland), pp. 229–256.
89. Moutinho-Pereira, J., Correia, C.M., Gonçalves, B., Bacelar, E.A., Coutinho, J.F., Ferreira, H.F., Lousada, J.L. and Cortez, M.I. (2012). Impacts of *leafroll-associated viruses* (GLRaV-1 and -3) on the physiology of the Portuguese grapevine cultivar ‘Touriga Nacional’ growing under field conditions. *Ann. Appl. Biol.* **160**, 237-249.
90. Naidu, R.A., Maree, H.J. and Burger, J.T. (2015). *Grapevine leafroll disease* and associated viruses: a unique pathosystem. *Annu. Rev. Phytopathol.* **53**, 613–634.
91. Naidu, R.A., Rowhani, A., Fuchs, M., Golino, D.A. and Martelli, G.P. (2014). *Grapevine leafroll*: a complex viral disease affecting a high-value fruit crop. *Plant Dis.* **98**(9), 1172–1185.
92. Nascimento, L.C., Pio-Ribeiro, G., Willadino, L. and Andrade, G.P. (2003). Stock indexing and *Potato virus* elimination from potato plants cultivated *in vitro*. *Scientia Agricola* **60**(3), 525-530.
93. Nguyen, C., Sagan, V., Maimaitiyiming, M., Maimaitijiang, M., Bhadrar and Kwasniewski, M.T. (2021). Early Detection of Plant Viral Disease Using Hyperspectral Imaging and Deep Learning. *Sensors* **21**(3), 742. <https://doi.org/10.3390/s21030742>.
94. Oliver, J.E. and Fuchs, M.F. (2011). *Fanleaf degeneration/decline disease* of grapevines. Integrated Pests Management. <https://ecommons.cornell.edu/server/api/core/bitstreams/ee6242d0-fabc-4085-88b2-19d1ea7fb805/content>.
95. Pacifico, D., Caciagli, P., Palmano, S., Mannini, F. and Marzachi, C. (2011). Quantitation of *Grapevine leafroll associated virus-1* and -3, *Grapevine virus A*, *Grapevine fanleaf virus* and *Grapevine fleck virus* in field-collected *Vitis vinifera* L. ‘Nebbiolo’ by real-time reverse transcription-PCR. *J. Virol. Methods* **172**, 1–7.
96. Panattoni, A., D’Anna, F., Cristani, C. and Triolo, E. (2007). Grapevine *virus A* eradication in *Vitis vinifera* explants by antiviral drugs and thermotherapy. *J. Virol. Methods* **146**, 129-135.
97. Panattoni, A., Luvisi, A. and Triolo, E. (2013). Review. Elimination of viruses in plants: twenty years of progress. *Span. j. agric. res.* **11**(1), 173–188. <https://doi.org/10.5424/SJAR/2013111-3201>.
98. Panattoni, A. and Triolo, E. (2010). Susceptibility of grapevine viruses to thermotherapy on *in vitro* collection of Kober 5BB. *Sci. Hortic.* **125**, 63-67.
99. Perrone, I., Chitarra, W., Boccacci, P. and Gambino, G. (2017). Grapevine-virus-environment interactions: an intriguing puzzle to solve. *New Phytol.* **213**(3), 983-987.
100. Pesqueira, A.M., García-Berrios, J.-J., Barrasa, M. and Cabaleiro, C. (2012). Economic Impact of Leafroll Disease in Vineyards of the Cultivar Albariño in Rías Baixas (Spain). Paper presented at: 17th ICVG (Davis, CA, USA).

101. Poojari, S., Moreau, D.L., Kahl, D., Ritchie, M., Ali, S. and Úrbez-Torres, J.R. (2020). Disease Incidence and Genetic Variability of Economically Important Grapevine Viruses in Nova Scotia. *Can. J. Plant Pathol.* **42**, 584–594.
102. Prosser, S.W., Goszczynski, D.E. and Meng, B. (2007). Molecular analysis of double-stranded RNAs reveals complex infection of grapevines with multiple viruses. *Virus Res.* **124**, 151–159.
103. Quak, F. (1987). Therapy of individual plants. In *Viruses of potatoes and seed-potato production*, De Bokx J.A., van der Want J.P.H. (Eds.), Wageningen: Pudoc, pp. 151–161.
104. Rayapati, N. (2017). Risk of virus problems in field-grafted vineyards. *Good Fruit Grower*/June 6, 2017. <https://www.goodfruit.com/rayapati-risk-of-virus-problems-in-field-grafted-vineyards/>.
105. Rayapati, N., Rowhani, A., Fuchs, M., Golino, D. and Martelli, G.P. (2014). Grapevine leafroll: a complex viral disease affecting a high-value fruit crop. *Plant Dis.* **98**, 1172–1185.
106. Ricketts, K.D., Gomez, M.I., Atallah, S.S., Fuchs, M.F., Martinson, T.E., Battany, M.C., Bettiga, L.J., Cooper, M.L., Verdegaal, P.S. and Smith, R.J. (2015). Reducing the economic impact of grapevine leafroll disease in California: identifying optimal disease management strategies. *Am. J. Enol. Vitic.* **66**, 138–147.
107. Rieger, T. (2016). GPGV *Pinot gris virus* in More Napa Vineyards. *Wines and Vines*, December 5.
108. Rowhani, A., Golino, D.A., Klaassen, V., Sim, S.T., Gouran, M. and Al Rwahnih, M. (2015). *Grapevine Leafroll associated virus 3*: Effects on rootstocks, vine performance, yield and berries. Paper presented at: 18th ICVG (Ankara, Turkey).
109. Rowhani, A., Uyemoto, J.K., Golino, D.A., Daubert, S.D. and Al Rwahnih, M. (2017). Virus involved in graft incompatibility and decline. In *Grapevine Viruses: Molecular Biology, Diagnostics and Management* (Springer: Cham, Switzerland), pp. 289–302.
110. Russo, M. (2005). Electron Microscopy of Grapevine Virus Infections. *Phytopathol. Mediterr.*, **24** (1/2), 144–47.
111. Saldarelli, P., Giampetruzzi, A., Maree, H.J. and Al Rwahnih, M. (2017). High-Throughput Sequencing: Advantages Beyond Virus Identification. In *Grapevine Viruses: Molecular Biology, Diagnostics and Management* (Springer: Cham, Switzerland), pp. 625–642.
112. Saldarelli, P., Giampetruzzi, A., Morelli, M., Malossini, U., Pirolo, C., Bianchedi, P. and Gualandri, V. (2015). Genetic variability of grapevine *Pinot gris virus* and its association with grapevine leaf mottling and deformation. *Phytopathology* **105**(4), 555–63.
113. Samaali, B., Toumi, A., Hadamane, A.M. and Kallel, S. (2019). Study of Distribution and Analysis of the Transmission of *Grapevine Fanleaf Virus* in Northern Tunisian Vineyards. *J. New Sci.* **64**, 4040–4047.
114. Savino, V., Di Terlizzi, B., Riveccio, S. and Di Silvio, F. (1991). Presence in clonal rootstocks of a graft-transmissible factor that induces stunting and bushy growth in European grapevines. Paper presented at: 10th ICVG. (Volos, Greece).
115. Saygac, S. and Önder, S. (2021). Effects of shoot tip size on in vitro regeneration and virus elimination of grapevine cv. superior seedless. *Plant Prot. Bull.* **61-3**. DOI: 10.16955/bitkorb.877123.
116. Staudt, G. and Kassemeyer, H.H. (1994). Elimination of *Grapevine leafroll associated virus type 1* in *Vitis vinifera* cv. Lemberger. *Vitis* **33**, 179–180.
117. Stellmach, G. (1980). Recherche sur les effets d'une réinfection virale de vignes ayant été libérée de virus pathogènes sur la sélection sanitaire et spécialement par thérapie. *Bul. OIV* **53**, 179–186.
118. Stewart, S. and Nassuth, A. (2001). RT-PCR based detection of *Rupestris stem pitting associated virus* within field-grown grapevines throughout the year. *Plant Dis.* **85**, 617–620.
119. Suzuki, N., Rivero, R.M., Shulaev, V., Blumwald, E. and Mittler, R. (2014). Abiotic and biotic stress combinations. *New Phytol.* **203**, 32–43. doi:10.1111/nph.12797.
120. Vişoiu, E. and Teodorescu, Al. (2001). *Biotehnologii de producere a materialului săditor viticol* (Bucharest, Romania: Ceres Publishing House).
121. Vondras, A.M., Lerno, L., Massonnet, M., Minio, A., Rowhani, A., Liang, D., Garcia, J., Quiroz, D., Figueroa-Balderas, R., Golino, D.A., Ebeler, S.E., Al Rwahnih, M. and Cantu, D. (2021). Rootstock influences the effect of *Grapevine leafroll-associated viruses* on berry development and metabolism via abscisic acid signalling. *Mol. Plant Pathol.* **22**(8), 984–1005. doi: 10.1111/mpp.13077.
122. Walter, B. (1988). Effects of *Grapevine fleck virus* on some American and French-American hybrid rootstocks. *Vitis* **27**(1), 13–22.
123. Walter, B. and Martelli, G.P. (1996). Sélection clonele de la vigne: sélection sanitaire et sélection pomologique. Influence des viroses et qualité. 1-ère Partie: Effets des viroses sur la culture de la vigne et ses produits. *Bulletin de l'O.I.V* **789-790**, 945–971.
124. Wang, Q., Mawassi, M., Li, P., Gafny, R., Sela, I. and Tanne, E. (2003). Elimination of *Grapevine virus A* (GVA) by cryopreservation of *in vitro*-grown shoot tips of *Vitis vinifera* L. *Plant Sci.* **165**, 321–327.
125. Youssef, S.A., Al-Dhaher, M.M.A. and Shalaby, A.A. (2009). Elimination of *Grapevine fanleaf virus* (GFLV) and *Grapevine leaf roll-associated virus-1* (GLRaV-1) from infected grapevine plants using meristem tip culture. *Int. J. Virol.* **5**(2), 89–99.

126. Zherdev, A.V., Vinogradova, S.V., Byzova, N.A., Porotikova, E.V., Kamionskaya, A.M. and Dzantiev, B.B. (2018). Methods for the Diagnosis of Grapevine Viral Infections: A Review. *Agriculture* **8(12)**, 195. <https://doi.org/10.3390/agriculture8120195>.
127. <https://eur-lex.europa.eu/legal-content/RO/TXT/PDF/?uri=CELEX:01968L0193-20050714&from=LV>.
129. <https://www.wineaustralia.com/getmedia/b1e5038d-cc30-48d8-b3c2-25844af8e795/201107-Grapevine-fleck-and-associated-viruses?ext=.pdf>.
129. <https://grapes.extension.org/grapevine-fanleaf-degeneration-disease/>.
130. <https://www.dpi.nsw.gov.au/agriculture/horticulture/grapes/publications/grapevine-pinot-gris-virus>.
131. <https://www.oiv.int/>

The effect of a modified DKW nutrient medium and antioxidant-adsorbent synergy on the *in vitro* regeneration of *Liriodendron tulipifera* L.

R.R. Khaydaraliyev* and Kh.Kh. Kushiev

Research Institute Agrobiotechnology and Biochemistry of Gulistan State University,
Uzbekistan

*Corresponding author email: rasuljonxaydaraliyev@gmail.com

ABSTRACT

The study aimed to improve the *in vitro* regenerative response of *Liriodendron tulipifera* L. by optimizing the mineral composition of the DKW nutrient medium and reducing phenolic oxidation, a major limitation in woody plant micropropagation. Agrochemical analysis of donor plant soils revealed deficiencies in phosphorus, zinc, and boron, which were corrected through targeted fertilization to enhance explant physiological quality. A modified DKW medium enriched with KH_2PO_4 (+30 mg L⁻¹), FeNaEDTA (+10.37 mg L⁻¹), and increased Mn and Zn concentrations significantly improved morphogenic performance compared with the standard DKW control. Among the hormonal treatments, the combination BAP 1.5 mg L⁻¹ + kinetin 0.5 mg L⁻¹ + GA₃ 0.2 mg L⁻¹ (RA variant) yielded the highest number of lateral buds, apical leaves, and lateral leaves after 21 days. Subsequent optimization with antioxidant-adsorbent combinations demonstrated that higher concentrations of ascorbic acid (150–250 mg L⁻¹) together with activated charcoal (0.5–1 g L⁻¹) markedly reduced phenolic oxidation and tissue necrosis. The V6 treatment (250 mg L⁻¹ ascorbic acid + 1 g L⁻¹ activated charcoal) achieved the highest explant viability (92%) and leaf formation. These results confirm that mineral-adjusted DKW medium combined with antioxidant-adsorbent synergy provides an effective protocol for the microclonal propagation of *L. tulipifera*, offering a reliable strategy for the regeneration of phenolic, woody species.

Keywords: activated charcoal, ascorbic acid, *Liriodendron tulipifera*, microclonal propagation, sterilization protocol

INTRODUCTION

Liriodendron tulipifera L., commonly known as the yellow poplar or tulip tree, is native to the eastern regions of North America - from southern New England west to southern Ontario and Michigan, extending south to Louisiana, and east to north-central Florida (Cao *et al.*, 2020). It is distributed throughout the Appalachian Mountain range, including Tennessee, North Carolina, Virginia, and Kentucky, as well as in the Ohio River Valley, Pennsylvania, New York, Alabama, Georgia, Mississippi, Illinois, and Arkansas (Meyer, 2020). The forests in these areas, particularly the Appalachian deciduous forests, represent the primary natural habitat of the tulip tree. This species grows best in deep, well-drained silt loam soils belonging mainly to the Inceptisol or Ultisol orders. Ultisol and Inceptisol soil types are generally characterized as acidic and low-fertility soils, with relatively low levels of nitrogen (N), phosphorus (P), and potassium (K) (Martínez *et al.*, 2007). These soils typically exhibit a slightly acidic pH ranging from 5.5 to 6.5. *Liriodendron tulipifera* naturally occurs in the Appalachian Mountains, the Ohio River Valley, and

the Near Eastern United States (Beck, 1962). Within such soil and climatic conditions, the species is distinguished from other forest trees by its rapid growth rate-reaching up to 40 meters in height within 50 years-its high-quality timber, and its ornamental characteristics (Russell, 1977). The straight trunk and conical crown shape make this species valuable for furniture, veneer, paper production, and as an ornamental tree in urban landscapes (Beck, 1962; Santamour and McArdle, 1984). Moreover, *L. tulipifera* is an important melliferous (honey-bearing) species and a natural source of ecologically and pharmacologically active compounds, including alkaloids, flavonoids, and phenolic acids (Parks *et al.*, 1995). However, its propagation through seeds is limited, with a germination rate of only about 10%. Vegetative propagation is also difficult due to lignification and phenolic oxidation. Therefore, the development of efficient *in vitro* microclonal propagation protocols is essential for conserving its genetic resources and for its use in breeding programs (Wilcox and Taft, 1969).

In vitro propagation protocols for various ornamental and valuable woody species have been developed using MS, WPM, and DKW nutrient media. However, the physiological characteristics of *L. tulipifera*-particularly its high susceptibility to phenolic exudation, tissue necrosis, and contamination-make the establishment of a stable protocol challenging. Consequently, adapting the mineral composition of the nutrient medium to the soil characteristics of its natural habitats, along with the use of antioxidants (ascorbic acid), adsorbents (activated charcoal), and optimized sterilization methods, is of critical importance for successful *in vitro* culture of this species.

This study aimed to develop an effective *in vitro* microclonal propagation protocol for *Liriodendron tulipifera* by using a modified DKW medium, determining the optimal phytohormone regime, reducing phenolic oxidation through the incorporation of ascorbic acid and activated charcoal into the medium, and improving sterilization protocols.

MATERIAL AND METHODS

To obtain donor plant material for the study, *Liriodendron tulipifera* trees cultivated under the soil and climatic conditions of the Fergana Valley and Syrdarya region were selected. Before explant collection, agrochemical analysis of the soils from these regions was carried out at the Scientific Research Institute of Agrobiotechnology and Biochemistry of Gulistan State University.

Experimental Site and Study Objective. The study was conducted in a controlled plant biotechnology laboratory dedicated to micropropagation research. The objective was to evaluate the *in vitro* regenerative performance of *Liriodendron tulipifera* explants cultivated on modified DKW nutrient media, assessing the influence of cytokinin–auxin balance, antioxidant supplementation, and activated charcoal on early regenerative responses.

Plant Material and Explant Establishment. Nodal segments of *Liriodendron tulipifera* were used as initial explants. Plant material was washed under running tap water, surface-sterilized using standard disinfection procedures (ethanol followed by sodium hypochlorite), then rinsed repeatedly with sterile distilled water. Explants were inoculated onto nutrient media under a laminar airflow hood to ensure aseptic conditions. Cultures were maintained at 24 ± 1 °C, under a 16 h photoperiod and a light intensity of $40\text{--}50 \mu\text{mol m}^{-2} \text{s}^{-1}$, conditions consistent with standard micropropagation protocols for woody species. The pH of all culture media was adjusted to 5.7 ± 0.1 before autoclaving at 121 °C for 20 minutes.

Culture Media Preparation. Three basal media were initially used for explant establishment: MS (Murashige and Skoog), WPM (Woody Plant Medium), and DKW (Driver and Kuniyuki Walnut medium). Based on internal optimization tests, DKW proved the most suitable and was subsequently modified according to the mineral profile of the species, following in situ soil analysis results.

Table 1. Composition of the modified DKW nutrient medium)

Component	Standard DKW	Modification applied	Final concentration
KH ₂ PO ₄	—	+30 mg·L ⁻¹	30 mg·L ⁻¹
FeNaEDTA	—	+10.37 mg·L ⁻¹	10.37 mg·L ⁻¹
MnSO ₄ ·H ₂ O	standard	+1.20 mg·L ⁻¹	increased
ZnSO ₄ ·7H ₂ O	standard	+1.00 mg·L ⁻¹	increased
Basal salts	5.6 g·L ⁻¹ DKW	unchanged	5.6 g·L ⁻¹
Sucrose	30 g·L ⁻¹	unchanged	30 g·L ⁻¹
Agar	7 g·L ⁻¹	unchanged	7 g·L ⁻¹
pH	adjusted	5.7 ± 0.1	—

All media used in both experimental stages contained sucrose (30 g·L⁻¹) and agar (7 g·L⁻¹), with identical cytokinin, auxin, and GA₃ concentrations unless otherwise specified.

The experiment included two stages: Stage 1 — Hormone and Antioxidant Treatments (RD, RA, RB, RC). This stage evaluated combinations of cytokinins, auxins, gibberellin, and an antioxidant, together with a treatment involving activated charcoal. Stage 2 — Antioxidant and Adsorbent Optimization (A1–A6).

This stage assessed combinations of ascorbic acid and activated charcoal under identical hormone conditions.

Table 2. Experimental variants for Stage 1 (RD, RA, RB, RC)

Experimental variant	Cytokinins BAP+Kinetin mg·L ⁻¹	Auxins IBA/ mg·L ⁻¹	Gibberellin GA ₃ /mg·L ⁻¹	Antioxidant ASA /mg·L ⁻¹	Activated charcoal
RD – Control	BAP 1.5 + Kinetin 0.5	0.1	—	—	—
RA	BAP 1.5 + Kinetin 0.5	0.1	—	100	—
RB	BAP 1.5 + Kinetin 0.5	0.1	0.2	100	—
RC	BAP 1.7 + Kinetin 0.6	0.2	—	100	300

Table 3. Experimental variants for Stage 2 (A1–A6)

Experimental Variant	Ascorbic acid (mg·L ⁻¹)	Activated charcoal (g·L ⁻¹)
V1	150	0.5
V2	200	0.5
V3	250	0.5
V4	150	1.0
V5	200	1.0
V6	250	1.0

Observation Stages and Measured Parameters

Explants were evaluated at 7, 14, and 21 days after inoculation. The following parameters were recorded: number of newly formed lateral buds; number of apical leaves; number of lateral leaves; coloration score (1–5), indicating explant physiological condition. For the color scoring assessment, the following scale was applied: 5 – fully saturated healthy green, indicating optimal growth; 4 – green but slightly lighter, suggesting minor stress or insufficient light; 3 – yellow-green, indicating nutrient deficiency or hormonal imbalance; 2 – completely yellow, associated with chlorosis (Fe or Mg deficiency) and physiological stress; 1 – brown or blackened, indicating necrosis or severe tissue degradation (George et al. 2008), (Bhojwani and Razdan 1996), and FAO/IAEA (2009)

Statistical Analysis

All quantitative data were processed using analysis of variance (ANOVA). Differences between treatments were considered statistically significant at $p < 0.05$. Means were presented with standard deviations, and statistically significant differences from the control were marked accordingly (* $p < 0.05$).

RESULTS AND DISCUSSIONS

Explants grown on WPM exhibited limited development, with reduced lateral bud activation and slower leaf expansion compared with those cultured on DKW medium (Figure 1). In contrast, DKW supported more vigorous early growth, characterized by enhanced shoot elongation, increased lateral bud formation, and improved overall explant vigor. These differences highlight the superior suitability of the DKW basal medium for initiating regenerative responses in *Liriodendron tulipifera* under antioxidant-free conditions (Figure 1).

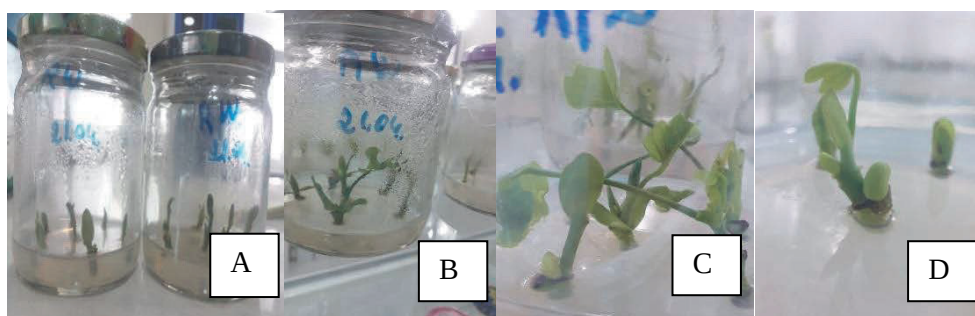


Figure 1. Comparative response of *Liriodendron tulipifera* explants cultured on WPM and DKW media in the absence of antioxidants and activated charcoal.

From left: (A) explants cultured on WPM medium on 21 April 2025, photographed on 2 May 2025; (B) explants on WPM medium photographed on 7 May 2025; (C) explants cultured on DKW medium photographed on 7 May 2025; (D) lateral shoots newly formed after 16 days of incubation on DKW medium.

Across all evaluation moments (7, 14, and 21 days), explants cultured in RA, RB, and RC variants exhibited significantly improved growth parameters compared with the RD control (Table 4). At 7 days, the antioxidant-supplemented media (RA, RB, RC) increased the number of lateral buds and early leaf initiation, indicating a reduction in oxidative stress during the initial adaptation period. By 14 days, all treated variants continued to outperform the control, particularly RA and RB, which induced higher numbers of lateral buds (1.26–1.33 vs. 1.06 in control) and lateral leaves (2.40–2.53 vs. 1.86). This demonstrates that both antioxidant (ASA) and GA₃ supplementation act synergistically in stimulating shoot and leaf development.

After 21 days, RA produced the strongest regenerative response, with the highest values for lateral bud formation (1.93) and lateral leaves (4.40), significantly exceeding the control. RB also maintained superior performance, while RC showed intermediate but still significant improvements, (Table 4).

According to the results of the study, the RA variant was identified as the most optimal, as it demonstrated the highest explant viability and new shoot formation rate. These findings provide a foundation for developing methods to propagate *Liriodendron tulipifera* using tissue culture technologies, applying biotechnology to its propagation process, and ensuring an alternative approach to regeneration, as well as for subsequent biotechnological work aimed at mass propagation and conservation.

Table 4. Schedule for the observation and recording of *in vitro* explant growth

Days	Container number	Number of new lateral bud(s)	Number of apical leaf/leaves	Number of lateral leaf/leaves	Color score (1-5)
7 day	RD control	0.33±0.48	0.46±0.51	0.8±1.01	4.86±0.35
	RA	0.4±0.5*	0.53±0.51*	1.06±1.03*	4.73±0.45
	RB	0.4±0.5*	0.66±0.48*	1.2±1.01*	4.8±0.41
	RC	0.46±0.51*	0.6±0.5*	0.93±1.03	4.73±0.45
14 day	RD control	1.06±0.45	0.86±0.63	1.86±0.91	4.8±0.41
	RA	1.26±0.88*	1.2±0.56*	2.53±1.4*	4.73±0.59
	RB	1.33±0.97*	1.13±0.63*	2.4±1.54*	4.66±0.48
	RC	1.13±0.99*	1±0.65*	2.13±1.59*	4.6±0.5
21 day	RD control	1.4±0.9	1.26±0.88	2.53±1.76	4.13±1.3
	RA	1.93±0.59*	2.06±0.59*	4.4±1.35*	4.53±0.63
	RB	1.86±0.74*	1.73±0.88*	3.86±2.06*	4.33±1.04
	RC	1.53±0.99*	1.53±0.83*	3.06±1.27*	4.2±1.08

*Note: statistical significance level compared to the control, $p < 0.05$. were considered statistically significant

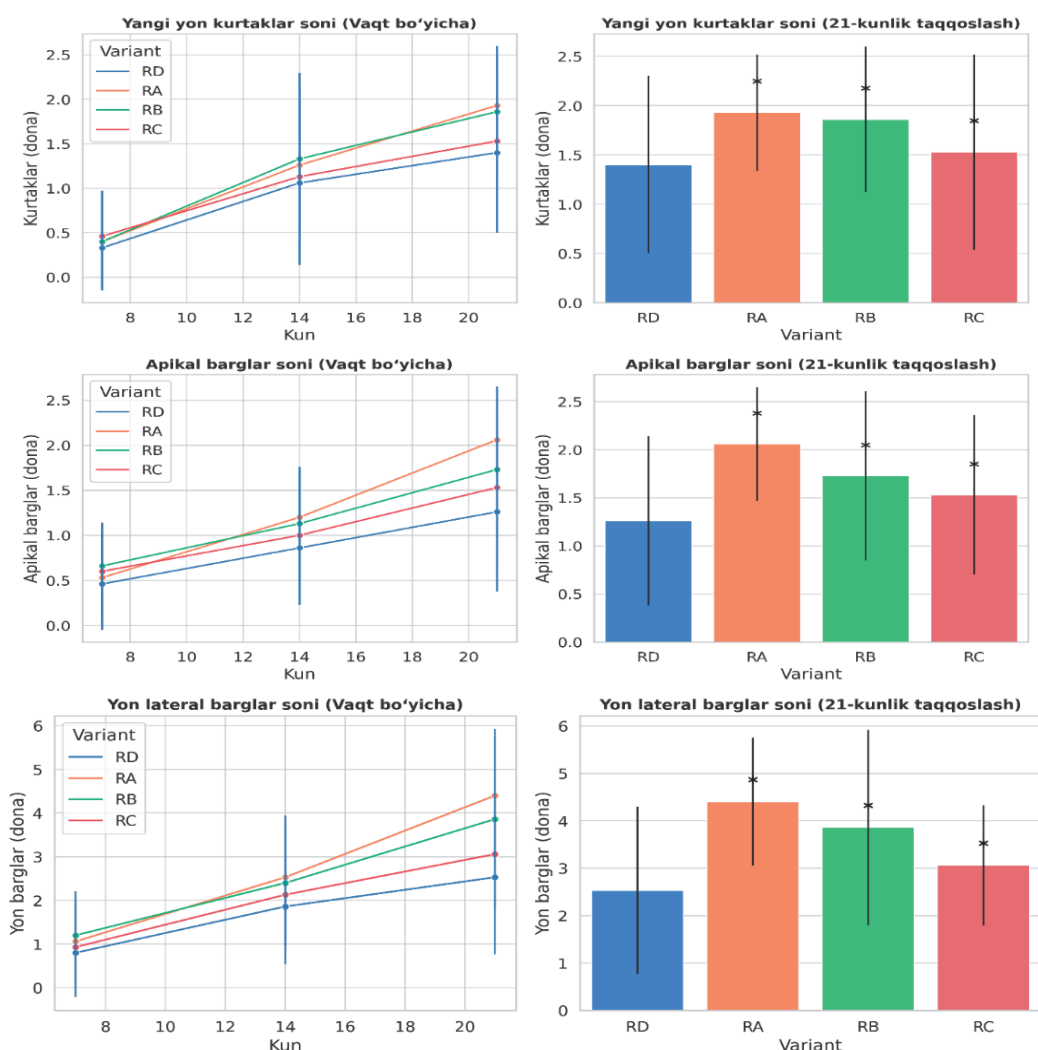


Figure 2. The line graphs illustrate the growth dynamics of newly formed lateral shoots, apical leaves, and lateral leaves in *Liriodendron tulipifera* explants during a 21-day incubation period, compared to the RD control



Figure 3. Days of growth stages of *Liriodendron tulipifera* explants (3, 7, 14 days)

Effect of increasing antioxidant and adsorbent concentrations on explant growth

In the above experiments, *Liriodendron tulipifera* explants were cultured *in vitro* using ascorbic acid (ASA) at a concentration of $100 \text{ mg}\cdot\text{L}^{-1}$ as the main antioxidant (Kang *et al.*, 2014; Zhong, 2019). The observations showed vigorous explant growth; however, since polyphenol oxidation and necrosis symptoms were not completely eliminated, activated charcoal (AC) and salicylic acid were additionally incorporated into the nutrient medium in the next experimental stage (Table 4). Polyphenol exudation at wounded sites often leads to necrosis and tissue browning (George *et al.*, 2008). Polyphenols are secondary metabolites widely distributed in woody plant species and are highly susceptible to rapid oxidation under *in vitro* conditions, leading to tissue browning and reduced regeneration efficiency (Krishnan and Siril, 2017).



Figure 4. Phenolic oxidation and medium browning observed upon the addition of $100 \text{ mg}\cdot\text{L}^{-1}$ ascorbic acid

To reduce the frequently observed phenolic oxidation, necrosis, and tissue browning in *in vitro* cultured explants (Gao *et al.*, 2019), as well as to decrease the formation of reactive oxygen species (ROS) (Şen, 2012), activate the systemic acquired resistance (SAR) mechanism, enhance stress tolerance, and stimulate bud differentiation and root induction, salicylic acid was added to the culture medium. Salicylic acid is known to enhance phenolic biosynthesis and increase the activity of antioxidant enzymes such as peroxidase (POD) and polyphenol oxidase (PPO) (Galal, 2012). Activated charcoal, on the other hand, adsorbs phenolic exudates and other toxic metabolites, but it can also partially absorb auxins and cytokinins from the medium (Weatherhead, 1978). Therefore, in the control RA variant, the concentrations of growth regulators were adjusted as follows: BAP increased from $1.5 \text{ mg}\cdot\text{L}^{-1}$ to $1.7 \text{ mg}\cdot\text{L}^{-1}$, kinetin from $0.5 \text{ mg}\cdot\text{L}^{-1}$ to $0.6 \text{ mg}\cdot\text{L}^{-1}$, and instead of $0.1 \text{ mg}\cdot\text{L}^{-1}$ IBA, $1 \text{ mg}\cdot\text{L}^{-1}$ IAA auxin was applied.

The response of *Liriodendron tulipifera* explants to different combinations of ascorbic acid (ASA) and activated charcoal (AC), (Table 4). Across all evaluation moments (7, 14, and 21 days), higher concentrations of antioxidants and adsorbents significantly influenced bud induction, leaf formation, and the intensity of phenolic oxidation.

At 7 days, low to moderate antioxidant levels ($150\text{--}200 \text{ mg}\cdot\text{L}^{-1}$ ASA) with $0.5 \text{ g}\cdot\text{L}^{-1}$ AC (variants V1 and V2) supported early bud formation and reduced phenolic exudation. Increasing ASA to $250 \text{ mg}\cdot\text{L}^{-1}$ (V3) slowed initial growth, whereas the addition of $1.0 \text{ g}\cdot\text{L}^{-1}$

AC (V4, V5, V6) inhibited elongation due to medium browning but further decreased phenolic oxidation. Medium darkening represents a serious problem in the *in vitro* culture of economically important woody plants, primarily due to high levels of lignification and accumulation of secondary metabolites. This phenomenon complicates the development of effective regeneration and genetic transformation systems across various species (Liu, et al.,2024). By 14 days, the treatments containing 200 mg·L⁻¹ ASA (V2, V5) resulted in the highest number of newly formed leaves and vigorous bud activity. The combination of 250 mg·L⁻¹ ASA + 1.0 g·L⁻¹ AC (V6) supported stable development and minimal phenolic oxidation, while 150 mg·L⁻¹ ASA + 1.0 g·L⁻¹ AC (V4) showed reduced elongation.

Table 4. *In vitro* growth of *Liriodendron tulipifera* explants in modified DKW nutrient medium supplemented with higher concentrations of ascorbic acid and different amounts of activated charcoal (observations at 7, 14, and 21 days.)

Days	Variant	Number of new lateral bud(s)	Number of apical leaf/leaves	Number of lateral leaf/leaves	Color score (1-5)
7 day	V1	0.53±0.51	0.66±0.61	1.0±0.53	4.5±0.5
	V2	0.6±0.50	0.73±0.45	1.06±0.59	4.7±0.5
	V3	0.66±0.61	0.8±0.86	1.13±0.74	4.6±0.6
	V4	0.66±0.48	0.53±0.91	1.06±0.79	4.4±0.6
	V5	0.73±0.45	0.80±1.01	1.4±0.82	4.8±0.4
	V6	0.66±0.48	0.76±0.94	1.26±0.79	4.7±0.5
14 day	V1	1.06±0.45	1.13±0.63	2.4±1.12	4.5±0.6
	V2	1.26±0.88	1.26±0.79	2.53±1.18	4.7±0.5
	V3	1.4±0.91	1.46±0.74	2.66±1.44	4.6±0.5
	V4	1.13±0.99	1.26±1.09	2.53±0.91	4.4±0.6
	V5	1.33±0.97	1.4±0.63	2.8±1.65	4.7±0.5
	V6	1.46±0.74	1.53±1.35	3.33±1.79	4.8±0.4
21 day	V1	1.73±0.59	2.13±0.83	3.73±1.27	4.3±0.8
	V2	1.8±0.67	2.00±0.92	4.00±1.51	4.6±0.5
	V3	1.93±0.70	2.53±1.35	4.53±1.18	4.5±0.6
	V4	1.53±0.99	2.46±0.55	4.26±1.48	4.2±0.8
	V5	2.13±0.51	2.66±1.04	4.66±0.97	4.7±0.5
	V6	2.26±0.70	3.06±0.27	4.93±1.27	4.5±0.6

After 21 days, the strongest responses were recorded in variants V2, V5, and V6, which reached the highest values for lateral bud formation and total leaf number. Viability reached 82% in V2, 90% in V5, and 92% in V6, indicating that high ASA combined with AC promotes long-term stability and reduces oxidative tissue damage, although excessive antioxidant levels (250 mg·L⁻¹) may slightly reduce growth rate despite high survival. These results confirm that ASA and AC play a synergistic role in reducing oxidative stress and improving explant viability under *in vitro* conditions.

CONCLUSION

The mineral modification of the DKW medium significantly enhanced the regenerative response of *Liriodendron tulipifera* explants, confirming the importance of adapting nutrient balance to species-specific ecological requirements. Among the hormonal treatments, the RA combination (BAP 1.5 mg L⁻¹, kinetin 0.5 mg L⁻¹, GA₃ 0.2 mg L⁻¹) proved to be the most effective, producing the highest number of lateral buds and leaves and achieving superior explant viability. Phenolic oxidation could not be fully controlled with 100 mg L⁻¹ ascorbic acid alone; however, the synergistic use of ascorbic acid and activated charcoal markedly reduced browning and

necrosis. The A6 treatment (250 mg L⁻¹ ASA + 1 g L⁻¹ AC) provided the best overall performance, achieving 92% viability and promoting vigorous leaf development. Overall, the optimized protocol offers a robust and efficient approach for the *in vitro* micropropagation of *L. tulipifera* and may be applied to other woody species prone to oxidative stress.

In the future, further optimization can focus on improving the rooting and ex vitro acclimatization stages, as well as applying this protocol to large-scale propagation and conservation programs for *Liriodendron tulipifera*

ACKNOWLEDGEMENTS

This research received no specific grant from any funding agency in the public, commercial, or not-for-profit sectors.

REFERENCES

1. Bhojwani, S.S. and Razdan, M.K. (1996) Plant Tissue Culture: Theory and Practice)
2. Beck, D.E. (1962). Yellow-poplar site index curves. USDA Forest Service, Research Note 180, Southeastern Forest Experiment Station, Asheville, NC. https://www.srs.fs.usda.gov/pubs/rn/rn_se180.pdf
3. Cao, Y, Feng J, Hwarari, D, Ahmad, B, Wu, H, Chen, J, Yang, L (2020). Alterations in Population Distribution of *Liriodendron chinense* (Hemsl.) Sarg. and *Liriodendron tulipifera* Linn. Caused by Climate Change. *Environmental Science and Ecology* 12(3):234–245. <https://doi.org/10.3390/f13030488>
4. Gao, J, Zhang, P, Xue, J, Xue, Y, Wang, S, 2019. Advances in phenolic substances and their effects on browning in woody plant tissue culture. *Acta Horticulturae Sinica* 46:1645–54 doi:10.16420/j.issn.0513-353x.2018-0698)
5. Galal, A., 2012. Improving effect of salicylic acid on the multipurpose tree *Ziziphus spina-christi* (L.) Willd tissue culture. *American Journal of Plant Sciences*, 3(7), pp.947–952. doi:10.4236/ajps.2012.37112)
6. Meyer, F. G. (2020) Fowells HA (1965). *Silvics of Forest Trees of the United States*. Agriculture Handbook No. 271. USDA Forest Service, Washington, D.C. 762 pp. http://floranorthamerica.org/Liriodendron_tulipifera
7. George, E.F., Hall, M.A., De Klerk, G. J. (2008) *Plant Propagation by Tissue Culture, Volume 1: The Background*) <https://investigacionfitopatologiaumar.wordpress.com/wp-content/uploads/2016/06/plant-propagation.pdf>
8. George, E.,F, Hall, M.A, De Klerk, G.J. (2008). *Plant Propagation by Tissue Culture*. Springer.
9. Kang, Y.F, Liu, C.M, Kao, C.L, Chen, C.Y. (2014). Antioxidant and anticancer constituents from the leaves of *Liriodendron tulipifera*. *Molecules* 19(4):42344245. <https://doi.org/10.3390/molecules19044234>
10. Krishnan, P.N, Siril, EA (2017). Control of phenolic exudation in *Garcinia* tissue culture. *Plant Biotechnology* 34(2):87–94. [https:// DOI:10.3844/ajbbsp.2006.97.104](https://doi.org/10.3844/ajbbsp.2006.97.104)
10. Liu, C., Fan, H., Zhang, J., Wu, J., Zhou, M., et al. 2024. Combating browning: mechanisms and management strategies in in vitro culture of economic woody plants. *Forestry Research* 4:e032 <https://doi.org/10.48130/forres-0024-0026>
12. Liu, C., Fan, H., Zhang, J., Wu, J., Zhou, M., et al. 2024. Combating browning: mechanisms and management strategies in in vitro culture of economic woody plants. *Forestry Research* 4:e032 <https://doi.org/10.48130/forres-0024-0026>
13. Parks, C.A., Miller, J.H., Voth, R.D. (1995). Silvicultural considerations for *Liriodendron tulipifera*. *Southern Journal of Applied Forestry* 19(2):78–83.
14. Russell, E.W. (1977). *Silvics of North America, Volume 2: Hardwoods – Liriodendron tulipifera*. USDA Forest Service, Agriculture Handbook 654.
15. Santamour, F.S. Jr., McArdle, A.J. (1984). Cultivar checklist for Liquidambar and *Liriodendron*. *Journal of Arboriculture* 10(11):309–312. <https://auf.isa-arbor.com/content/isa/10/11/309.full.pdf>
16. Martínez, V. (2007) Enzyme activities as affected by soil properties and land use in a tropical watershed). <https://doi.org/10.1016/j.apsoil.2006.05.012>
17. Wilcox, JR, Taft, KA, (1969). Genetics of yellow-poplar. USDA Forest Service Research Paper WO-6:12 pp
18. Weatherhead, M. A. (1978). Some effects of activated charcoal as an additive to plant tissue culture media (adsorption of auxin and cytokinin).
19. Zhong, J. 2019. Preliminary study of tissue culture system of *Sapindus mukorossi* Gaertn. Thesis. Beijing Forestry University, China. pp. 32–36.
20. Şen, A., 2012. Oxidative Stress Studies in Plant Tissue Culture October 2012. DOI:10.5772/48292)

Biotechnological potential of aquatic plant ecdysteroids

A. J. Jumaniyozova* and N.A. Khojamshukurov

Institute of Tashkent Chemical- technology, Tashkent, Uzbekistan

Corresponding author email: asoloyjumaniyozova@gmail.com

ABSTRACT

Ecdysteroids are polyhydroxylated sterols initially identified in insects but later reported in numerous terrestrial plants, including *Ajuga*, *Rhaponticum*, and *Cyanotis*. These metabolites display diverse biological properties such as anabolic, adaptogenic, antioxidant, antimicrobial, and antitumor effects, making them highly relevant for pharmaceutical and nutraceutical applications. However, their occurrence in aquatic plants remains poorly studied. This review highlights the potential of fast-growing macrophytes, particularly *Lemna minor* (duckweed), as promising sources of ecdysteroids and other bioactive compounds including flavonoids and amino acids. Available literature is critically analyzed to compare existing findings and to assess their implications for food technology, pharmacy, and biotechnology. By integrating current evidence, this article positions *Lemna minor* as a model system for exploring aquatic plant-based metabolites. The review also outlines future research priorities and industrial opportunities for sustainable production of nutraceutical and pharmaceutical agents from aquatic sources.

Keywords: aquatic plants, *Lemna minor*, ecdysteroids

INTRODUCTION

Aquatic macrophytes are widely distributed in freshwater ecosystems, where they play essential ecological roles in water purification, nutrient cycling, and food web stability. Beyond their ecological significance, these plants represent valuable reservoirs of biologically active compounds, including flavonoid glycosides, vitamins, amino acids, and macroelements, which have been increasingly recognized as potential sources for human and animal health applications (Seferli *et al.*, 2024). Flavonoids isolated from aquatic plants exhibit potent antioxidant and antimicrobial properties, underscoring their potential in the development of biotechnological products (Alharthi *et al.*, 2024). Additionally, minerals such as calcium, magnesium, iron, and zinc contribute not only to plant metabolism but also enhance their nutritional and pharmacological relevance (Seferli *et al.*, 2024).

Among these bioactive constituents, ecdysteroids have attracted particular attention. Initially discovered in insects, these polyhydroxylated sterols have also been detected in aquatic plants, where they are implicated in stress tolerance and metabolic regulation [Todorova *et al.*, 2024]. Alongside, vitamins (B, C, and E) and amino acids further enhance the nutritional and biomedical potential of aquatic macrophytes.

Within the duckweed family, *Lemna minor* stands out as a fast-growing, small floating plant with remarkable ecological and biotechnological applications. It functions effectively as a bioindicator and phytoremediator, particularly in the absorption of heavy metals and wastewater treatment (Zhou *et al.*, 2023). Moreover, its protein content may reach up to 45% of dry weight, and it is enriched with pigments such as β -carotene and xanthophylls at concentrations significantly higher than those found in many terrestrial plants. These

attributes position *L. minor* as a promising candidate for alternative feed in poultry and aquaculture, as well as a sustainable source of nutraceuticals (Ullah *et al.*, 2022).

Taken together, the phytochemical diversity of aquatic plants highlights their dual ecological and biotechnological importance. In this context, the present review systematically examines bioactive compounds of aquatic macrophytes, with particular emphasis on ecdysteroids and their biotechnological significance using *Lemna minor* as a model species.

Literature review methodology

Study Design and Aim

This study was conducted as a structured literature review aimed at summarizing and systematizing current knowledge on the bioactive compounds of aquatic plants and macrophytes, including ecdysteroids, vitamins, amino acids, proteins, and mineral elements. The research strategy involved comprehensive searches in major scientific databases, namely PubMed, Scopus, Web of Science, ScienceDirect, and Google Scholar.

Literature Search Strategy

The literature search covered publications from 1995 to 2024, focusing on peer-reviewed journal articles, conference proceedings, and relevant monographs in the fields of phytochemistry, biotechnology, pharmacology, food chemistry, and environmental sciences. The following keywords and their combinations were used: “*ecdysteroids in aquatic plants*,” “*bioactive compounds in Lemna and Azolla*,” “*proteins and amino acids in macrophytes*,” “*vitamin content of duckweed*,” “*minerals in Eichhornia and Pistia*,” and “*biotechnological applications of aquatic plants*.”

Inclusion and Exclusion Criteria

Inclusion criteria were established to ensure scientific relevance: Studies reporting quantitative or qualitative data on bioactive compounds in aquatic plants and macrophytes; Research focusing on species such as *Lemna*, *Azolla*, *Eichhornia*, and *Pistia*; Articles providing insights into the potential applications of these compounds in biotechnology, pharmacology, nutraceuticals, or environmental protection. Exclusion criteria included publications lacking primary data, non-peer-reviewed sources, and articles not available in English or Russian.

Data Extraction and Analysis

The extracted data were categorized into thematic groups: ecdysteroids and their pharmacological activity; vitamins and antioxidant potential; proteins and amino acids with nutritional and therapeutic value; minerals with physiological significance; biotechnological and pharmaceutical applications. All information was critically analyzed, cross-compared, and synthesized to provide an integrated perspective on the biotechnological significance of aquatic plants as renewable bioresources.

Occurrence and Biosynthesis of Ecdysteroids in Aquatic Plants

Ecdysteroid Content in Aquatic and Terrestrial Plants

The systematic analysis of published data revealed that aquatic plants represent a promising and underexplored source of bioactive compounds, including ecdysteroids, proteins, amino acids, vitamins, and mineral elements. Although terrestrial species such as

Ajuga turkestanica, *Rhaponticum carthamoides*, and *Cyanotis arachnoidea* remain the most studied ecdysteroid-producing plants, several aquatic species have alsodemonstrated measurable concentrations of these metabolites. In *Lemna minor*, ecdysteroid levels were reported in the range of 0.2–0.4 mg/g dry weight, which, although lower than terrestrial species, is noteworthy given the plant’s rapid growth and high biomass yield.

These compounds were detected using chromatographic and spectroscopic techniques such as HPLC and LC-MS.

The reported concentrations in *Lemna minor* range from 0.2–0.4 mg/g DW depending on nutrient and light conditions.

Table 1 summarizes the ecdysteroid content of selected terrestrial and aquatic plants. As expected, terrestrial adaptogenic species such as *Ajuga turkestanica* (0.8–1.2 mg/g DW) and *Cyanotis arachnoidea* (0.7–1.1 mg/g DW) demonstrated the highest concentrations, confirming their well-documented role as major sources of pharmacologically relevant ecdysteroids. *Rhaponticum carthamoides* also showed moderate levels (0.5–0.9 mg/g DW), supporting previous reports of its traditional use as a tonic and performance-enhancing plant.

In contrast, aquatic macrophytes such as *Lemna minor* exhibited lower ecdysteroid content (0.2–0.4 mg/g DW). However, this relatively modest concentration must be interpreted in the context of the plant’s exceptionally high growth rate and short harvesting cycle, which allows continuous biomass production. From a biotechnological perspective, even small concentrations of ecdysteroids may be commercially viable when combined with large-scale cultivation systems.

Interestingly, *Vitex glabrata* contained 0.3–0.6 mg/g DW, overlapping with the lower range of aquatic species. This finding suggests that certain macrophytes may reach levels comparable to terrestrial plants under optimized growth conditions. Moreover, variations in ecdysteroid content across species are likely influenced by environmental conditions, nutrient availability, and ecological adaptation strategies, which could be harnessed in controlled cultivation systems to enhance metabolite yield.

Table 1. Ecdysteroid content in selected terrestrial and aquatic plants (mg/g dry weight).

Plant species	Ecdysteroid content (mg/g DW)	References
<i>Ajuga turkestanica</i>	0.8–1.2	(Guibout <i>et al.</i> , 2015)
<i>Rhaponticum carthamoides</i>	0.5–0.9	(Roumanille <i>et al.</i> , 2020)
<i>Cyanotis arachnoidea</i>	0.7–1.1	(Hunyad <i>et al.</i> 2016)
<i>Vitex glabrata</i>	0.3–0.6	(Thiem <i>et al.</i> 2017).
<i>Lemna minor</i>	0.2–0.4	(Todorova <i>et al.</i> , 2024)

These findings suggest that aquatic plants, although secondary producers of ecdysteroids, could complement terrestrial sources by offering a renewable, sustainable supply of these metabolites. Variations in ecdysteroid content among species are likely driven by environmental factors, nutrient status, and ecological adaptation mechanisms, which could be optimized in future cultivation systems.

Nutritional and Biochemical Composition of Aquatic Plants

Table 2 presents a comparison of the major nutritional and biochemical components of selected aquatic macrophyte plants. Among the analyzed species, *Lemna minor* exhibited the highest protein concentration (35–45% of dry weight), surpassing common terrestrial crops

such as soybeans (typically 30–35%). This exceptional protein yield, combined with a balanced amino acid profile reported in earlier studies, underscores the potential of *Lemna* as a sustainable plant-based protein source for both nutraceutical and pharmaceutical applications.

Azolla spp. are particularly notable for their symbiotic relationship with *Anabaena azollae*, which enables atmospheric nitrogen fixation, enriching the plant with nitrogenous compounds and enhancing its protein content. This makes *Azolla* a dual-purpose resource, valuable both as animal feed and as a natural biofertilizer. *Eichhornia crassipes* (water hyacinth) and *Pistia stratiotes* showed relatively lower protein concentrations (18–30%), yet their biochemical composition remains noteworthy. *Eichhornia* was particularly enriched in Ca and Mg, minerals that play essential roles in bone health, enzymatic activity, and cellular metabolism. *Pistia* contained higher levels of Zn and K, which are critical for immune function and electrolyte balance. These mineral accumulations indicate that aquatic macrophytes may serve as functional foods or nutraceutical additives, beyond their role as protein sources.

Vitamin content analysis revealed that *Lemna minor* and *Azolla* spp. possess high levels of vitamins A, C, and E, as well as B-complex vitamins, which provide strong antioxidant potential. The antioxidant-rich profile of these plants suggests potential for use in the development of dietary supplements aimed at reducing oxidative stress and supporting metabolic health. *Pistia*, although lower in protein, contained notable amounts of vitamin C, reinforcing its antioxidant significance.

Taken together, (Table 2) indicates that aquatic plants represent a multifunctional biomass resource: *Lemna minor* is optimal for protein and micronutrient recovery. *Azolla* spp. offer nitrogen-fixing capacity and balanced nutritional properties. *Eichhornia* and *Pistia* provide specific mineral and vitamin enrichments valuable for targeted applications.

Thus, while each species exhibits unique strengths, *Lemna minor* emerges as the most versatile aquatic macrophyte, combining high protein, vitamin richness, and trace element accumulation with rapid growth, making it particularly suitable for biotechnological exploitation.

Table 2. Major nutritional and biochemical components of aquatic plants (% dry weight).

Species	Protein (%)	Vitamins (A, C, E, B)	Predominant minerals	References
<i>Lemna minor</i>	35–45	High	Fe, Mn	(Bethancourt-Dalmasi <i>et al.</i> , 2025).
<i>Azolla</i> spp.	20–30	B-complex, carotenoids	N, P	(Varun <i>et al.</i> , 2024)
<i>Eichhornia</i> spp.	20–30	Moderate	Ca, Mg	(Bethancourt-Dalmasi <i>et al.</i> , 2025).
<i>Pistia</i> spp.	18–25	Vitamin C	Zn, K	(Varun <i>et al.</i> , 2024)

Table 2 presents the major nutritional and biochemical constituents of selected aquatic macrophytes. Among the analyzed species, *Lemna minor* exhibited the highest protein concentration (35–45% DW), surpassing common terrestrial crops such as soybean (30–35%). *Azolla* spp. and *Eichhornia* spp. displayed moderate protein levels (20–30%), while *Pistia stratiotes* contained 18–25%, confirming the nutritional versatility of aquatic species. Overall, the results demonstrate that aquatic plants, particularly *Lemna minor*, combine high biomass productivity with significant levels of bioactive compounds, thereby offering potential for application in pharmaceuticals, nutraceuticals, and environmental biotechnology.

DISCUSSION

Comparative Role of Aquatic and Terrestrial Species

The findings of this review confirm that aquatic macrophytes are an underexplored yet significant reservoir of bioactive compounds. While terrestrial species such as *Ajuga turkestanica* and *Cyanotis arachnoidea* remain the richest sources of ecdysteroids, aquatic plants, including *Lemna minor* and *Azolla spp.*, also synthesize measurable concentrations of these metabolites. Although the absolute ecdysteroid content is lower in aquatic species, their rapid growth rate and continuous biomass production offer clear biotechnological advantages. As noted by Dinan and Lafont (2022), total yield and scalability of cultivation can outweigh metabolite concentration when assessing commercial potential.

Nutritional and Biotechnological Potential

Beyond ecdysteroids, aquatic macrophytes demonstrate remarkable nutritional versatility. In addition to ecdysteroids, aquatic macrophytes revealed remarkable nutritional profiles (Table 2). The protein content of *Lemna minor* (35–45% DW) rivals or even exceeds that of soybeans and other conventional crops, suggesting its potential as a sustainable alternative protein source. Previous studies (Hafiz Ullah et al., 2022) confirmed that *Lemna* can be effectively used as a poultry and aquaculture feed ingredient due to its balanced amino acid composition. Similarly, *Azolla spp.*, while slightly lower in protein, provide additional ecological benefits through nitrogen fixation, making them a dual-purpose resource for both agriculture and nutrition.

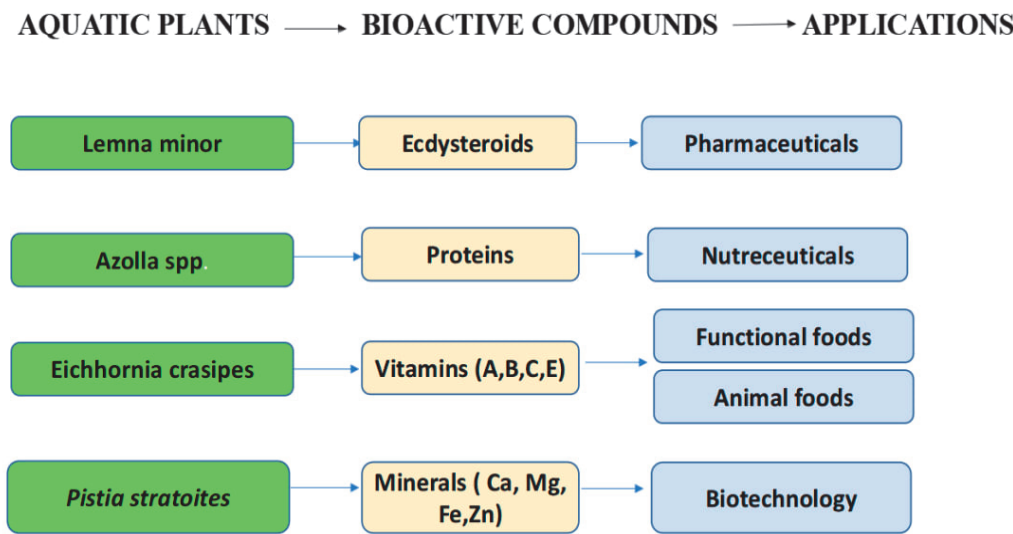


Figure 1. Bioactive compounds derived from aquatic plants and their applications. *Lemna minor* produces ecdysteroids with pharmaceutical potential; *Azolla spp.* is rich in proteins applied in nutraceuticals; *Eichhornia crassipes* contains vitamins (A, B, C, E) useful in functional and animal foods; while *Pistia stratiotes* provides minerals (Ca, Mg, Fe, Zn) applied in biotechnology.

Mineral and vitamin accumulation patterns further support the multifunctional value of aquatic plants. *Eichhornia crassipes*, rich in calcium and magnesium, and *Pistia stratiotes*, enriched with zinc and potassium, demonstrate species-specific metabolic adaptations that can be harnessed for targeted nutritional applications.

These findings are consistent with Seferli et al. (2024), who reported that macrophytes contribute significantly to micronutrient cycling within aquatic ecosystems. Moreover, the antioxidant-rich vitamin profile of *Lemna minor* and *Azolla* spp. suggests that these species could be valuable in the formulation of nutraceuticals aimed at oxidative stress management.

Pharmacological and Biotechnological Relevance

Although aquatic plants contain modest levels of ecdysteroids, recent *in vitro* and molecular docking studies (Mamadaliyeva *et al.*, 2020; Dinan and Lafont, 2022) suggest that even small doses can exhibit anabolic, antioxidant, and immunomodulatory effects when bioavailable. Therefore, aquatic species could complement terrestrial sources and contribute to sustainable production pipelines for ecdysteroid-based pharmaceuticals.

Ecdysteroids, particularly 20-hydroxyecdysone (20E), have attracted growing pharmacological interest due to their wide spectrum of biological activities in animals and humans. Numerous studies have demonstrated that phytoecdysteroids exert anabolic, adaptogenic, antioxidant, anti-inflammatory, immunomodulatory, and hypoglycemic effects without exhibiting the adverse side effects typical of synthetic steroids (Parr, *et al.*, 2014).

Experimental investigations have shown that ecdysteroids enhance protein synthesis and muscle growth by stimulating ribosomal activity and increasing mRNA translation efficiency. For this reason, 20E has been described as a natural anabolic agent with potential application in sports nutrition and muscle regeneration therapies. In addition, ecdysteroids regulate glucose metabolism by modulating PI3K/Akt and AMPK signaling pathways, thereby improving insulin sensitivity and cellular energy balance.

The antioxidant properties of ecdysteroids are linked to their polyhydroxylated structure, which allows free radical scavenging and stabilization of cellular membranes under oxidative stress. In aquatic macrophytes, this property may provide physiological protection against light-induced oxidative damage, while in humans it contributes to neuroprotective and hepatoprotective effects (Kumpun *et al.*, 2011).

Furthermore, several reports have emphasized the cytoprotective and anticancer potential of ecdysteroids. *In vitro* assays revealed that 20E inhibits the proliferation of certain tumor cell lines by inducing apoptosis and suppressing mitogenic signaling cascades. Such activities make ecdysteroids promising candidates for the development of plant-derived therapeutic agents.

Given their low toxicity and high bioactivity, ecdysteroids are also explored as natural components of cosmeceuticals, functional foods, and dietary supplements. However, their clinical application is still limited by poor bioavailability and rapid metabolism in mammals, which calls for further research into delivery systems such as nanoencapsulation and biopolymer conjugation (Dinan and Lafont, 2022).

Environmental and Research Perspectives

Nevertheless, several limitations should be acknowledged. First, the biochemical composition of aquatic plants is strongly influenced by environmental conditions, including nutrient availability, water quality, and light intensity. As noted by Van Dyck *et al.* (2021), ecdysteroid concentrations in *Lemna* and *Azolla* fluctuate considerably under different ecological settings. Second, large-scale utilization may raise ecological concerns, particularly with invasive species such as *Eichhornia crassipes*, which require controlled cultivation to avoid environmental risks. Future studies should integrate metabolomic and transcriptomic approaches to clarify biosynthetic

pathways, optimize cultivation for metabolite yield, and assess the bioavailability of aquatic-derived compounds in clinical and nutritional contexts.

CONCLUSION

This review demonstrates that aquatic macrophytes, particularly *Lemna minor*, represent a promising yet underutilized resource for the production of bioactive compounds, including ecdysteroids, proteins, vitamins, and essential minerals. While terrestrial plants such as *Ajuga turkestanica* and *Cyanotis arachnoidea* continue to be the richest sources of ecdysteroids, the rapid growth rate, ease of cultivation, and high biomass productivity of aquatic species provide significant biotechnological advantages. The comparative analysis of available data (Tables 1 and 2) highlights that *Lemna minor* combines moderate ecdysteroid concentrations with exceptional nutritional value, including high protein content and antioxidant-rich vitamins. These features position it as a versatile candidate for applications in pharmaceuticals, nutraceuticals, functional foods, and sustainable animal feed production. Moreover, the ability of certain macrophytes, such as *Azolla*, to fix atmospheric nitrogen adds further ecological and agricultural value. However, it must be emphasized that the biochemical composition of aquatic plants is highly dependent on environmental factors, and standardized cultivation protocols are required to ensure consistent metabolite yields. Addressing these limitations through controlled cultivation systems, metabolic engineering, and biorefinery approaches could significantly enhance the commercial potential of aquatic plants. In conclusion, aquatic macrophytes bridge the gap between ecological sustainability and biotechnological innovation. By combining moderate pharmacological potential with exceptional nutritional attributes, species such as *Lemna minor* offer a sustainable platform for the development of future pharmaceutical and nutraceutical products. Further interdisciplinary research integrating plant biotechnology, pharmacology, and environmental science will be critical to fully exploit their potential.

REFERENCES:

1. Alharthi, F., Althagafi, H. A., Jafri, I., Oyouni, A. A. A., Althaqafi, M. M., Al-Hijab, L. Y. A., Al-Hazmi, N. E., Elagib, S. M., and Naguib, D. M. (2024). Phytochemical Composition and Bioactivities of Some Hydrophytes: Antioxidant, Antiparasitic, Antibacterial, and Anticancer Properties and Mechanisms. *Plants*, 13(15), 2148. <https://doi.org/10.3390/plants13152148>
2. Bethancourt-Dalmasi, H., Viuda-Martos, M., Lucas-González, R., Borrás, F., & Fernández-López, J. (2025). Exploring the Applications of *Lemna minor* in Animal Feed: A Review Assisted by Artificial Intelligence. *Applied Sciences*, 15(12), 6732. <https://doi.org/10.3390/app15126732>
3. Dinan, L., and Lafont, R. (2022). Chapter Two - Ecdysteroids as defensive chemicals, Editor(s): Russell Jurenka, *Advances in Insect Physiology*, Academic Press, Volume 63: 107-154, ISSN 0065-2806, ISBN 9780323952644, <https://doi.org/10.1016/bs.aiip.2022.09.001>.
4. Guibout, L., Mamadalieva, N., Balducci, C., Girault, J. P., & Lafont, R. (2015). The minor ecdysteroids from *Ajuga turkestanica*. *Phytochemical analysis : PCA*, 26(5), 293–300. <https://doi.org/10.1002/pca.2563>
5. Hunyadi, A., Herke, I., Lengyel, K. 2016. Ecdysteroid-containing food supplements from *Cyanotis arachnoidea* on the European market: evidence for spinach product counterfeiting. *Sci Rep* 6, 37322 <https://doi.org/10.1038/srep37322>
6. Kumpun, S., Girault, J.-P., Dinan, L., Blais, C., Maria, A., Dauphin-Villemant, C., Yingyongnarongkul, B., Suksamrarn, A., & Lafont, R. (2011). *The metabolism of 20-hydroxyecdysone in mice: Relevance to pharmacological effects and gene switch applications of ecdysteroids*. *Journal of Steroid Biochemistry and Molecular Biology*, 126(1–2), 1–9.

7. Mamadalieva, N. Z., Hussain, H., Mollica, A., Zengin, G., Mamadalieva, R. Z., Elhady, S. S., Fadil, S. A., Ashour, M. L., & Youssef, F. S. (2022). Ecdysteroids as Potent Enzyme Inhibitors and Verification of Their Activity Using In Vitro and In Silico Docking Studies. *Life* (Basel, Switzerland), 12(6), 824. <https://doi.org/10.3390/life12060824>
8. Roumanille, R., Vernus, B., Brioché, T., Descossy, V., Van Ba, C. T., Campredon, S., Philippe, A. G., Delobel, P., Bertrand-Gaday, C., Chopard, A., Bonnieu, A., Py, G., & Fañça-Berthon, P. (2020). Acute and chronic effects of *Rhaponticum carthamoides* and *Rhodiola rosea* extracts supplementation coupled to resistance exercise on muscle protein synthesis and mechanical power in rats. *Journal of the International Society of Sports Nutrition*, 17(1), 58. <https://doi.org/10.1186/s12970-020-00390-5>
9. Seferli, M., Kotanidou, C., Lefkaki, M., Adamantidi, T., Panoutsopoulou, E., Finos, M. A., Krey, G., Kamidis, N., Stamatis, N., Anastasiadou, C., and Tsoupras, A. (2024). Bioactives of the Freshwater Aquatic Plants, *Nelumbo nucifera* and *Lemna minor*, for Functional Foods, Cosmetics and Pharmaceutical Applications, with Antioxidant, Anti-Inflammatory and Antithrombotic Health Promoting Properties. *Applied Sciences*, 14(15), 6634. <https://doi.org/10.3390/app14156634>
10. Thiem, B., Kikowska, M., Maliński, M. P., Kruska, D., Napierała, M. & Florek, (2017). *Ecdysteroids: production in plant in vitro cultures*. *Phytochemistry Reviews*, 16(4), 603–622. <https://doi.org/10.1007/s11101-016-9483-z>
11. Todorova, V., Ivanova, S., Chakarov, D., Kraev, K., & Ivanov, K. (2024). Ecdysterone and Turkesterone—Compounds with Prominent Potential in Sport and Healthy Nutrition. *Nutrients*, 16(9), 1382. <https://doi.org/10.3390/nu16091382>
12. Ullah, H., Gul, B., Khan, H. et al. Effect of growth medium nitrogen and phosphorus on nutritional composition of *Lemna minor* (an alternative fish and poultry feed). *BMC Plant Biol* 22, 214 (2022). <https://doi.org/10.1186/s12870-022-03600-1>
13. Varun, S. T., Prasad, R. M. V., Jayalaxmi, P., and Sandhya Rani, M. S. (2021). *A study on nutrient analysis of Azolla pinnata*. *Indian Journal of Animal Production and Management*, 37(1-4), 102-106. <https://doi.org/10.48165/ijapm..37.1-4.15>
14. Van-Dyck, I. Vanhoudt, N., Vives, J., Battle, I., Horemans, N., Nauts, R., Van Gompel, A., Claesen, Vangronsveld, J., (2021). Effects of environmental parameters on *Lemna minor* growth: An integrated experimental and modelling approach, *Journal of Environmental Management*, Volume 300, 2021, 113705, ISSN 0301-4797, <https://doi.org/10.1016/j.jenvman.2021.113705>.
15. Zhou, Y., Stepanenko, A., Kishchenko, O., Xu, J., & Borisjuk, N. (2023). Duckweeds for Phytoremediation of Polluted Water. *Plants* (Basel, Switzerland), 12(3), 589. <https://doi.org/10.3390/plants12030589>



Vegetable, Aromatic and medicinal plants

Growth and yield performance of Cucumber (*Cucumis sativus*) under different intercropping patterns with Water spinach (*Ipomoea aquatica*)

R. Agustina *, A.F. Indrazatmiko, S. Budi and E.S. Redjeki

**Department of Agrotechnology, Faculty of Agriculture, Universitas Muhammadiyah
Gresik, East Java, Indonesia**

*Corresponding author email: rohmatin@umg.ac.id

ABSTRACT

Intercropping has emerged as a sustainable agronomic strategy to enhance land-use efficiency and crop productivity through improved resource utilization and ecological balance. This study investigated the growth and yield performance of cucumber (*Cucumis sativus*) under different intercropping patterns with water spinach (*Ipomoea aquatica*). A field experiment was conducted using a randomized complete block design (RCBD) consisting of four treatments—monoculture, single-row intercropping, double-row intercropping, and alternating single-row intercropping—with three replications. Growth parameters (plant height and leaf number) and yield components (fruit length, diameter, weight, and number per plant) were measured. Results showed that intercropping patterns had a significant impact on cucumber growth and yield. The double-row intercropping system produced the highest plant height, number of leaves, and yield per hectare, statistically comparable to monoculture but superior to single-row and alternating patterns. Enhanced performance under the double-row system was attributed to improved light distribution, nutrient uptake, and soil moisture retention, which reduced interspecific competition and promoted photosynthetic efficiency. Conversely, single-row intercropping resulted in lower yield due to higher competition for resources. These findings suggest that the double-row intercropping pattern offers an ecologically efficient and productive alternative to monoculture, contributing to sustainable vegetable production systems. Implementing row-based intercropping could optimize land productivity while supporting agroecological resilience and resource-use efficiency in intensive vegetable cultivation.

Keywords: cucumber, water spinach, intercropping pattern, crop productivity, resource efficiency, agroecological management.

INTRODUCTION

Cucumber (*Cucumis sativus*) is among the most widely cultivated vegetable crops due to its high nutritional value and economic significance. However, the efficiency of cucumber production is frequently challenged by factors such as land-use limitations, nutrient depletion, and pest infestations, which can adversely affect growth and yield. One sustainable solution to address these challenges is intercropping, a well-recognized agronomic practice that enhances land productivity, improves resource utilization efficiency, and reduces the occurrence of pests and diseases (Chadfield *et al.*, 2022; Toker *et al.*, 2024). Water spinach (*Ipomoea aquatica*), a fast-growing leafy vegetable, has been

considered a potential companion crop for cucumbers in intercropping systems due to its capacity to improve soil structure, suppress weeds, and optimize nutrient cycling. Nevertheless, the impact of various intercropping patterns on cucumber growth and yield remains inadequately explored, making it crucial to investigate how different planting arrangements influence cucumber performance.

Existing research on intercropping systems has shown that various planting arrangements can significantly affect plant physiological responses, nutrient uptake efficiency, and overall productivity (Chen *et al.*, 2019; Toker *et al.*, 2024). Monocropping, while offering ease of management, often leads to nutrient depletion, higher susceptibility to pests, and inefficient resource use. In contrast, intercropping systems can boost biodiversity, enhance soil fertility, and create a more balanced agroecosystem, leading to greater sustainability in crop production (Glaze-Corcoran *et al.*, 2020; Li *et al.*, 2023; Maitra *et al.*, 2021; Rodriguez *et al.*, 2020). However, despite the acknowledged benefits, a gap remains in understanding how intercropping patterns, particularly row-based and alternating systems, affect cucumber growth and yield compared to monoculture. This study aims to fill this gap by evaluating various intercropping patterns of cucumber and water spinach, as well as their impact on plant growth parameters, leaf production, and yield performance.

The rationale for conducting this research arises from the need for innovative cropping systems that maximize land-use efficiency while ensuring high productivity. Previous studies have primarily focused on intercropping systems that feature cereal and legume crops, whereas research on vegetable intercropping, particularly involving cucumber and water spinach, is limited. Moreover, past findings have emphasized that row-based intercropping may offer improved light distribution, nutrient uptake efficiency, and better soil moisture conservation, resulting in enhanced growth and yield outcomes (Feng *et al.*, 2019; Jianxiong *et al.*, 2020; Lu *et al.*, 2023). By comparing various intercropping arrangements, this study seeks to identify the most effective planting pattern that can sustainably boost cucumber production.

This research is of significant importance to the scientific community as it contributes to sustainable agricultural practices by identifying optimal intercropping arrangements that improve productivity and resource efficiency. The study also provides valuable insights into plant-plant interactions, soil fertility management, and ecological advantages associated with diversified cropping systems. By closing the knowledge gap in vegetable intercropping, the findings from this study can inform future research and agricultural policies aimed at enhancing food safety while reducing environmental degradation.

MATERIALS AND METHODS

The research was conducted at the Agrotechnology Study Program Laboratory, Faculty of Agriculture, Universitas Muhammadiyah Gresik, located in Klitih Hamlet, Wajik Village, Lamongan District, with geographical coordinates of 7°06'11"S and 112°22'32"E. A tropical climate characterizes this study. Wajik is situated at an altitude of 8 meters above sea level, with a mean temperature of 26 °C. The site receives an annual precipitation of 1600 mm. The soil is classified as heavy clay Vertisol. The study employs a random group design with four intercropping pattern treatments: (1) monoculture, (2) single-row intercropping, (3) double-row intercropping, and (4) alternating single-row intercropping, with each treatment repeated three times (Figure 1). The measured variables include the growth and yield of cucumbers under different intercropping patterns. The experimental plot size for each treatment was 3 m × 1 m. The cucumber plant spacing was 50 cm x 70 cm, while the distance for water spinach was 15 cm x 20 cm. Results are expressed as the mean of three replications for each individual. Data were analyzed using two-way analysis of variance

(ANOVA) with SmartStat XL V. software (Setiawan, 2023), and multiple treatments were compared using the Tukey test at a 5% significance level.

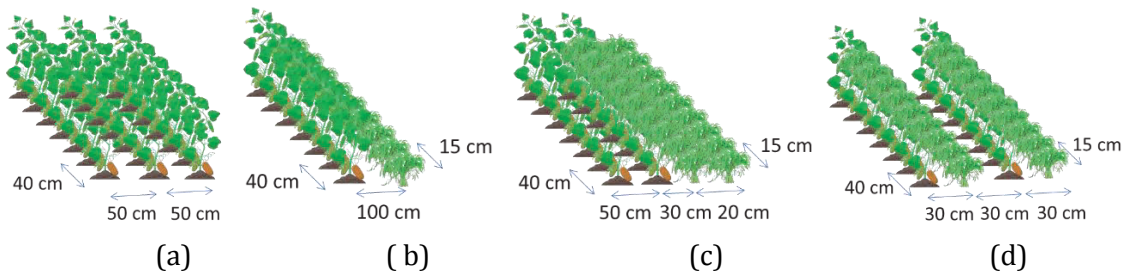


Figure 1. An intercropping pattern of cucumber and water spinach. Plot size: 3 m x 1 m.
 (a) Monoculture, population density of 24 cucumber plants; (b) single-row intercropping, population density of 5 cucumber plants and 22 water spinach plants; (c) double-row Intercropping, population density of 10 cucumber plants and 44 water spinach plants; (d) Alternating single-row intercropping, population density of 10 cucumber plants and 44 water spinach plants.

RESULTS AND DISCUSSIONS

System intercropping is employed to enhance land efficiency and optimize plant interactions. In this study, long-plant cucumbers in monoculture were compared to various intercropping patterns with water spinach at 14, 28, 42, and 56 days after planting (DAP).

Table 1. Influence of different intercropping patterns with water spinach (*Ipomoea aquatica*) on cucumber plant length.

Treatment	14 DAP	28 DAP	42 DAP	56 DAP
Monoculture	12.2 ± 1.2 ^b	127.8 ± 6.3 ^b	178.5 ± 8.6 ^{ab}	224.8 ± 18.4 ^a
Single-row Intercropping	7.5 ± 1.0 ^a	116.2 ± 8.4 ^{ab}	152.0 ± 2.0 ^a	214.2 ± 11.3 ^a
Double-row Intercropping	8.3 ± 0.7 ^a	109.3 ± 9.3 ^{ab}	195.7 ± 3.2 ^b	254.7 ± 17.6 ^b
Alternating single-row intercropping	7.5 ± 0.5 ^a	105.2 ± 5.5 ^a	178.6 ± 20.0 ^{ab}	220.7 ± 11.0 ^a
CV (%)	7.08	6.08	6.36	4.44
Tukey test	1.78	19.71	31.70	28.66
LS	**	*	*	*

DAP stands for Days After Planting, LS denotes Level of Significance, SD refers to Standard Deviation, CV indicates Coefficient of Variation, Different letters within a column indicate significant differences according to Tukey's test ($p < 0.05$).

The results indicate significant differences between treatments, marked by different letters at each observation time. The research results highlight significant differences in plant height, depending on the intercropping patterns with water spinach, compared to monoculture (Table 1). At 14 days after planting, cucumbers grown in the monoculture system showed the highest growth (12.2 cm), results that differed significantly from plants grown in the intercropping treatments. Cucumber plants in monoculture do not face substantial competition for light and nutrients in their initial growth phase (Chaudhary *et al.*, 2022; Gou *et al.*, 2017). However, as the plants developed, differences emerged between the cropping systems, highlighting the influence of complex ecological interactions in intercropping systems. In the advanced growth phase (42 DAP and 56 DAP), the two-row intercropping system showed the highest growth. At 42 DAP, the height of cucumbers in the -row intercropping system reached 195.7 cm and was significantly different from the single-row intercropping system (152 cm). The same differences were evident at 56 days after

planting (DAP), where the length of cucumbers in the double-row intercropping system (254.7 cm) was significantly higher than in all other treatments (Table 1). The benefits of the double-row intercropping system for increasing long-plant cucumbers can be explained from physiological and agronomic perspectives. From a physiological standpoint, the double-row intercropping pattern enables more even light distribution among plants, reduces shade competition, and enhances photosynthesis efficiency (Liu *et al.*, 2018, 2017; Lu *et al.*, 2023). From an agronomic perspective, this pattern promotes root stratification, which reduces direct nutrient competition, increases nutrient absorption efficiency, and improves soil moisture retention (Andersen, 2005; Glaze-Corcoran *et al.*, 2020; Qiang *et al.*, 2024). The results showed that the single-row intercropping system produced the lowest growth rates of cucumber plants, especially after 28, 42 and 56 days after planting. This can be attributed to intense interspecific competition in dense planting patterns. Greater spacing between cucumbers and water spinach in the single-row intercropping system significantly increases competition for light, nutrients, and soil moisture, ultimately impeding optimal cucumber growth.

The study's results indicate that selecting an appropriate intercropping pattern, such as the double-row system, can significantly optimize the growth of primary plants without compromising land efficiency and sustainable production systems. The observed differences between treatments reflect the complex interactions among ecological, physiological, and agronomic factors in the double-row planting system. However, to provide comprehensive and practical recommendations, the study will further integrate other aspects such as harvest outcomes, product quality, pest and disease dynamics, along with economic analysis.

Table 2. Effect of different intercropping patterns with water spinach (*Ipomoea aquatica*) on the average number of leaves in cucumber (*Cucumis sativus*) at various growth stages.

Treatment	14 DAP	28 DAP	42 DAP	56 DAP
Monoculture	2.7 ± 0.8 ^a	23.5 ± 0.6 ^{ab}	13.3 ± 2.4 ^a	193.9 ± 3.5 ^a
Single-row Intercropping	2.0 ± 0.5 ^a	22.2 ± 1.9 ^a	32.3 ± 1.5 ^b	199.2 ± 7.0 ^{ab}
Double-row Intercropping	2.0 ± 0.5 ^a	27.2 ± 1.1 ^b	36.6 ± 4.1 ^b	218.8 ± 7.1 ^b
Alternating single-row intercropping	2.2 ± 0.3 ^a	26.4 ± 1.6 ^b	33.6 ± 4.3 ^b	218.8 ± 7.8 ^b
CV (%)	22.95	5.25	11.56	3.38
Tukey test	1.43	3.68	9.47	19.87
F-test	-	**	**	**

DAP stands for Days After Planting, LS denotes Level of Significance, SD refers to Standard Deviation, CV indicates Coefficient of Variation, Different letters within a column indicate significant differences according to Tukey's test ($p < 0.05$).

The results of leaf number showed significant differences between the various intercropping patterns with water spinach and the monoculture system (Table 2). During the early growth phase (14 DAP), number of leaves did not show a significant difference between treatments, ranging from 2.0 to 2.7 leaves per plant. At 28 DAP, the double-row intercropping and the alternating single-row intercropping systems showed the highest leaf number (27.2 and 26.4 leaves) compared to the single-row intercropping system (22.2) and monoculture (23.5). This suggests that specific intercropping systems can enhance leaf production compared to monoculture systems, as supported by improved light efficiency and optimized nutrient utilization in the double-row intercropping pattern sequence (Gou *et al.*, 2017). At 42 DAP, the number of cucumbers leaves in the double-row intercropping and alternating single-row

intercropping reached 36.6 and 33.6 leaves, respectively, compared to 13.3 leaves in cucumber grown in the monocropping system (13.3) and single-row intercropping (32.3). The same significant differences were maintained at 56 DAP. The number of leaves in the two-row intercropping and single-row intercropping (218) was significantly higher than that in the monoculture (193.9) and single-row intercropping (199.2). The increase in the number of leaves in the two-row intercropping and single-row alternating intercropping can be attributed to several factors, including reduced competition, improved access to light (Gou *et al.*, 2017), improved nutrient uptake by roots (Qiang *et al.*, 2024), and more efficient groundwater management compared to monoculture systems.

The significant difference in the increase in the number of leaves on cucumber plants in double-row intercropping and alternating single-row intercropping after 42 DAP, compared to monoculture, can be attributed to various synergistic agronomic and physiological factors. These factors include improved light management, enhanced efficiency, better nutrient uptake through root zone differentiation, reduced allelopathic effects, and a more stable groundwater balance (Fu *et al.*, 2019; Nweke, 2020; Yuhua *et al.*, 2017). Consequently, these two intercropping patterns offer greater agronomic benefits than monoculture systems, especially in promoting the vegetative growth of cucumber plants during advanced growth phase.

The results of the study revealed significant differences in several parameters of cucumber harvest results being influenced by the types of intercropping patterns with water spinach (Table 3). Regarding fruit length, the single-row intercropping showed significantly greater fruit length (23 cm).

This indicates that intercropping patterns can influence the development of cucumber fruit length, potentially through mechanisms related to light competition and the distribution of different nutrients in each system (Frąc *et al.*, 2023). However, for fruit diameter, no significant differences were observed between treatments (5.1 - 5.6 cm), suggesting that fruit diameter is more affected by genetic factors than by environmental influences (Kumar *et al.*, 2022).

Table 3. Effect of different intercropping patterns with water spinach (*Ipomoea aquatica*) on yield and fruit characteristics of cucumber (*Cucumis sativus*).

Treatment	Fruit Length (cm)	Fruit Diameter (cm)	Fruit Weight (t ha ⁻¹)	Fruit Weight Plant ⁻¹ (kg)	Fruit Weight Fruit ⁻¹ (g)	Number of Fruits Plant ⁻¹
Monoculture	19.4 ± 0.3 ^a	5.3 ± 0.4	26.9 ± 7.2 ^b	2.8 ± 1.2 ^a	260.8 ± 51.2 ^b	11.3 ± 4.3 ^a
Single-row Intercropping	23.0 ± 0.6 ^b	5.6 ± 0.0	14.7 ± 5.1 ^a	2.3 ± 1.1 ^a	193.4 ± 52.3 ^a	11.3 ± 4.3 ^a
Double-row Intercropping	21.3 ± 1.0 ^{ab}	5.1 ± 0.0	25.9 ± 5.6 ^b	4.1 ± 1.2 ^b	235.9 ± 51.2 ^b	17.0 ± 4.4 ^c
Alternating single-row intercropping	19.7 ± 1.1 ^a	5.1 ± 0.1	23.0 ± 5.4 ^{ab}	3.9 ± 1.2 ^b	238.7 ± 50.5 ^b	14.3 ± 4.4 ^b
CV (%)	3.77	3.87	16.35	10.72	5.46	4.12
Tukey test	2.22	0.58	10.46	0.99	35.87	1.57
F-test	**	NS	*	**	**	**

DAP stands for Days After Planting, LS denotes Level of Significance, SD refers to Standard Deviation, CV indicates Coefficient of Variation, Different letters within a column indicate significant differences according to Tukey's test ($p < 0.05$).

In terms of fruit production per hectare, the monoculture and double-row intercropping recorded the highest yields (26.9 t.ha⁻¹ and 25.9 t.ha⁻¹), respectively and differed significantly from the yields recorded for cucumber grown in single-row intercropping (14.7 t.ha⁻¹). These findings suggest that a double-row intercropping model can enhance

cucumber productivity compared to single-row intercropping, likely due to the more efficient use of light and nutrient resources.

In terms of fruit weight per plant, double-row intercropping (4.1 kg) and single-row alternating intercropping (3.9 kg) were significantly higher compared to monoculture (2.8 kg) and single-row intercropping (2.3 kg). These results indicate that double-row intercropping and single-row alternating can increase the productivity of each cucumber plant, possibly due to improved management and reduced competition between plants compared to denser monoculture systems (Stefan *et al.*, 2022).

When considering fruit weight per fruit, the monoculture (260.8 g) and double-row intercropping (235.9 g) showed significantly higher fruit weights compared to single-row intercropping (193.4 g). The single-row intercropping (238.7 g) showed no significant difference from either the double-row intercropping or monoculture. These findings suggest that specific intercropping systems can enhance cucumber fruit size, with double-row intercropping yielding results comparable to monoculture in terms of fruit weight per plant. The number of fruits per plant was significantly higher in double-row intercropping (17) compared to all other crop types. The alternating single-row intercropping (14.3) also showed higher yields than monoculture and single-row intercropping (11.3). This significant difference indicates that double-row intercropping can increase the number of fruits produced per plant, which may be due to nutrient uptake and more optimal conditions (Li *et al.*, 2021). The study's results showed that double-row intercropping yielded comparable results to monoculture in terms of fruit weight per hectare and fruit weight per plant. Still, it had a significantly higher number of fruits per plant.

Additionally, alternating single-row intercropping also demonstrated promising results, particularly in terms of the number of fruits per plant and the weight of the fruits per plant. In contrast, single-row intercropping had the lowest yield compared to the other systems, which may have been caused by increased competition for resources due to the closer planting distance between cucumber and water spinach. Therefore, the intercropping system with the double-row pattern can be recommended as a more effective planting pattern for increasing cucumber productivity compared to other systems.

CONCLUSIONS

The results of this study demonstrate that different intercropping patterns with water spinach (*Ipomoea aquatica*) significantly influenced the growth and yield performance of cucumber (*Cucumis sativus*). Among the evaluated systems, the double-row intercropping consistently exhibited superior performance, with the highest plant height (254.7 cm at 56 DAP), leaf number (218.8), and fruit yield (25.9 t.ha⁻¹). These results indicate that this arrangement optimizes light distribution, enhances nutrient uptake, and reduces competition between plants.

The alternating single-row intercropping also showed notable improvements in vegetative growth and yield components compared to monoculture, suggesting its potential as a productive and land-efficient alternative for small-scale farmers. In contrast, the single-row intercropping recorded the lowest growth and yield parameters, likely due to greater interspecific competition for light, nutrients, and soil moisture.

Overall, this study confirms that double-row intercropping represents the most effective and sustainable pattern for improving cucumber productivity while maintaining ecological balance. The findings highlight the potential of optimized intercropping arrangements as a viable strategy for enhancing land-use efficiency and promoting sustainable vegetable production systems.

REFERENCES

1. Andersen, M.K., 2005. Competition and complementarity in annual intercrops - the role of plant available nutrients, in: *The Royal Veterinary and Agricultural University, Copenhagen, Denmark, Department of Soil Science*. p. 123.
2. Chadfield, V., Hartley, S., Redeker, K., 2022. Associational resistance through intercropping reduces yield losses to soil-borne pests and diseases. *The New Phytologist*. 235: 2393–2405.
3. Chaudhary, M., Kumar, R., Yadav, A., Maurya, S.K., Sahu, S., Tiwari, A., 2022. Best Row Ratio Combinations of Agronomic Crops in the Intercropping System: An Overview. *International Journal of Plant & Soil Science*. 34(22): 645–654.
4. Chen, P., Song, C., Liu, X.-M., Zhou, L., Yang, H., Zhang, X., Zhou, Y., Du, Q., Pang, T., Fu, Z.-D., Wang, X.-C., Liu, W., Yang, F., Shu, K., Du, J., Liu, J., Yang, W., Yong, T., 2019. Yield advantage and nitrogen fate in an additive maize-soybean relay intercropping system. *The Science of the total environment*. 657: 987–999.
5. Feng, L., Raza, M., Chen, Y., Khalid, M., Meraj, T., Ahsan, F., Fan, Y., Du, J., Wu, X., Song, C., Liu, C., Bawa, G., Zhang, Z., Yuan, S., Yang, F., Yang, W., 2019. Narrow-wide row planting pattern improves the light environment and seed yields of intercrop species in relay intercropping system. *PLoS ONE*. 14(2): e0212885.
6. Frąc, M., Panek, J., Gryta, A., Oszust, K., Pertile, G., Siegieda, D., Mącik, M., Pylak, M., Imran Pathan, S., Pietramellara, G., 2023. Legume-cereal intercropping as a strategy of regenerative agriculture supporting reverse of biodiversity loss - relevance of microbiome-based research. *ARPHA Conference Abstracts*. 6: e108886.
7. Fu, Z.-D., Zhou, L., Chen, P., Du, Q., Pang, T., Song, C., Wang, X., Liu, W., Yang, W., Yong, T., 2019. Effects of maize-soybean relay intercropping on crop nutrient uptake and soil bacterial community. *Journal of Integrative Agriculture*. 18(9): 2006–2018.
8. Glaze-Corcoran, S., Hashemi, M., Sadeghpour, A., Jahanzad, E., Keshavarz Afshar, R., Liu, X., Herbert, S.J., 2020. Understanding intercropping to improve agricultural resiliency and environmental sustainability. *Advances in Agronomy*. 162: 199–256.
9. Gou, F., Ittersum, M., Simon, E., Leffelaar, P., Putten, P., Zhang, L., Werf, W., 2017. Intercropping wheat and maize increases total radiation interception and wheat RUE but lowers maize RUE. *European Journal of Agronomy*. 84: 125–139.
10. Jianxiong, H., Jian, P., Zhou, L., Dinghua, Z., Yuan, S., Chen, J.-F., Li, J., Qing, G., Lin, W., 2020. An improved double-row rubber (*Hevea brasiliensis*) plantation system increases land use efficiency by allowing intercropping with yam bean, common bean, soybean, peanut, and coffee: A 17-year case study on Hainan Island, China. *Journal of Cleaner Production*. 263: 121493.
11. Kumar, R., Bhushan, A., Samnotra, R., Sharma, S., Naik, R., Chheepa, M.S., Naga, V.K., 2022. Heritability Analysis for Horticultural Traits in Tomato under Low Cost Polyhouse Conditions of Jammu Subtropics. *International Journal of Environment and Climate Change*. 12(10): 737–741.
12. Li, C., Stomph, T., Makowski, D., Li, H., Zhang, C., Zhang, F., Van Der Werf, W., 2023. The productive performance of intercropping. *Proceedings of the National Academy of Sciences of the United States of America*. 120(2): e2201886120.
13. Li, X.-F., Wang, Z.-G., Bao, X.-G., Sun, J.-H., Yang, S.-C., Wang, P., Wang, C.-B., Wu, J.-P., Liu, X.-R., Tian, X.-L., Wang, Yu, Li, J.-P., Wang, Yan, Xia, H.-Y., Mei, P.-P., Wang, X.-F., Zhao, J.-H., Yu, R.-P., Zhang, W.-P., Che, Z.-X., Gui, L.-G., Callaway, R., Tilman, D., Li, L., 2021. Long-term increased grain yield and soil fertility from intercropping. *Nature Sustainability*. Nat Sustai(4): 943–950.
14. Liu, X., Rahman, T., Song, C., Su, B., Yang, F., Yong, T., Wu, Y., Zhang, C., Yang, W., 2017. Changes in light environment, morphology, growth and yield of soybean in maize-soybean intercropping systems. *Field Crops Research*. 200: 38–46.
15. Liu, X., Rahman, T., Song, C., Yang, F., Su, B., Cui, L., Bu, W., Yang, W., 2018. Relationships among light distribution, radiation use efficiency and land equivalent ratio in maize-soybean strip intercropping. *Field Crops Research*. 224: 91–101.
16. Lu, J., Dong, Q., Lan, G., He, Z., Zhou, D.-M., Zhang, H., Wang, X.-G., Liu, X., Jiang, C.-J., Zhang, Z., Wan, S., Zhao, X., Yu, H., 2023. Row ratio increasing improved light distribution, photosynthetic characteristics, and yield of peanut in the maize and peanut strip intercropping system. *Frontiers in Plant Science*. 14(14 Juli).
17. Maitra, S., Hossain, A., Brestič, M., Skalický, M., Ondrisik, P., Gitari, H., Brahmachari, K., Shankar, T., Bhadra, P., Palai, J.B., Jena, J., Bhattacharya, U., Duvvada, S.K., Lalichetti, S., Sairam, M., 2021. Intercropping—A Low Input Agricultural Strategy for Food and Environmental Security. *Agronomy*. 11(2): 343.
18. Nweke, I., 2020. Potentials of Intercropping Systems to Soil - Water - Plant-Atmosphere. *Agricultural Science*. 2(1): 31–38.
19. Qiang, B., Fan, Z., Tang, N., Asad, M.S., Timbang, B.C., Ren, X., Chen, X., 2024. Improving the productivity of intercropping through above and below ground separation: A case study on photosynthetic characteristics and root distribution. *Industrial Crops and Products*. 222: 119506.
20. Rodriguez, C., Carlsson, G., Englund, J., Flöhr, A., Pelzer, E., Jeuffroy, M., Makowski, D., Jensen, E., 2020. Grain legume-cereal intercropping enhances the use of soil-derived and biologically fixed nitrogen in temperate

- agroecosystems. A meta-analysis. *European Journal of Agronomy*. 118: 126077.
21. Setiawan, A., 2023. SmartstatXL Versi 3.6.5.4 [software].
 22. Stefan, L., Engbersen, N., Schöb, C., 2022. Rapid transgenerational adaptation in response to intercropping reduces competition. *eLife*. 11(Januari): 40.
 23. Toker, P., Canci, H., Turhan, I., Isci, A., Scherzinger, M., Kordrostami, M., Yol, E., 2024. The advantages of intercropping to improve productivity in food and forage production – a review. *Plant Production Science*. 27(3): 155–169.
 24. Yuhua, Fu, S., Zhang, X., Zhao, K., Chen, H., 2017. Intercropping improves soil nutrient availability, soil enzyme activity and tea quantity and quality. *Applied Soil Ecology*. 119: 171–178.

Review on ethnobotanical knowledge and socio-economic value of Anchote (*Coccinia abyssinica*) cultivation and processing in major growing areas of Ethiopia

B.T. Abebe* and A.F. Dejenu

Ethiopian Biodiversity Institute, Ethiopia

*Corresponding author email: bachatamiru12@gmail.com

ABSTRACT

Anchote (*Coccinia abyssinica* (Lam.) Cogn.) is an indigenous and commonly cultivated root crop in Ethiopia that is highly valued for its nutritional, medicinal, and socioeconomic benefits. Despite its importance, the crop is still underutilized and understudied in terms of indigenous knowledge and traditional processing techniques. This review synthesizes existing indigenous knowledge on *C. abyssinica* cultivation, traditional food processing methods, socioeconomic role, and opportunities for enhancing its utilization. By systematically compiling published literature, technical reports, and academic works, this review provides a consolidated overview of the *C. abyssinica*'s ethnobotanical and socio-economic dimensions in its major growing regions of Ethiopia, particularly Oromia. The analysis focuses on its major growing regions in Ethiopia, particularly areas within the Oromia region, to draw context-specific conclusions and identify prevailing research gaps. The analysis confirms that *C. abyssinica* has deep cultural and traditional importance among local communities and has been cultivated for centuries using indigenous farming techniques. The crop is rich in essential nutrients, particularly calcium, and is an important dietary component. *C. abyssinica* plays an important role in household food security, income production, and cultural identity. Traditional processing procedures, such as boiling and drying improve its flavor and nutritional content. However, issues such as limited market access, a lack of superior cultivars, and dwindling cultivation all pose threats to its long-term viability. Strengthening conservation efforts and boosting value addition can help *C. abyssinica* play a bigger role in rural livelihoods and food security.

Keywords: cultural practices, traditional food system, tuber, nutritional value, value addition.

INTRODUCTION

Ethiopia has diversified agro-ecological conditions that are suitable for the production of various types of crops (Ayalew, 2020). Farmers in Ethiopia, like many other developing countries, have developed and maintained several diverse and locally adapted root and tuber cultivars of crops because no single cultivar can satisfy their basic needs (AbaDura *et al.*, 2021; Mariutti *et al.*, 2021). Among these, a root and tuber crop *C. abyssinica* is widely cultivated in different parts of Ethiopia (Duresso, 2018). Anchote [*Coccinia abyssinica* (Lam.) Cogn.] is a trailing vine tuberous crop, belongs to the Cucurbitaceous family and is grown annually (AbaDura *et al.*, 2021). It is an indigenous root crop extensively produced and highly employed in the socio-economic area across the South and South Western in Wollega,

Ilubabor, Kafa, and Sidamo in Ethiopia (Regessa, 2022). It is a subsistence crop commonly produced to fill food security gaps during the hunger seasons (June to September) in Ethiopia (Abera and Haile, 2015).

Coccinia abyssinica here after called (*C. abyssinica*) is a climate-resilient indigenous root crop that grows in a cultivar of agro-ecological zones in Ethiopia (Ashebir, 2022). Proper agronomic management and cultivation techniques are required to increase yield, improve tuber quality, and ensure long-term production (Wakjira *et al.*, 2024). It flourishes best in mid to high-altitude locations with evenly distributed rainfall and mild temperatures, as well as well-drained, fertile loamy or sandy loam soils rich in organic matter (Leta *et al.*, 2017; Gudeta, 2022).

C. abyssinica production has deep cultural ties with the Oromo people; it is utilized as a cultural meal during the Meskel Festival, which celebrates the discovery of the genuine cross (Temam, 2021; Anbesse and Hailegeorgis, 2021). According to Negassa and Amenu (2016), *C. abyssinica* tubers are high in nutritious content and extremely important. *C. abyssinica* tuber and leaf, like other root crops, provide significant nutrients and are being promoted for increased consumption to improve human nutrition. *C. abyssinica* is rich in minerals and fiber. It contains a lot of calcium, which is essential for our bones and teeth (Regessa, 2022). *C. abyssinica* is also thought to improve the health and strength of breastfeeding women (Temam, 2021). *C. abyssinica* juice contains saponin, which is used to cure gonorrhea, TB, and cancer (Regasa *et al.*, 2018). *C. abyssinica* is high in potassium and iron. As a result, it has the potential to help the country's food security (Gebeyehu *et al.*, 2024).

C. abyssinica tuber produces around 27 culinary products, which are classified as boiled, dehydrated, or soup (Parmar *et al.*, 2017). Another study discovered that “defeki” (paste), “moqa” (soup), and “chufata” (boiled and peeled *C. abyssinica* tuber cut and eaten with kochkocha) are traditional *C. abyssinica* recipe preparation methods in western Oromia (Gebeyehu *et al.*, 2024). Different cultivars of traditional foods made from *C. abyssinica* tubers and leaves need to be thoroughly explored and documented, including preparation methods, ingredients, and motivations for consuming *C. abyssinica* food products (Keno, 2024).

Industrialization is now rising at a considerably higher rate, and so are food processing sectors (Regessa, 2022). As a result, it is necessary to process a cultivar of locally available raw materials such as potato, carrot, beetroot, sweet potato, yam, garlic, and others (Habtamu, 2013). Among these several root crops, *C. abyssinica* plants are included in this category (Regessa, 2022) and selected for this review work. *C. abyssinica* flour contains more nutrients and crude fiber than wheat flour (Gebeyehu *et al.*, 2024). Biscuits made from a blend of *C. abyssinica* and wheat may have good potential for a cultivar of reasons, including increasing *C. abyssinica* consumption and encouraging farmers; the products will be a more acceptable healthy alternative because they are enriched with dietary fiber and minerals (Regessa, 2022). Despite its promise, *C. abyssinica* is still a neglected and underutilized crop (Abdena and Aga, 2023). Documenting the indigenous knowledge surrounding its cultivation and utilization is crucial for preserving cultural heritage and enhancing food security. This review explores indigenous knowledge surrounding its cultivation, processing, and socio-economic contributions to communities in its major growing regions.

Indigenous Knowledge in *Coccinia abyssinica* Cultivation

C. abyssinica cultivars are chosen based on tuber yield, disease resistance, and environmental adaptability (Duresso, 2018). Landrace cultivars are widely planted in Ethiopia and nearby areas, where farmers choose high-yielding plants with ideal tuber size, texture, and storage quality (Negash, 2019). Gutema (2019) found that 97.8% of growers in Gimbi and 98.3% in Ayira district prefer to plant white *C. abyssinica* (Fig. 1). This is because

white *C. abyssinica* has a soft texture, is easy to cook, contains less fiber, has a white tuber color, has a good taste, and has a higher market demand (Parmar *et al.*, 2017).

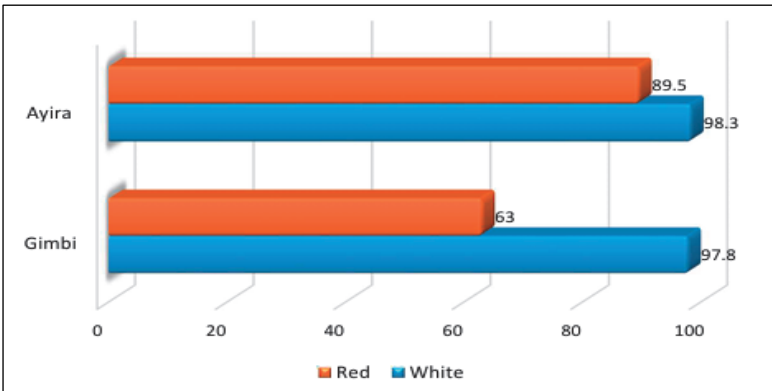


Figure 1. Local cultivars of *C. abyssinica* preference for growers (Source: Gutema, 2019)

Ethiopian farmers have produced and maintained multiple different and regionally adapted crop cultivars on their small piece of land (Belay *et al.*, 2017). Research is undertaken to improve the crop's genetic diversity and breeding potential, with the goal of generating cultivars that are more resistant to pests and diseases while keeping high nutritional value (Duresso, 2018). Choosing the proper cultivar results in increased output and adaptability to certain agro-ecological circumstances (Feyissa, 2020).

Abdena and Aga (2023) researched the evaluation of underutilized cultivated six (6) *C. abyssinica* accessions and identified higher storage root yield above 101 tons/ha. They also reported higher marketable storage root yield (ton/ha), and higher dry matter content production, indicating good performance and adaptation in Ethiopia's West Shewa central highland. The report focused mostly on agronomic performance, but there is limited information on post-harvest attributes, consumer preferences, and value-added processing capabilities, all of which are critical for market expansion and food security.

A genetic diversity analysis of 12 *C. abyssinica* populations using Inter Simple Sequence Repeat (ISSR) markers revealed that the Illubabor and Gimbi populations exhibited the highest genetic variation, underscoring their significance as key reservoirs for conservation and the selection of superior landraces. As interest in the preservation and sustainable utilization of traditional crops continues to grow, there is an increasing imperative to document, enhance, and promote high-yielding and resilient cultivars to ensure food security and biodiversity conservation (Ficiciyan *et al.*, 2018; Karri and Nalluri, 2024).

C. abyssinica L. is typically propagated through both seeds and vegetative means (Sisay *et al.*, 2020). However, seed propagation is less common due to its slow and inconsistent germination rates, making vegetative propagation via tuber or stem cuttings the preferred method for achieving faster and more uniform plant establishment (AbaDura *et al.*, 2021). Farmers carefully select quality fruits for seed production depending on their size and tuber composition. *C. abyssinica* plants with larger fruits generate smaller tubers; hence, smaller fruits are used as planting material (Feyissa, 2020). This is because using smaller fruits as planting material may lower the number of resources allocated to fruit formation, freeing up more energy for tuber growth. Tuber propagation entails selecting healthy, disease-free tubers that are sliced into portions with at least one viable bud and then planted in moist, well-prepared soil (Saraswathi and Thingalmaniyan, 2017). This method ensures genetic uniformity, promotes rapid growth, and facilitates early tuber formation, making it a highly efficient strategy for *C. abyssinica* cultivation.

Land Preparation and Intercropping Practices

Land preparation for *C. abyssinica* aims for a fine tilth for optimal tuber expansion. Tillage is the mechanical manipulation of soil with tools and implements to generate ideal soil conditions for better seed germination and subsequent crop growth. The frequency of tillage is determined by the type of soil, climate, and planting material size (Belissa, 2021). Tillage frequency for *C. abyssinica* is not recommended at this time; however, experiments on *C. abyssinica* show that the land should be crushed and a fine seed bed developed (Abera and Haile, 2015; Duresso, 2018). Seeds are commonly utilized for planting (Belissa, 2021). Farmers traditionally plant *C. abyssinica* by broadcasting and mixing with soil (Mekbib and Deressa, 2016), but in experimental plots, row planting is used. For example, in an experiment conducted in Jimma University College of Agriculture and Veterinary Medicine (JUCAVM) and East Wollega, where the spacing between plants is 10 cm and 40 cm between rows (Gudeta, 2022). Similarly, Anbesse and Hailegeorgis (2021) observed that increasing intra-row separation from 10 to 30 cm reduces root yield by 137%, while increasing row spacing from 40 to 100 cm results in a 37% loss in production.

Intercropping *C. abyssinica* with other crops is a widely adopted practice that enhances land productivity, improves soil fertility, and reduces pest pressure (Zerihun, 2022). *C. abyssinica* is frequently intercropped with cereal crops like maize and sorghum, as well as legumes like beans and peas and various crops like cereals (such as wheat, “teff”, maize, sorghum) (Gurmu, 2019). Leguminous intercrops in particular give nitrogen to the soil via biological nitrogen fixation, lowering the demand for synthetic fertilizers (Kebede, 2021). Proper spacing and crop arrangement are critical for reducing competition and ensuring that all crops receive enough sunlight and nutrients (Yimer and Babege, 2018). Successful *C. abyssinica* intercropping systems are intended to maximize resource utilization while avoiding excessive shade or root competition (Gebremedhin *et al.*, 2015). Rotational intercropping of deep-rooted and shallow-rooted crops can improve soil structure while lowering the danger of nutrient depletion (Shekhar *et al.*, 2024). Farmers frequently use mixed cropping tactics, in which *C. abyssinica* is produced alongside fast-growing vegetables such as cabbage or onions, allowing for early harvests before *C. abyssinica* matures (Bekele, 2019). This method not only boosts farm productivity but also improves food security by varying harvests throughout the growing season.

C. abyssinica cultivation also benefits from integrated soil fertility and moisture management. The crop thrives in well-drained loamy soils high in organic matter and with moderate soil moisture levels (Gebeyehu *et al.*, 2024). Organic fertilizers, such as compost and farmyard manure, improve soil structure and nutrient availability, resulting in increased tuber bulking and quality (Belissa, 2021). Mulching and the installation of soil conservation features such as ridges and bunds assist in retaining moisture and reducing erosion in moisture-stressed areas.

To summarize, adequate site preparation and intercropping are critical for increasing the growth, productivity, and sustainability of *C. abyssinica* farming. Fine seedbed preparation, proper spacing, and the use of organic fertilizers all improve soil fertility and tuber quality. Intercropping with grains, legumes, or vegetables increases land productivity, conserves moisture, and promotes sustainable farming practices.

Weeding and Fertilization

Weeding is an important agronomic practice in the cultivation of *C. abyssinica* because it reduces competition for nutrients, moisture, and sunlight, ensuring optimal plant growth and tuber formation (Belissa, 2021). The first weeding is usually done two to three weeks after planting, when the weeds are still small and simpler to handle (Wakjira *et al.*, 2024). Manual weeding using hand tools like hoes is the most frequent method, however, mulching with organic materials can also help inhibit weed development while conserving soil moisture.

Research on soil nutrient management for *C. abyssinica* is limited, despite observations that the crop requires nutrient-rich soil for optimal growth and development (Duresso, 2018). According to Duresso (2018), research conducted in Gimbi and Ayira revealed that 71.1% and 59.7% of growers, respectively, rely on natural fertilizers such as manure and compost. Farmyard manure (FYM) and blended fertilizer (NPSB) have been shown to alter days to physiological maturity, vine number, vine length, marketable root production, and other characteristics (Belissa, 2021). The highest overall root production was recorded at 175 kg ha⁻¹ NPSB and 10 t/ha FYM (Biratu, 2019). However, Abera and Guteta (2007) reported that the maximum root yield was recorded at 8 t/ha of farm yard manure (FYM), and the yield began to fall when the FYM rate was increased to 10 t/ha. Inorganic fertilizer application resulted in the highest yield at a nitrogen rate of 46 kg/ha and a phosphorus rate of 20 kg/ha (Abera and Haile, 2015), respectively.

C. abyssinica responds well to integrated nutrient management, which blends organic and inorganic fertilizers to promote long-term productivity. Organic inputs that increase soil structure and moisture retention, and mineral fertilizers like NPSB provide necessary nutrients for strong growth and tuber development (Belissa, 2021; Biratu, 2019). Balanced fertilization improves yield and soil health, although efficiency varies depending on soil type, moisture, and planting density, highlighting the importance of site-specific recommendations (Abera and Haile, 2015; Wakjira *et al.*, 2024).

Crop protection and Harvesting Techniques

Agronomic research on *C. abyssinica* in Ethiopia has primarily concentrated on aspects such as fertilization, spacing, and earthing-up to improve growth and yield. However, there is a notable lack of studies addressing pest and disease management. As highlighted in a review by Belissa (2021), despite the crop's commercial importance, formal agronomic research has largely overlooked the protection of *C. abyssinica* from diseases and insect pests.

Traditionally, farmers rely on indigenous knowledge to manage pests and protect their crops. For instance, to control bacterial leaf blight and powdery mildew, farmers often use botanical extracts from neem (*Azadirachta indica*), garlic (*Allium sativum*), and chili pepper (*Capsicum annuum*), which have antifungal and antibacterial properties. To deter insect pests like beetles, growers commonly practice intercropping with repellent plants such as marigold (*Tagetes* spp.) or Ethiopian mustard (*Brassica carinata*), which help reduce pest infestations naturally (Negash, 2019).

Farmers typically sow *C. abyssinica* seeds in April and harvest the tubers between July and August, making the growing period approximately four to five months (Belissa, 2021). Unlike most tubers, *C. abyssinica* is not harvested for home storage; instead, farmers leave the tubers in the field, harvesting only to meet immediate needs. One of the *C. abyssinica*'s unique characteristics is its long-term edibility compared to other local tubers such as yams and Oromo potatoes, whose underground organs become inedible after a year or so (Wayessa, 2018). Farmers strategically adjust harvesting periods based on household food needs and storage techniques, relying on tubers when other food sources are scarce (Abera and Haile, 2015). This significant increase in yield indicates that postponing harvest permits tubers to accumulate more biomass and minerals, increasing their nutritional value. Such approaches are consistent with traditional methods, in which farmers alter harvesting periods based on household food requirements and storage strategies, using tubers when other food sources are scarce. As a result, *C. abyssinica* is available all year, and leaving *C. abyssinica* tubers unharvested for several years increases its medicinal and nutritional worth (Mosissa *et al.*, 2019). It has been reported that the date of harvest and in situ storage affect tuber yield. Studies indicate that prolonging the harvest period from the fourth to the seventh month can increase fresh and dry tuber yield by an average of 450%, a trend also observed in crops like sugar beet (Abera and Haile, 2015).

People have traditionally believed that appropriate land management (adhering to taboos and prohibitions in land preparation procedures) appeases the spirit of the land (“*dachee*”) and results in a good crop (Wakeway *et al.*, 2023). The Oromo also observe the “*Irreessa*” festival (Oromo’s national Thanksgiving Day), during which people congregate in sacred sites to thank God (“*Waaqa*”) for the good crop and other favors they think they got over the previous year (Bulcha 2015). During “*Irreessa*” events, friends, family, and relatives gather in joyful celebration, bringing people closer together and strengthening social bonds. *C. abyssinica* is a ritualized food offered on this auspicious day (Wayessa, 2018).

Traditional Food Processing of *Coccinia abyssinica*

C. abyssinica is deeply culturally and nutritionally significant in Ethiopia, notably among the Oromo, who have grown and consumed it for generations. The crop is both a staple and a ceremonial food, prized for its high energy content and medicinal characteristics believed to promote bone health and disease recovery (Belissa, 2021). Despite their importance, traditional processing methods are typically local and family-based, passed down through generations. These indigenous procedures are intended to increase edibility, nutrient preservation, and tuber flavor and digestibility before consumption (Wayessa, 2018).

Harvested *C. abyssinica* tubers require further preparation for cooking, which includes peeling the outer inedible layer using a knife and cleaning them (Wayessa, 2018). Boiling is the most frequent way to prepare *C. abyssinica* tubers since it softens the harsh texture and increases nutritional availability (Abdisa, 2021). Traditional preparation frequently involves boiling *C. abyssinica* with little water to retain its high nutritious content, which includes carbohydrates, calcium, and iron (Bikila *et al.*, 2020). After boiling, the tubers can be peeled and eaten simply, mashed, or combined with other ingredients like butter, spices, or milk to add taste.

In Wollega, tubers are cooked in clay pots (Fig. 2). In this region, pottery-making is the domain of women, and men’s participation is restricted by taboos. Women employ axes and hoes in both agriculture and clay mining, and the two activities are frequently described using the same etymology (Wayessa, 2018). According to Wayessa (2018), each ethnolinguistic group in the southern Ethiopian highlands has a distinct technical style, separate material identity based on parts of or suites of technological decisions.

Several factors influence how potters choose and use raw materials to manufacture pots. The most important of these is their learning network, which fosters and sustains relationships between potters and their daughters (Wayessa, 2018). Daughters learn pottery-making from their moms and other female relatives (apprenticeships outside the family are uncommon); therefore, technological choices are taught and passed down through generations.



Figure 2. *C. abyssinica* tuber harvested and being cooked (source: Wayessa, 2018)

The ethnic community prefers to utilize pots manufactured by its own potter. It has been noted that Oromo women frequently steam tubers in pots created by Oromo potters. Furthermore, until recently, *C. abyssinica* consumption was limited to Oromo people; non-Oromo people rarely

ate *C. abyssinica*. The coupling of a certain technological style with specific tuber residues can aid in linking tuber-use to various social groupings (Wayessa, 2018). Similarly, culinary traditions, such as ceramic vessel types and heat treatments for processing culturally unique food products, leave signs of the cultural group's identity. In Wollega, *C. abyssinica* is steamed in a pot filled one-third with water. When the water in the pot boils appropriately, washed tubers are placed in it, and the mouth is covered with huge leaves and a clay lid, which is carefully sealed with a layer of fresh cow dung to reduce heat loss. Extremely huge tubers are cut to fit inside the pot and for uniform cooking (Wayessa, 2018) (Fig. 3).

Wollega has three types of cooking pots: stew, porridge, and steaming pots. Cooking pots undergo a number of use-related changes over time, the most frequent of which are oxidation, carbonization, and residue encrustation. Oxidation and carbonization are the most prevalent use-related changes observed on the external walls of all cooking pots in Wollega. Carbon accretion gradually covers the pot from the bottom to the top. People in the study region prefer to steam tubers until the water in the pot has completely evaporated. This is primarily because the presence of water is thought to make the steamed tuber oily and less appetizing. Steaming tubers until the moisture has been completely cooled off causes scorching and encrustation of the contents on the internal walls of the steaming pots (Wayessa, 2018).

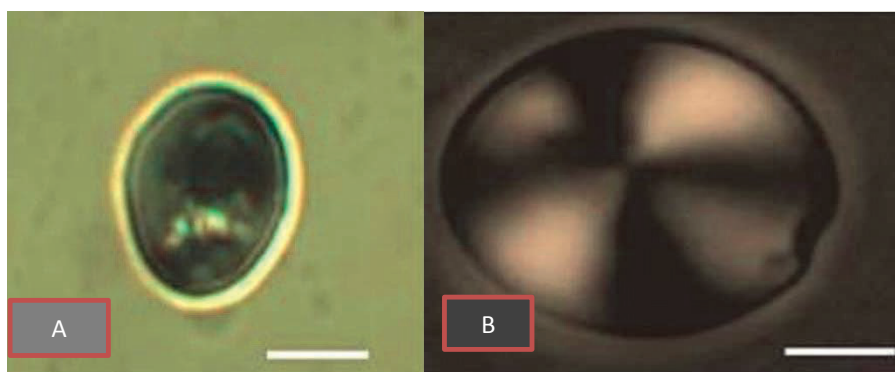


Figure 3. Morphology of cooked (A) and uncooked (B) of *C. abyssinica* starch under Microscope (Source: Wayessa, 2018).

Drying and Powdering

Post-harvest processing of *C. abyssinica* is crucial for increasing shelf life, improving food safety, and market value. Drying and powdering fresh tubers can extend their shelf life and increase their use in diets (Fufa and Bada, 2017). Erku *et al.* (2024) found that using these approaches increases the crop's versatility, enabling it to be used in a cultivar of food products and creating opportunities for rural value-added initiatives.

Drying and Powdering *C. abyssinica* is traditionally preserved in a way that increases its shelf life and makes it more useful in a cultivar of dishes (Shebabaw, 2013). To speed up the drying process, the tubers are washed, peeled, and sliced into thin pieces. Sun drying is the most typical way, however newer approaches like solar or mechanical drying can help retain more nutrients (Wayessa, 2018). *C. abyssinica* powder is highly prized for its ease and adaptability, since it may be rehydrated for porridge, blended into soups, or used in baked goods. This processing procedure preserves the tuber's high nutritional value, which includes carbohydrates, calcium, iron, and dietary fiber, making it a viable option for food security and value-added products.

C. abyssinica's rich nutrient composition, particularly its high levels of carbohydrates, calcium, and iron, make it suitable for powder processing. This transformation preserves the crop's essential nutritional qualities, but also improves its shelf stability, transportability, and adaptability in the development of value-added food products such as porridges, soups, and baked goods (Gebeyehu *et al.*, 2024).

Food products from *Coccinia abyssinica* and consumption

C. abyssinica based food products are well-known in major growing areas (Table 1). According to different literatures, *C. abyssinica* tubers and leaves are source of various food types (Table 2). There are approximately seven different cultivars of *C. abyssinica* products, six of which are food types manufactured from its tuber (Negassa *et al.*, 2024). According to a survey report by (Parmar *et al.*, 2017), *C. abyssinica* roots and leaves can be used to produce around 27 different cuisines. Those literatures also classified *C. abyssinica* items into four categories: soups, boiled roots, flour, and other primary *C. abyssinica* dishes.

Table 1. Major Anchote (*Coccinia abyssinica*) Growing Areas, Cultivars, and References

Anchote Growing Areas	Cultivars Grown / Local Landraces	References
Western Oromia (East Wollega, West Wollega, Jimma)	White, red, and mixed-flesh landraces (local cultivars distinguished by tuber color and size)	Duresso (2018); Gudeta (2022)
Kellem Wollega and West Shoa Zones	Local cultivars with high moisture tolerance and good tuber quality	Abdena andAga (2023); Temam (2021)
East Wollega (Nedjo, Gimbi, Leka Dulecha)	White and light-red cultivars are often produced for their great yield and market value.	Negassa and Amenu (2016); Wakjira et al. (2024)
South Western Ethiopia (Masha, Bonga)	Locally improved selections adapted to humid conditions	Anbesse and Hailegeorgis (2021); Gutema (2019)
Central Oromia (Ambo, Dendi, Toke Kutaye)	Farmers’ cultivars selected for early maturity and drought resistance	Abdena and Aga (2023); Belissa (2021)
Ilubabor and Kafa Zones	Local red and white landraces	Mekbib and Deressa (2016); Bekele (2019)

In addition, *C. abyssinica* has the potential for innovative food products that fulfill urban and commercial demands. Processing tubers into powder, flour, or packaged ready-to-cook forms extends shelf life and convenience, providing for more market access (Erku *et al.*, 2024). Such value-added products not only diversify household meals but also create small-scale enterprises and income generation in rural areas. Furthermore, adding *C. abyssinica* into modern foods can help to preserve its cultural significance while promoting nutrition-sensitive food solutions.

Table 2. Different types of food products prepared from *C. abyssinica*

No	Food Type (Local Name)	Preparation Method	Consumption Context	References
1	Chufana (Cuffannaa)	Washed, boiled, peeled, and sliced lengthwise.	Consumed during Meskel festival and as a staple.	Keno (2024)
2	Murmura (Murmuraa)	Boiled, peeled, and cut into large pieces.	Eaten as a regular dish.	Keno (2024)
3	Defeki (“Dafaqqii”)	Boiled, peeled, made into a paste with ginger/garlic, and mixed with spiced butter.	Eaten during Meskel; also, for those with malaria or bone problems.	Parmar <i>et al.</i> (2017); Desta <i>et al.</i> (2021)
4	Kori Anchote (“Qorii Ancootee”)	Boiled, peeled, diced, and mixed with ingredients while heating.	Served with bread or injera during Meskel and other times.	Keno (2024)
5	Stew (“Ittoo Ancootee”)	Boiled, peeled, diced, and cooked in a stew with pulses.	A regular household dish and common at gatherings.	Keno (2024)
6	Rafu Anchote (“Raafuu Ancootee”)	Immature buds are collected, washed, chopped, and boiled.	Consumed as a vegetable when the leaves are immature.	Negash (2019); Keno (2024)
7	Soup (“Moqa”)	Washed, sliced, dried, milled, and mixed with other flours for soup.	Specifically for postpartum women and people with bone fractures.	Wayessa (2018); Keno (2024)

Some authors have attempted to characterize *C. abyssinica* foods as “kitifo”, “lankata” (finely ground tuber), “wot”, soup, and “murmura” (cooked tuber sliced into pieces) (Fufa, 2012, Duresso, 2018). Other authors have suggested that *C. abyssinica* stew (locally known as “Ittoo Anchote”) is one of the *C. abyssinica* products (Parmar *et al.*, 2017, Keno, 2024). According to Temam (2021), *C. abyssinica* stew, also known as “Ittoo Anchote”, is prepared on special occasions using only sliced *C. abyssinica* and enough butter. Research report by Keno (2024), remarked that these reports do not provide any information regarding the preparation process, substances, or occasions of their preparation.

Boiled *C. abyssinica* is typically served with “kochkocha”, a fermented side dish made from ground green pepper (Temam, 2021). Amazingly, there is no fermenting procedure required for the manufacture of “kochkocha” (Keno, 2024). Pregnant women and breastfeeding mothers use local traditional medicines (Parmar *et al.*, 2017).

Nutritional Contents of *Coccinia abyssinica*

C. abyssinica is a highly nutritious indigenous crop in Ethiopia that contributes to food security and dietary diversity (Buko, 2024). Plant parts such as tubers, leaves, and fruits contain essential macro and micronutrients, which make them an excellent source of energy, protein, vitamins, and minerals (Regessa, 2022). *C. abyssinica*'s nutritional density supports its use in traditional diets and highlights its potential for generating nutrient-rich food products that can address local malnutrition concerns while improving overall health outcomes (Erku *et al.*, 2024).

When considering a crop as a food source, nutritional content is the primary issue, and *C. abyssinica* plant sections, such as tuber, leaf, and fruit, have valuable nutrients (Regessa, 2022). *C. abyssinica* tubers have much more nutrients than equal weights of potato (*Solanum tuberosum* L.), yam (*Dioscorea abyssinica*), and cassava (*Manihotes culenta*) tubers, Swiss chard, and cucumber (Abera and Haile, 2015).

According to different researchers, *C. abyssinica* demonstrates remarkable nutrients, particularly protein and ash content than most other root and tuber crops (Table 2). About 2.05g of protein per 100g, *C. abyssinica* contains more protein than cassava (0.53g), Enset (0.6g) and sweet potato (13.65g), making it a valuable source of protein in regions where protein deficiency is a concern. And also, in comparison to other tropical root and tuber crops, *C. abyssinica* tubers are richer in protein as well as various minerals such as Ca, Mg, P, Fe, and S (Parmar *et al.*, 2017). The comparative analysis highlights *C. abyssinica* as nutritionally valuable root crops for high protein and mineral content

C. abyssinica tubers are more nutritious compared to several commonly cultivated root and tuber crops, particularly in terms of protein and mineral content (Parmar *et al.*, 2017). This unique nutrient profile not only promotes human nutrition but also establishes *C. abyssinica* as a functional food with potential applications in value-added goods (Mosissa *et al.*, 2019; Gemedé and Urugo, 2025).

In addition to protein, *C. abyssinica* is also rich in macro and micronutrients including calcium (Ca), magnesium (Mg), phosphorus (P), iron (Fe), and sulfur (S), exceeding many other tropical root and tuber crops in mineral density (Gebeyehu *et al.*, 2024). This combination of protein and minerals emphasizes *C. abyssinica*'s potential as a functional food and its importance in food security and nutrition initiatives (Regassa *et al.*, 2024).

Table 3. Proximate nutrient contents of *C. abyssinica* compare with other root and tuber crops

Root and Tuber crops	Nutrient content (g/100g)						References
	Carbohydrate	Gross energy (Kcal/100g)	Fiber	Protein	Fat	Ash	
Potato	21.47	96.78	0.43	1.6	0.1	0.83	Temam (2021)
Beet root	6.99	42	2.56			1.4	Chandrasekara and Josheph (2016)
Cassava	86.25	364.6	1.53	0.53	0.17	1.89	Tiruneh <i>et al.</i> (2021)
Anchote	7.32	111.77	5.95	2.05	15.4	1.1	Gebeyehu <i>et al.</i> (2024), Paulos (2025)
Enset	97.71	394.24	1.04	0.6	0.3	1.05	Wakeway <i>et al.</i> (2023)
Sweet potato	85.97	336.72	7.19	13.65	2.28	7.06	Akomolafe (2025)
Yam	84.07	353.64	4.39	3.3	0.98	1.76	Akalu and Geleta (2019)

Limitations in *C. abyssinica* food preparation

Despite its nutritional and culinary potential, *C. abyssinica* offers various cooking challenges that limit its convenience and acceptability (Desalegn, 2018). The tubers are typically hard and fibrous, which increases cooking time and fuel consumption, especially when using traditional boiling methods (Bikila *et al.*, 2022).

C. abyssinica tuber takes longer to boil due to its hardness. It is stated that old *tuber* takes a long time to boil. The presence of a considerable number of fibers in *C. abyssinica* tubers may also influence the boiling time (Desalegn and Kibr, 2021). Boiling *C. abyssinica* tuber requires a significant amount of fuel wood and may take up to three hours. A large volume of fuel wood is required. However, boiling *C. abyssinica* tubers in a pressure cooker takes only around 45 minutes. Therefore, the white *C. abyssinica* is preferred over the orange because it is easier to boil. The tubers that are difficult to boil are known as “*hiddoo*” (fibrous Anchote) (Keno, 2024). Consumers typically reject and discard this form of *C. abyssinica* since it is difficult to boil. *C. abyssinica* can be used in many ways and has similarities with potatoes. Climate, soil type, and agricultural techniques may all have an impact on *C. abyssinica* harden. *C. abyssinica* cultivars cultivated on fertile, well-aerated soils with adequate rainfall are not as difficult to boil.

Addressing these agronomic and processing constraints through research, improved cultivation practices, cultivar selection, and innovative cooking techniques has the potential to considerably increase *C. abyssinica* production, processing, and consumption (Keno, 2024; Paulos, 2025). Such activities have the potential to increase food security and nutritional value, while also preserving the cultural heritage and promoting sustainable livelihoods in Ethiopia (Erku *et al.*, 2024; Gebeyehu *et al.*, 2024).

Socio-Economic Importance of *Coccinia abyssinica*

Income Generation

C. abyssinica cultivation and sale provide major income-generating opportunities for smallholder farmers, notably in Ethiopia, where the crop is culturally and nutritionally significant (Ashebir, 2022, Fekadu 2011, Guma *et al.*, 2015; Keno, 2024). Due to the high demand in local markets, producers benefit from selling fresh tubers directly to customers, restaurants, and food processors. The growing recognition of *C. abyssinica*'s health benefits, such as its high calcium and iron content, has created a demand for value-added goods such as dried *C. abyssinica* slices and *C. abyssinica* powder (Tiruneh *et al.*, 2021). Farmers and

businesses can increase their profit margins and expand their client base by processing and packaging their products, which includes serving urban consumers and international markets. Furthermore, *C. abyssinica* promotes women's economic empowerment because many women are involved in its production, processing, and marketing. *C. abyssinica*'s promotion as a functional food, combined with improved farming techniques and stronger market links, has the potential to increase its position as a sustainable income source (Negassa *et al.*, 2024).

Contribution to Food Security

C. abyssinica is an important food security crop in Ethiopia, where it is a traditional staple (Zerihun, 2022). Its tubers are high in carbs, dietary fiber, and vital minerals like calcium and iron, making it a very nutritious food item that contributes to a healthy diet (Bikila *et al.*, 2020). *C. abyssinica* has a somewhat long shelf life when stored properly, and its capacity to be dried and processed into powder increases its availability beyond harvest seasons, minimizing food shortages (Abdisa, 2021).

Aside from its immediate nutritional benefits, *C. abyssinica* promotes food security by creating economic opportunities for smallholder farmers (Ashebir, 2022). The crop's expanding market demand encourages farmers to produce it as both a subsistence and cash crop, allowing households to earn money to buy other foods. Furthermore, its compatibility with intercropping systems enables diverse farming, lowering the risks associated with crop failure.

The tubers can be stored for extended periods of time, increasing availability beyond the harvest season and minimizing vulnerability to food shortages (Gebeyehu *et al.*, 2024). The increasing market demand for *C. abyssinica* provides income-generating opportunities for smallholder farmers, allowing households to purchase other food items and diversify their diets (Ashebir, 2022).

Cultural Significance and Medicinal Value

C. abyssinica has significant cultural and medicinal properties, particularly among Ethiopia's Oromo people, who associated it with strength, health, and wealth (Wayessa, 2018). Beyond its nutritional value, *C. abyssinica* is used in social behaviors such as supplementing breastfeeding women's diets and being served at weddings, holidays, and community events (Duresso, 2018; Keno, 2024). As interest in indigenous and traditional cuisines rises, *C. abyssinica* remains a source of cultural pride and a representation of Ethiopia's rich agricultural legacy (Wayessa, 2016).

C. abyssinica is also thought to have medical benefits, including the treatment of bone fractures and back discomfort, most likely due to its high calcium concentration (Parmar *et al.*, 2017; Abdisa, 2021). Consumption is encouraged for young people, pregnant women, and the elderly since seniors associate it with physical power and endurance (Olkaba and Ayalew, 2024). *C. abyssinica* is associated with traditional medicine and indigenous knowledge systems (Temam, 2021; Abdena and Aga, 2023).

In addition to its medical and dietary benefits, *C. abyssinica* encourages community bonding and intergenerational information transfer. Local traditions guide production, preparation, and consuming practices, preserving indigenous agricultural expertise and cultural heritage (Gebremedhin and Admassu, 2022; Temam, 2021). Despite its acknowledged relevance, there is limited scientific study proving *C. abyssinica*'s health advantages and social roles, emphasizing the necessity for ethnographic and qualitative investigations to capture and conserve this traditional knowledge (Abdena and Aga, 2023; Wayessa, 2016).

CONCLUSION

C. abyssinica is a widely used indigenous root crop that has substantial nutritional, cultural, and economic value, particularly in Ethiopia. Its production and use are intimately

connected to traditional knowledge and practices, reflecting the inventiveness and heritage of local communities. Traditional cultural techniques linked with its cultivation, such as land preparation, intercropping, weeding, fertilizing, crop protection, and harvesting, are critical to increasing yield and guaranteeing long-term production. However, obstacles such as a lack of established agronomic techniques, inadequate knowledge on nutrient management, and vulnerability to pests and diseases reduce crop output. Furthermore, traditional food processing procedures including boiling, drying, and powdering, contribute to increased nutritional content and shelf life, while varied preparation techniques reflect profound cultural traditions among the Oromo people. Despite its advantages, restrictions like long cooking periods, high fiber content, and fuelwood consumption prevent widespread use. Addressing these limits through research and innovation can assist in improving *C. abyssinica* production, processing, and consumption levels. Further research is needed to determine the appropriate tillage frequency, planting spacing, and fertilization rates for *C. abyssinica* productivity. Field trials should focus on identifying best practices for various agro-ecological zones. Future research should be directed towards the development of new *C. abyssinica* based food products, such as fortified flour, snacks, and ready-to-eat meals, which can boost market demand and broaden dietary choices.

REFERENCES

1. AbaDura, N. S., Beyene, T. M., and Legesse, A. (2021). Assessment of *C. abyssinica* Production and Its Physiological Adaptive Mechanisms for Moisture Stress: Implication for Conservation. *J Horti*, 8, 266.
2. Abdena, Z. T., and Aga, M. C. (2023). Evaluation of underutilized cultivated “Anchote” (*C. abyssinica* (Lam.) Cogn.) accessions in West Shewa Zone, Oromia Regional State, Central Ethiopia. *Daagu International Journal of Basic and Applied Research (DIJBAR)*, 5(1), 144–163.
3. Abdisa, R. H. (2021). *Investigation and Optimization of Drying Process Parameters on Nutritional Quality of “Anchote” (C. Abyssinica Tuber Flour)*.
4. Abera, G., and Guteta, H. (2007). Response of “Anchote” (*C. abyssinica*) to organic and inorganic fertilizers rates and plant population density in western Oromia, Ethiopia. *East African Journal of Sciences*, 1(2), 120–126.
5. Abera, G., and Haile, D. (2015). Yield and nutrient concentration of “Anchote” [*C. abyssinica* (Lam.) Cogn.] affected by harvesting dates and in-situ storage. *African Journal of Crop Science*, 3(5), 156–161.
6. Akalu, Z. K., and Geleta, S. H. (2019). Comparative analysis on the proximate composition of tubers of *Colocasia Esculenta*, *L. Schott* and *Dioscorea alata* cultivated in Ethiopia. *American Journal of Bioscience and Bioengineering*, 7(6), 93–101.
7. Akomolafe, S. F. (2025). Nutritional composition of three cultivars of sweet potato (*Ipomea batata* L.) based diet commonly consumed in Nigeria: a comparative study. *Food Chemistry Advances*, 101067.
8. Anbesse, D., and Hailegeorgis, A. (2021). Growth, Yield and Yield Components of “Anchote” (*C. abyssinica* L.) in Response to Farmyard Manure and Phosphorus Fertilizer Rates at Masha, South Western Ethiopia. *South Western Ethiopia*.
9. Ashebir, S. F. (2022). Review of the role of orphan crops in food security. *Research on World Agricultural Economy*, 3(2), 1–14.
10. Ayalew, M. (2020). The role of Rainfall amount and distribution on Agriculture Systems and Crop Cropping systems of different agro-ecological regions of Ethiopia: a review. *International Journal of Research in Agriculture and Forestry*, 7(5), 26–40.
11. Bekele, Y. (2019). *Determinants Of Farmers’ adoption on Sustainable Agricultural Practices; The Case of Kafa Zone, Gimbo District, Ethiopia*. St. Mary’s University.
12. Belay, A., Recha, J. W., Woldeamanuel, T., and Morton, J. F. (2017). Smallholder farmers’ adaptation to climate change and determinants of their adaptation decisions in the Central Rift Valley of Ethiopia. *Agriculture and Food Security*, 6, 1–13.
13. Belissa, S. (2021). *Agronomy Practices of “Anchote” (Coccinia Abyssinica L.) in Ethiopia . A Review*. 8(1000), 8–11. <https://doi.org/10.35248/2376-0354.22.9.301>
14. Bikila, A. M., Tola, Y., Esho, T. B., and Forsido, S. F. (2020). Effect of pre drying treatment and drying temperature on proximate composition, mineral contents, and thermophysical properties of “Anchote” (*C. abyssinica* (Lam.) Cogn.) flour. *Food Science and Nutrition*, 8(10), 5532–5544.
15. Biratu, A. E. (2019). *Effect of blended fertilizer (NPSB) and farmyard manure rates on yield and yield components of “Anchote” [C. abyssinica (Lam.) (Cogn.)] at Jimma, South Western Ethiopia*. 81.
16. Buko, D. H. (2024). High Nutritional and Low Antinutritional Factors in “Anchote” [*Coccinia Abyssinica* (Lam.)

- Cogn.]: Review of a Potential Future Food and Nutritional Security Tuberous Root Crop of Ethiopia. *Ethiopian Journal of Crop Science*, 12(2), 1-14.
16. Bulcha, M. (2015). Oromia's "Irreecha" Festival—A Revival of an Ancient African Culture—An Attempt to Understand and Explain.
 17. Chandrasekara, A., and Josheph Kumar, T. (2016). Roots and tuber crop as functional foods: A review on phytochemical constituents and their potential health benefits. *International Journal of Food Science*, 2016. <https://doi.org/10.1155/2016/3631647>
 18. Desalegn, A., and Kibr, G. (2021). Effect of pretreatment and drying methods on the quality of "Anchote" (*C. abyssinica* (Lam.)) flour. *Journal of Food Quality*, 2021(1), 3183629.
 19. Duresso, M. E. (2018). Study on ethnobotany and phenotypic diversity in "Anchote" (*C. abyssinica* (Lam.) Cogn.) landraces in western Ethiopia. *International Journal of Agricultural Sciences*, 8(2), 1402–1427.
 20. Erku, E. G., Kassa, M. G., Belachew, M. T., Teferi, D. A., and Gelan, K. M. (2024). "Anchote" (*Coccinia abyssinica*): a tuber crop with enhanced potential for innovative food products and sustainable packaging. *Cogent Food and Agriculture*, 11(1), 2544952.
 21. Feyissa, T. (2020). "Anchote" (Lam.) Cogn. (*C. abyssinica*) biology, productivity, and prospects of genetic improvement using biotechnological tools. *Journal of Horticultural Research*, 28(2).
 22. Ficiyan, A., Loos, J., Sievers-Glotzbach, S., and Tschardt, T. (2018). More than yield: Ecosystem services of traditional versus modern crop cultivars revisited. *Sustainability*, 10(8), 2834.
 23. Fufa, T. W. (2012). Genetic variability and association in yield and yield related traits in "Anchote" [*C. abyssinica* (Lam.) COGN]. *Unpublished Msc Thesis, Jimma University, Jimma, Ethiopia*.
 24. Gebeyehu, H. R., Jalata, D. D., Beza, D. M., Asefa, B. G., Sherge, K. T., and Mijena, D. F. (2024). Nutritional and anti-nutritional composition of "Anchote" (*C. abyssinica* (Lam.) Cogn.) accessions in Ethiopia. *Journal of Agriculture and Food Research*, 15, 100980.
 25. Gebremedhin, G., and Admassu, H. (2022). Effects of heat-moisture treatment and hydroxypropylation on the physical, physicochemical, thermal, and functional properties of "Anchote" (*C. abyssinica*) starch. *Journal of Food Measurement and Characterization*, 16(6), 4771–4787.
 26. Gebremedhin Woldegiorgis, T. T., Gurmu, F., Chindi, A., and Seid, A. (2015). Root and tuber crops research in Ethiopia: achievements and future prospects. *Agricultural Research for Ethiopian Renaissance*.
 27. Gemede, H. F., and Urugo, M. M. (2025). "Anchote" (*Coccinia abyssinica*) endemic tuber to Ethiopia: nutritional composition, antinutritional constituents and value addition. *Journal of Food Composition and Analysis*, 108163.
 28. Gudeta, A. (2022). Production Status and Relevance of "Anchote" (*C. abyssinica* (Lam.) in Ethiopia: A Review. *International Journal of Agriculture Extension and Rural Development Studies*, 9(4), 20–27. <https://doi.org/10.37745/ijaerds.15/vol9n42027>
 29. Gurmu, F. (2019). Sweetpotato research and development in Ethiopia: a comprehensive review. *Journal of Agricultural and Crop Research*, 7(7), 106–118.
 30. Gutema, A. O. (2019). *Growers' Indigenous Knowledge and the Contribution of Neglected and Underutilized Tuber Crops to the Household Food Security in Southwestern Ethiopia* [Addis Ababa University].
 31. Habtamu, S. (2013). *Effect of Processing on Physicochemical and Antinutritional Factors of C. abyssinica (C. abyssinica) and Development of Value-Added Biscuit*. September, 1–113.
 32. Karri, V., and Nalluri, N. (2024). Enhancing resilience to climate change through prospective strategies for climate-resilient agriculture to improve crop yield and food security. *Plant Science Today*, 11(1), 21–33.
 33. Kebede, E. (2021). Contribution, utilization, and improvement of legumes-driven biological nitrogen fixation in agricultural systems. *Frontiers in Sustainable Food Systems*, 5, 767998.
 34. Keno, O. L. (2024). Nutritional Composition of "Anchote" (*C. abyssinica* (Lam.) Cogn.) and its Products: A Tip for Future Research and Development. *Ethiopian Journal of Agricultural Sciences*, 34(2), 104–114.
 35. Leta, G., KEIBORO, G., Stellmacher, T., and Hornidge, A.-K. (2017). *The agricultural extension system in Ethiopia: operational setup, challenges and opportunities*.
 36. Mariutti, L. R. B., Rebelo, K. S., Bisconsin-Junior, A., de Moraes, J. S., Magnani, M., Maldonado, I. R., Madeira, N. R., Tiengo, A., Marostica Jr, M. R., and Cazarin, C. B. B. (2021). The use of alternative food sources to improve health and guarantee access and food intake. *Food Research International*, 149, 110709.
 37. Mekbib, Y., and Deressa, T. (2016). *Exploration and collection of root and tuber crops in East Wollega and Ilu Ababora zones: Rescuing declining genetic resources*.
 38. Mosissa, A., Bekele, Y., and Berhanu, T. (2019). Food and Nutrition Security Potential of "Anchote" (*Coccinia abyssinica*); A Tuberous Crop Indigenous to Ethiopia: A Review. *International Journal of Food Engineering and Technology*, 7(2), 34–38.
 39. Negash, G. (2019). "Anchote" (*C. abyssinica* (Lam.) Cogn.): A nutrient rich root tuber crop of Ethiopia. *Agr. Sci. Res. J*, 9, 1–11.
 40. Negassa, B., Nebiyu, A., Garedew, W., Abbey, Lord, Ofue, R., Mousavi, N. S., Nutsukpo, B., Astatkie, T., and Kuyu, C. G. (2024). Evaluation of "Anchote" (*C. abyssinica*) genotypes and processing methods for mineral and phytochemical composition. *Journal of Agriculture and Food Research*, 18, 101415.
 41. Negassa, J., and Amenu, D. (2016). Socio-Economic Importance of "Anchote" (*C. abyssinica*) Cultural Foods of Western Oromia. The Case of Nedjo Woreda. *J. Biol. Chem. Research*, 33(1), 420–425.

42. Olkaba, G. H., and Ayalew, A. (2024). Phytochemical Screening of “Anchote” (*C. Abyssinica* (Lam.)) And Effect of Extract Via Aerobic Exercise on Endurance Performance. *Stadium-Hungarian Journal of Sport Sciences*, 7(1).
43. Parmar, A., Gebre, B. A., Legesse, A., Demelash, Y., Fladung, K., and Hensel, O. (2017). Nutritional comparison of white and red *C. abyssinica* (Lam.) Cong. accessions: an under-utilised edible tuber of the Ethiopian highlands. *Foods*, 6(8), 71.
44. Paulos, M. (2025). Anchote Research in Ethiopia and Achievements: A Comprehensive Review. *International Journal of Advanced Pharmaceutical Sciences and Research (IJAPSR)*, 5(5), 12-20.
45. Regasa, M. B., Fayisa, K. G., and Woldegebriel, H. H. (2018). Bioactive phytochemical screening and antioxidant potential of different solvent extracts of *C. abyssinica*: the underutilized delicious cultural food of Oromo people, Ethiopia. *Food Science and Technology*, 6(4), 81-90.
46. Regessa, M. D. (2022). Cultural Practices, Nutritional and Anti-nutritional Composition of “Anchote” (*C. abyssinica*). *International Journal of Nutrition and Food Sciences*, 11(5), 116-121.
47. Saraswathi, T., and Thingalmaniyan, K. S. (2017). Ivy/Little Gourd. In *Vegetable Crop Science* (pp. 489-500). CRC Press.
48. Shebabaw, H. (2013). Effect of processing on physicochemical and antinutritional factors of “Anchote” (*C. abyssinica*) and development of value-added biscuit. *Addis Ababa University*, 1-102.
49. Shekhar, M., Shivashankar, E., and Pandey, S. K. (2024). Crop rotation and intercropping techniques. *Modern Concept in Agronomy (Edition I)*. ND Global Publication House. <https://doi.org/10.5281>.
50. Sisay, S. A., Mekbib, F., and Gebre, E. (2020). An investigation of meristem culture in “Anchote” [*C. abyssinica* (Lam.) Cong.] under the influence of plant growth regulators and media strength. *African Journal of Biotechnology*, 19(11), 806-813.
51. Temam, W. T. N. (2021). Analysis on Production Potential of “Anchote” (*C. abyssinica*) In East Wellega Zone, Oromia, Regional State, Ethiopia. *Int. J. Adv. Res. Biol. Sci*, 8(12), 8-17.
52. Tiruneh, A. T., Negatu, A. A., and Satheesh, N. (2021). Effect of “Anchote” (*C. abyssinica*) and potato starch addition on colloidal stability of pineapple juice. *International Journal of Food Science*, 2021(1), 6615273.
53. Wakeway, W., Bejitual, T., and Gebremeskel, T. (2023). Exploring natural resource conservation mechanisms in Oromo orature: Ecopoetic study of selected Maccaa Oromo agricultural rites. *Social Sciences and Humanities Open*, 8(1), 100544.
54. Wakjira, H., Garedew, W., Nebiyu, A., and Shifaraw, G. (2024). Effect of Earthing-Up Frequency on Growth and Yield of “Anchote” (*C. abyssinica*) Cultivars at Gimbi District, Western Ethiopia. *International Journal of Agronomy*, 2024(1), 3918033.
55. Wayessa, B. S. (2016). Toward a history of the Oromo of Wallaga in southwestern Ethiopia: an ethnoarchaeological study of ceramic technological style and tuber crop domestication. *PhD Diss., University of Calgary*.
56. Wayessa, B. S. (2018). “Anchote” (*C. abyssinica*): A tuber viewed as a relative of women in the Wallaga Region of southwestern Ethiopia. *Ethnoarchaeology*, 10(1), 34-55.
57. Yimer, S., and Babege, T. (2018). Evaluation of constraints in production of root and tuber crops in Ethiopia: Overview of policy neglected climate resilient food security crops. *Journal of Plant Breeding and Crop Science*, 10(8), 210-217.
58. Zerihun, J. (2022). Production, challenges, potential and prospects of Oromo potato (*Plectranthus edulis*) and “Anchote” (*C. abyssinica*) in Ethiopia: A review. *Agricultural Reviews*, 43(3), 348-354.

Control of damage agents on cabbage and cauliflower crops under high plastic tunnels

A.E. Cenușă*, S.Ș. Hogeă, G. Șovărel and M. Costache

Research and Development Institute for Vegetable
and Flower Growing Vidra, Romania

*Corresponding author email: ema_april@yahoo.com

ABSTRACT

The study was conducted in 2022, under high plastic tunnel-conditions, at the Research and Development Institute for Vegetable and Flower Growing Vidra, aiming to evaluate the effectiveness of an integrated pest and disease management program in cabbage and cauliflower crops. Seven commercial hybrids were tested under two variants: a treated variant (V1), which received a complete sequence of chemical and biological plant protection products, and an untreated control (V2). The cabbage hybrids included 'Delight Ball F1', 'Vettel F1', 'Zarissima RZ F1' and 'Magnus Cresco F1', while the cauliflower hybrids were 'Freedom F1', 'Arienzo RZ F1' and 'Venoosa F1'. In cabbage, the main pathogens identified were *Xanthomonas campestris*, *Alternaria brassicae* and *Sclerotinia sclerotiorum*, together with the pests *Brevicoryne brassicae*, *Phyllotreta atra* and *Pieris brassicae*. In cauliflower, *X. campestris* and *A. brassicae* were present, along with *P. atra*, *P. brassicae* and *Plutella maculipennis*. The protection program ensured high control efficacy, ranging from 78–89% for pathogens and pests in cabbage and 84–97% in cauliflower. Yield results reflected the effectiveness of the treatments. In cabbage, yields increased significantly in all hybrids, with the highest value recorded in "Delight Ball F1" (9.160 kg/m²). In cauliflower, the highest yield was obtained in "Freedom F1" (6.110 kg/m²), with significant and distinctly significant differences from the untreated control. Overall, the integrated protection program demonstrated high efficiency in reducing disease and pest incidence and significantly improving yields under high plastic tunnel cultivation.

Keywords: fungicides, insecticides, pathogens, pests, efficacy.

INTRODUCTION

Cabbage and cauliflower crops under high plastic tunnels are frequently attacked by the pathogens *Xanthomonas campestris*, *Alternaria brassicae*, *Sclerotinia sclerotiorum* and the pests *Brevicoryne brassicae*, *Phyllotreta atra*, *Pieris brassicae* and *Plutella maculipennis* which reduce yield quantitatively and it depreciates qualitatively (Costache *et al.*, 2023).

Both "bio" products (different microorganisms with antagonistic effect, plant extracts) and chemical products are used to control these damage agents.

The biological agents *Azotobacter chroococcum*, *Bacillus megaterium*, *Agrobacterium radiobacter*, *Pseudomonas fluorescens*, *Pseudomonas auerofaciens*, *Bacillus subtilis* and *Trichoderma lignorum* have been studied to control the pathogens *Xanthomonas campestris* pv. *campestris*, *Alternaria brassicae* and *Sclerotinia sclerotiorum* on cabbage. They effectively limited

the development of bacterial and fungal diseases on cabbage, their protective effect against bacterial diseases being 65-79% and against fungal diseases 45-62% (Serhiienko *et al.*, 2023).

The products azoxystrobin 25% and azoxystrobin 20% + difenconazole 12.5% were the most effective in completely inhibiting the mycelial growth of *Alternaria brassicae* in cauliflower (Chandel *et al.*, 2023).

Experiments undertaken under laboratory conditions with chemical-based (lambda cyhalothrin and emamectin benzoate) and "bio" products (*Azadirachta indica* neem seed extracts, *Dodonaea angustifolia* fresh hop leaves and *Cymbopogon citratus* lemon grass) had positive performances in control of the *Brevicoryne brassicae* pest (Shiberu and Negeri, 2016). The cabbage moth (*Plutella maculipennis*) is one of the most important pests of cruciferous crops, causing economic losses of over 90% (Tolessa *et al.*, 2021).

Cabbage white butterfly (*Pieris brassicae*) is an important pest of plants in the Brassicaceae family (Shikha *et al.*, 2023), attacking and causing significant damage on cabbage, cauliflower, mustard and radish (Iabloncik *et al.*, 2022).

Fleas of the genus *Phyllotreta* are major pests of brassica vegetable crops, therefore their control is mainly based on conventional methods, available insecticides having limited effectiveness due to the development of resistance in pest populations (Li *et al.*, 2024).

In the paper, the efficacy of treatments with chemical and "bio" products used to control pathogens and pests specific to cabbage and cauliflower crops were tracked.

MATERIALS AND METHODS

Experimental site and growing conditions

The experiments were carried out in 2022 at the Research and Development Institute for Vegetable and Flower Growing Vidra (R.D.I.V.F.G.), under high plastic tunnel conditions. The tunnel measured 50 m × 10 m × 5 m, covered with anti-condensation polyethylene and equipped with frontal and lateral ventilation.

Temperature and relative humidity were automatically monitored using a CEM DT-172 thermohygrometer, recording averages of 9.7°C (minimum), 21.2°C (mean), 36.0°C (maximum) and 32.7%, 63.7%, 90.3%, respectively, during the experimental period.

Plant material. The biological material used in this study consisted of seven commercial Brassica hybrids, belonging to two crop types cultivated under protected conditions: Cabbage (*Brassica oleracea* var. *capitata*) with the cultivars 'Delight Ball F1', 'Vettel F1', 'Zarissima RZ F1', 'Magnus Cresco F1' and Cauliflower (*Brassica oleracea* var. *botrytis*), with the 'Freedom F1', 'Arienzo RZ F1', 'Venoosa F1'.

Seeds of all hybrids were sown on 24 February 2022 in alveolar trays filled with a commercial horticultural substrate enriched with a balanced fertilizer mixture. Seedlings were maintained under controlled protected conditions until transplanting.

Transplanting into the high plastic tunnel was carried out on 29 March 2022, using uniform and healthy seedlings. All hybrids were grown under the same environmental and cultural conditions to ensure the comparability of experimental data.

Experimental design. The study was conducted using a comparative two-variant design, applied separately to each of the cabbage and cauliflower hybrids included in the experiment. The two experimental variants were: V1 – Treated - plants protected using the complete plant protection program, and V2 – Untreated (Control): plants grown without any plant protection treatments (Table 1).

The Treated variant (V1) consisted of a fully integrated protection program, combining chemical and "bio" products applied sequentially throughout the vegetation period.

Table 1. Description of experimental variants and treatments applied

Variant	Description	Treatments applied	Purpose
V1 – Treated	Plants protected using complete plant protection program	Amistar 0.1%, Funres 0.3%, Decis Expert 100 EC 0.0075%, Mavrik 2F 0.05%, Flipper 2.0%, Neemex 0.3% + Break-Thru S240 0.02%	Evaluation of the integrated control program for pathogens and pests
V2 – Untreated (Control)	Plants without any plant protection treatments	(Control variant)	Baseline reference to assess attack severity and yield reduction

Depending on crop needs and the presence of pathogens and pests, the treatments included: chemical fungicides, bio - fungicides/bactericides, chemical insecticides, bio insecticides, all applied with Break-Thru S240 adjuvant (0.02%). The products used in V1—active substances, concentrations, action spectrum, and pre-harvest intervals (Table 2).

Table 2. Products used to control pathogens and pests on cabbage and cauliflower crops

Product	Active substance	Concentration (%)	Action spectrum	Break time (days)
a. Chemical-based fungicides				
Amistar	azoxystrobin 250g/l	0.1	<i>Alternaria brassicae</i> , <i>Sclerotinia sclerotiorum</i>	14
b. "Bio" fungicides				
Funres	<i>Mimosa tenuifolia</i> extract 60% + citrus extract 20%	0.3	<i>Xanthomonas campestris</i> , <i>Alternaria brassicae</i> , <i>Sclerotinia sclerotiorum</i>	0
c. Chemical based insecticides				
Decis Expert 100 EC	deltamethrin 100g/l	0.0075	<i>Pieris brassicae</i> , <i>Plutella maculipennis</i> , <i>Phyllotreta atra</i>	7
Mavrik 2F	tau-fluvalinate 240g/l	0.05	<i>Pieris brassicae</i> , <i>Plutella maculipennis</i>	7
d. "Bio" insecticides				
Flipper	potassium salts of fatty acids C7-C18, 479,8 g/l	2.0	<i>Brevicoryne brassicae</i>	0
Neemex	<i>Neem saponi</i> seed extract	0.3	<i>Brevicoryne brassicae</i> , <i>Pieris brassicae</i>	0

The treatments in the Treated variant (V1) followed a sequential application scheme (T1–T6), implemented according to the incidence and intensity of pest and pathogen attacks. Each treatment code corresponds to a specific product, concentration, application date, and biological target.

Chemical treatments were applied at 7–10 day intervals, whereas “bio” products were applied at 4–6 day intervals, in accordance with recommended use in protected cultivation systems, (Table 3).

Untreated variant (V2 – Control). The control variant consisted of plants that did not receive any chemical or biological plant protection treatments throughout the entire crop cycle. Only standard cultural practices (ventilation, irrigation, fertilization, and hygiene) were applied, and the natural development of pathogens and pests was allowed.

Table 3. Fungicide, bio-fungicide, insecticides and bio-insecticides treatments applied for pathogens and pests control

Treatment	Product	Concentration	Dates applied	Target pathogen(s)	Crop
T1	Amistar Decis Expert 100 EC	0.1% 0.0075%	05 May 20 April	<i>Alternaria brassicae</i> , <i>Sclerotinia sclerotiorum</i> <i>Pieris brassicae</i> , <i>Plutella maculipennis</i> , <i>Phyllotreta atra</i>	Cabbage Cauliflower
T2	Amistar Mavrik 2F	0.1% 0.05%	13 May 02 May	<i>A. brassicae</i> , <i>S. sclerotiorum</i> <i>P. brassicae</i> , <i>P. maculipennis</i>	Cabbage Cauliflower
T3	Funres Decis Expert 100 EC	0.3% 0.0075%	20 May 12 May	<i>Xanthomonas campestris</i> , <i>A. brassicae</i> , <i>S. sclerotiorum</i> <i>P. brassicae</i> , <i>P. maculipennis</i> , <i>P. atra</i>	Cabbage Cauliflower
T4	Funres Flipper	0.3% 2.0%	25 May 24 May	<i>X. campestris</i> , <i>A. brassicae</i> , <i>S. sclerotiorum</i> , <i>Brevicoryne brassicae</i>	Cabbage Cauliflower
T5	Funres Flipper	0.3% 2.0%	30 May 31 May	<i>X. campestris</i> , <i>A. brassicae</i> , <i>S. sclerotiorum</i> , <i>B. brassicae</i>	Cabbage Cauliflower
T6	Funres Neemex	0.3% 0.3%	08 June 10 June	<i>X. campestris</i> , <i>A. brassicae</i> , <i>S. sclerotiorum</i> , <i>B. brassicae</i> , <i>P. brassicae</i>	Cauliflower

This variant provided the baseline reference for evaluating the degree of attack by pathogens and pests, and the difference in yield between protected and unprotected plants.

RESULTS AND DISCUSSIONS

The temperature dynamics recorded inside the high plastic tunnels showed a clear increasing trend from late March to mid-June (Figure 1). Minimum temperatures ranged from approximately 5–7°C in late March and early April to 14–16°C in June. Mean temperatures followed a similar pattern, rising gradually from 15–20°C in March–April to 24–27°C in June. Maximum temperatures were consistently high throughout the entire experimental period, oscillating between 32°C and 39°C, with peak values recorded in June. These elevated maximum temperatures are characteristic of protected cultivation systems during late spring and early summer. Overall, the progressive increase in both minimum and mean temperatures ensured favourable thermal conditions for vegetative growth, while the periodically elevated maximum temperatures may have contributed to enhanced pest and pathogen activity, supporting the natural pressure required for evaluating the effectiveness of the protection program.

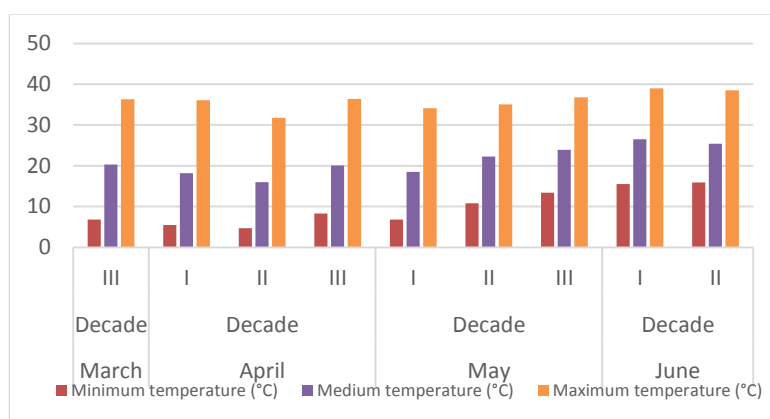


Figure 1. Temperature during experimental trials under a high plastic tunnel

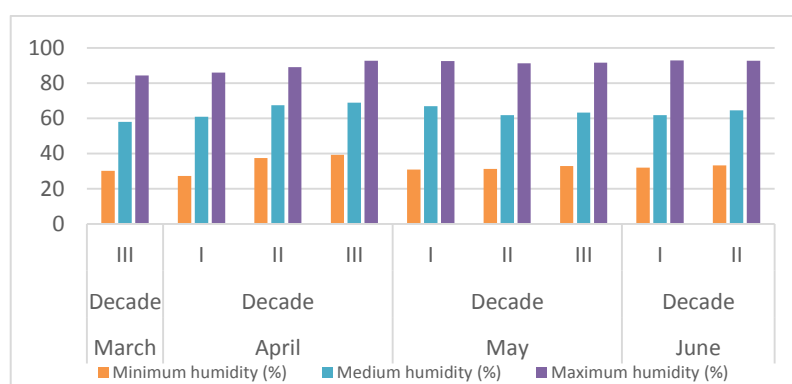


Figure 2. Relative air humidity during experimental trials under a high plastic tunnel

The relative humidity values recorded inside the high plastic tunnels showed moderate variation across the experimental period (Figure 2). Minimum humidity fluctuated between 27–40%, with the lowest values observed in late March and early April, and a slight increase toward late April and June. Mean humidity ranged between 58–70%, maintaining relatively stable levels throughout the season, which are characteristic of spring–early summer conditions in protected environments. Maximum humidity remained consistently high, ranging from 85–93%, with the highest values recorded in April and June. These elevated levels are typical of early morning periods in high tunnels and create favourable microclimatic conditions for the development of fungal pathogens such as *Alternaria brassicae* and *Sclerotinia sclerotiorum*. Overall, the combination of moderate mean humidity and persistently high maximum humidity provided suitable conditions for natural disease pressure, allowing for an accurate assessment of the efficacy of the applied protection program.

In the cabbage crop under high plastic tunnel, the following pathogens and pests were identified: *Xanthomonas campestris* (black rot, Figure 3, 4), *Alternaria brassicae* (dark spot of crucifers), *Sclerotinia sclerotiorum* (white rot, Figure 5) and the pests *Brevicoryne brassicae* (cabbage aphid, Figure 6), *Phyllotreta atra* (cabbage flea beetle; Figure 7), *Pieris brassicae* (cabbage white butterfly; Figure 8).

In the cauliflower crop, the pathogens identified were *Xanthomonas campestris* (black rot) and *Alternaria brassicae* (dark spot of crucifers, Figure 9) and the pests *Phyllotreta atra* (cabbage flea beetle, Figure 10), *Pieris brassicae* (cabbage white butterfly), *Plutella maculipennis* (diamondback moth, Figure 11).



Figure 3. Attack of *Xanthomonas campestris* on cabbage



Figure 4. Attack of *Xanthomonas campestris* on cabbage leaf



Figure 5. Attack of *Sclerotinia sclerotiorum* on cabbage



Figure 6. Attack of *Brevicoryne brassicae* on cabbage



Figure 7. Attack of *Phyllotreta atra* on cabbage



Figure 8. Attack of *Pieris brassicae* on cabbage



Figure 9. Attack of *Alternaria brassicae* on cauliflower



Figure 10. Attack of *Phyllotreta atra* on cauliflower



Figure 11. *Plutella maculipennis* - larvae, on cauliflower

For cabbage, the products Amistar 0.1%, Funres 0.3%, Decis Expert 100 EC 0.0075%, Mavrik 2F 0.05%, Flipper 2.0% and Neemex 0.3% provided good protection against the pathogens *Xanthomonas campestris* (E=79.3%), *Alternaria brassicae* (E=78.8%), *Sclerotinia sclerotiorum* (E=78.7%) as well as the pests *Brevicoryne brassicae* (E=88.6%), *Phyllotreta atra* (E=83.5%) and *Pieris brassicae* (E=85.8%) (Table 4).

For cauliflower, the same products ensured high protection against the pathogens *Xanthomonas campestris* (E=91.1%), *Alternaria brassicae* (E=93.6%) and pests *Phyllotreta atra* (E=79.8%), *Pieris brassicae* (E=84.7%), and *Plutella maculipennis* (E =84.0%) (Table 5).

Table 4. Control of damage agents in the cabbage crop under high plastic tunnel (Vidra, 2022)

Damage agents	Degree of attack (%) / hybrid												Average efficacy (%)
	‘Delight Ball F1’			‘Vettel F1’			‘Zarissima RZ F1’			‘Magnus Cresco F1’			
	treated	untreated	E (%)	treated	untreated	E (%)	treated	untreated	E (%)	treated	untreated	E (%)	
<i>X. c.</i>	2.2	10.8	79.6	3.1	21.8	85.8	4.0	17.4	77.0	2.9	11.5	74.8	79.3
<i>A. b.</i>	3.3	14.7	77.5	3.4	12.9	73.6	1.4	11.4	87.7	2.5	10.6	76.4	78.8
<i>S. s.</i>	0.7	3.4	79.4	1.1	3.8	71.0	1.0	6.2	83.9	0.8	4.1	80.5	78.7
<i>B.b.</i>	4.1	31.1	86.8	2.0	33.1	93.9	3.5	26.3	86.7	4.8	36.6	86.9	88.6
<i>P. a.</i>	0.2	2.8	92.9	0.4	3.5	88.6	1.1	4.9	77.5	0.6	2.4	75.0	83.5
<i>P. b.</i>	3.6	20.4	82.3	4.7	31.6	85.1	5.5	42.7	87.1	2.6	23.1	88.7	85.8

X. c. - *Xanthomonas campestris*, *A. b.* - *Alternaria brassicae*, *S. s.* - *Sclerotinia sclerotiorum*, *B. b.* - *Brevicoryne brassicae*, *P. a.* - *Phyllotreta atra*, *P. b.* - *Pieris brassicae*

Table 5. Control of damage agents in the cauliflower crop under high plastic tunnel conditions (Vidra, 2022)

Damage agents	Degree of attack (%) / hybrid									Average efficacy (%)
	'Freedom F1'			'Arienzo RZ F1'			'Venoosa F1'			
	treated	untreated	E (%)	treated	untreated	E (%)	treated	untreated	E (%)	
<i>X. c.</i>	0.4	5.3	92.4	0.5	6.2	91.9	0.8	7.3	89.0	91.1
<i>A. b.</i>	0.2	7.2	97.2	0.7	6.8	89.7	0.4	6.5	93.8	93.6
<i>P. a.</i>	2.7	10.6	74.5	2.5	11.4	78.1	1.6	12.2	86.9	79.8
<i>P. b.</i>	1.8	10.9	83.5	2.1	12.5	83.2	1.5	11.8	87.3	84.7
<i>P. m.</i>	2.9	20.1	85.6	3.3	21.7	84.8	3.8	20.8	81.7	84.0

X. c. - *Xanthomonas campestris*, *A. b.* - *Alternaria brassicae*, *P. a.* - *Phyllotreta atra*,
P. b. - *Pieris brassicae*, *P. m.* - *Plutella maculipennis*

Regarding the yields obtained, on cabbage, they were 9.160 kg/m² for the hybrid 'Delight Ball F1', 8.980 kg/m² for the hybrid 'Vettel F1', 7.840 kg/m² for the hybrid 'Zarissima RZ F1' and 8.050 kg/m² for the hybrid 'Magnus Cresco F1' (treated variants) and respectively 8.530 kg/m² for the 'Delight Ball F1' hybrid, 8.100 kg/m² for the 'Vettel F1' hybrid, 7.090 kg/m² for the 'Zarissima RZ F1' hybrid and 7.140 kg/m² for the 'Magnus Cresco F1' hybrid, for the variants untreated control – V2 (table 6). The 'Delight Ball F1' hybrid had the highest yield (9.160 kg/m²). In the experience with cabbage hybrids, the yield differences obtained in addition to the untreated control variant were very significantly positive.

Referring to the cauliflower yields, they were 6.110 kg/m² for the 'Freedom F1' hybrid, 5.740 kg/m² for the 'Arienzo RZ F1' hybrid and 4.510 kg/m² for the 'Venoosa F1' hybrid (treated variants) and 5.780 kg/m² in the 'Freedom F1' hybrid, 5.260 kg/m² in the 'Arienzo RZ F1' hybrid and 4.120 kg/m² in the 'Venoosa F1' hybrid, respectively the untreated control variants (table 7). The 'Freedom F1' hybrid had the highest yield (6.110 kg/m²). In the experience with cauliflower hybrids, the yield differences obtained in addition to the untreated control variant were significantly and distinctly positive.

Table 6. The obtained yields from the cabbage crop under high plastic tunnel conditions (Vidra, 2022)

Hybrid	kg/m ²		Difference from untreated control (kg/m ²)	% compared to the untreated control	Significance
	treated	untreated			
Delight Ball F1'	9.160	8.530	+0.630	107.4	***
'Vettel F1'	8.980	8.100	+0.880	110.9	***
'Zarissima RZ F1'	7.840	7.090	+0.750	110.6	***
'Magnus Cresco F1'	8.050	7.140	+0.910	112.7	***

LD 5%=0.10; LD 1%=0.14; LD 0,1%=0.20

Table 7. The obtained yields from the cauliflower crop under high plastic tunnel conditions (Vidra, 2022)

Hybrid	kg/m ²		Difference from untreated control (kg/m ²)	% compared to the untreated control	Significance
	treated	untreated			
'Freedom F1'	6.110	5.780	+0.330	105.7	*
'Arienzo RZ F1'	5.740	5.260	+0.480	109.1	**
'Venoosa F1'	4.510	4.120	+0.390	109.5	*

LD 5%=0.31; LD 1%=0.44; LD 0,1%=0.64

CONCLUSIONS

In the cabbage crop, the pathogens *Xanthomonas campestris*, *Alternaria brassicae* and *Sclerotinia sclerotiorum*, together with the pests *Brevicoryne brassicae*, *Phyllotreta atra* and *Pieris brassicae*, were consistently present. In cauliflower, the predominant pathogens were *X. campestris* and *A. brassicae*, while the main pests identified were *Phyllotreta atra*, *Pieris brassicae* and *Plutella maculipennis*.

The integrated sequence of treatments applied in the Treated variant (V1) provided high and consistent protection in both cabbage and cauliflower. Control efficiencies exceeded 78% for all pathogens and 83–89% for pests in cabbage, and reached 89–97% for pathogens and 74–87% for pests in cauliflower.

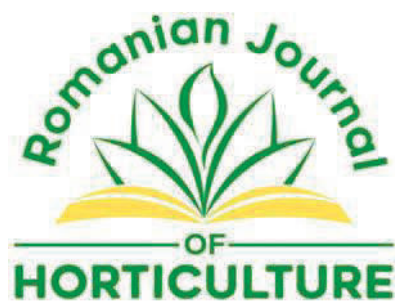
The positive impact of the protection program was also reflected in yields. All hybrids showed significantly higher production in the Treated variant (V1) compared with the untreated control (V2). The highest yields were obtained for the cabbage hybrid 'Delight Ball F1' (9.160 kg/m²) and the cauliflower hybrid 'Freedom F1' (6.110 kg/m²). Overall, the results demonstrate that the integrated protection program used in this study is highly effective under high plastic tunnel conditions, ensuring strong suppression of key pathogens and pests and leading to significant yield improvements in both cabbage and cauliflower hybrids.

ACKNOWLEDGEMENTS

The scientific paper is part of the project „Modern technologies for control pathogens and pests in vegetable crops under high plastic tunnels, with minimal impact on the environment”, no. 1022/12.10.2018, which is supported by Academy of Agricultural and Forestry Sciences "Gheorghe Ionescu-Șișești", for phase 5 „Integrated management for the control of damage agents in cabbage and cauliflower crops”.

REFERENCES

1. Chandel, S., Sharma, A., Sharma, M. (2023). Management of *Alternaria* leaf spot of cauliflower caused by *Alternaria brassicicola* through fungicides. Current Agriculture, Vol. 40, Issue 1, p. 21
2. Costache, M., Șovărel, G., Hoge, S., Cenușă, E. (2023). Bolile și dăunătorii culturilor de legume din spații protejate și câmp. Recunoaștere și combatere, Editura PIM, Iași.
3. Iablonec, A.R., Ienovan, A., Sperlea, M.O., Cărăbeș, A., Grozea, I., Ștef R., (2022). The influence of chemical and biological substances in reducing *Pieris brassicae* population in *Brassica oleracea* culture. Research Journal of Agricultural Science, 54 (3), 2022; ISSN: 2668-926X
4. Li, Z., Costamagna, A.C., Beran, F., You, M. (2024). Biology, Ecology and Management of Flea Beetles in Brassica Crops. Annual Review of entomology, vol. 69.
5. Serhiienko, V., Borzykh, O., Tkalenko, H., Balan, H. (2023). Control of white cabbage diseases using biologicals, Vegetable and Melon Growing, (73), 81-88. <https://doi.org/10.32717/0131-0062-2023-73-81-88>
6. Shiberu, T., Negeri, M., (2016). Effects of synthetic insecticides and crude botanicals extracts on cabbage aphid, *Brevicoryne brassicae* (L.) (Hemiptera: Aphididae) on cabbage. Journal of Fertilizers and Pesticides, 7:1 DOI: 10.4172/2471-2728.1000162
7. Shikha, D., Kataria, S.K., Chandi, R.S. (2023). Biology of *Pieris brassicae* (L.) on cauliflower. Indian Journal of Entomology Online published Ref. No. e23060. DoI. No.: 10.55446/IJE.2023.1060
8. Tolessa, T., Lombamo, T., Chiko, S. (2021). Evaluation of insecticides for the management of diamondback moth (*Plutella xylostella* (L.) (Lepidoptera: Plutellidae) on cabbage under field conditions. Journal of Biology, Agriculture and Healthcare www.iiste.org ISSN 2224-3208 (Paper) ISSN 2225-093X (Online) Vol. 11: 23



Potato growing

Potato (*Solanum tuberosum* L.) growth and yield response to inflorescence removal and seed tuber size in Girar Jarso District, Central Ethiopia

B.Tamiru Abebe^{1*} and G.Megersa Geleta²

¹Ethiopian Biodiversity Institute, Crop and Horticultural Directorate,
Addis Ababa, Ethiopia

²Salale University Agricultural and Natural Science College, Ethiopia

*Corresponding author email: bachatamiru12@gmail.com

ABSTRACT

Potato (*Solanum tuberosum* L.) is an important food security crop in Ethiopia. The low yield is caused in part by farmers using non-essential seed tuber size and poor agronomic management. This study aimed to examine the effects of seed tuber size and inflorescence removal on yield components and potato yield. A field experiment was carried out at the experimental site of General Tadesse Biru Campus, Salale University. Three levels of inflorescence removal and seed tuber sizes each comprised the treatments. The RCBD with a 3x3 factorial arrangement and 3 replications was used for the study. The R software version 4.03 was used to experiment with data. The experimental results showed that the interaction of big seed tuber size and flower bud removal led to the highest total tuber yield (34.64 t ha⁻¹). Large seed tuber size (46-55 mm) and inflorescence removal led to the highest marketable tuber yield (28.59 t ha⁻¹). Marketable tuber yield correlated positively with steam number, plant height, tuber number, tuber yield, tuber weight, tuber diameter, tuber length, and total tuber, but negatively with days to emergence. As a result, big seed tuber size and inflorescence removal were preferable in terms of all yield-contributing characteristics and tuber yield, and medium seed tuber size was beneficial to the economic and agronomical feasibility of potato production. Because the study was limited to a single season and location, more research is needed to make viable recommendations.

Keywords: Gudenie cultivar, flower, source-sink, assimilate partitioning, tuber yield

INTRODUCTION

Potatoes originated in the Andean region of South America, specifically Peru, Bolivia, and Northern Argentina (Fuentes *et al.*, 2022). Potatoes are the world's third most consumed food crop, behind rice and wheat (FAOSTAT, 2019; Salim *et al.*, 2023). Potatoes are farmed extensively in more than 164 countries and are used by over a billion people almost daily, either raw or prepared (Eid *et al.*, 2020). It is Ethiopia's primary food crop, particularly in high and middle-altitude areas, and one of the most widely planted vegetables (Yazie *et al.*, 2017). Ethiopia has tremendous potential because 70% of its arable land is suitable for potato production, primarily in highland regions above 1500 meters of elevation (Gebregwergis *et al.*, 2019). Over a total area of approximately 85,988.43 hectares, 1,141,871.725 tons were harvested (CSA 2020/21). Global potato production is estimated by FAOSTAT (2019) to be 388.19 million tons annually. Production grew to 29 million tons from 3.65 million tons overall (CSA, 2016). Among all the horticultural crops farmed in Ethiopia, potatoes are the second most cultivated crop with the current area coverage, after red peppers (CSA, 2020).

Ethiopia's national potato output of 13.27 tons per hectare is still low when compared to the average global productivity of 21.77 tons per hectare (FAOSTAT, 2019). Potato productivity remains low in Ethiopia despite its wide cultivation, due to insects and disease pressure, poor soil fertility, climate variability, shortage of water, and weak postharvest practices (Adem *et al.*, 2021; Hailu *et al.*, 2017). Selection of seed tuber size and inflorescence removal also the constraints of yield (Gebregwergis *et al.*, 2019; Habtie, 2022). Because flowers and fruits act as strong sinks, they divert minerals, sugars, and amino acids away from other plant parts (Engels *et al.*, 2012), creating competition among developing parts under assimilate limited conditions (Hassen *et al.*, 2013). Removing reproductive organs can redirect assimilates toward vegetative growth and tuber development (Hasani *et al.*, 2022). Although flowers serve as assimilate sinks, and they have limited economic relevance in potato production (Gebregwergis *et al.*, 2019). The yield of potatoes is also limited by the size of the seed tuber. Ebrahim *et al.* (2018) reported that seed tubers of the improved varieties (*Jalene* and *Gudenie*) that were between 46 and 55 mm (big) or 35 and 45 mm (medium) in diameter produced the maximum total tuber. As many parts of the country, seed tuber size similarly constrains potato productivity in the study area. Moreover, there is no information on potato production trends in the study area. The effects of seed tuber size and inflorescence removal on potato yield and yield components have not been previously investigated in the study area. Therefore, the present study evaluated the combined influences of inflorescence removal and seed tuber size on tuber yield and yield components potato.

MATERIALS AND METHODOLOGY

Description of the study area

The study was conducted in the Girar Jarso districts of the north Shewa zone of Oromia regional state (Fig. 1). The administrative towns of the districts, Fitcha, are located 112 kilometers north of Addis Abeba. From 9°37'0" N to 38°5'0" E and 2,738 m.a.s.l., the districts are distinguished by their longitudinal, latitudinal, and altitudinal locations. The mean annual temperature and rainfall of Girar Jarso are 15°C and 1,200 mm and 18°C and 1,400 mm.

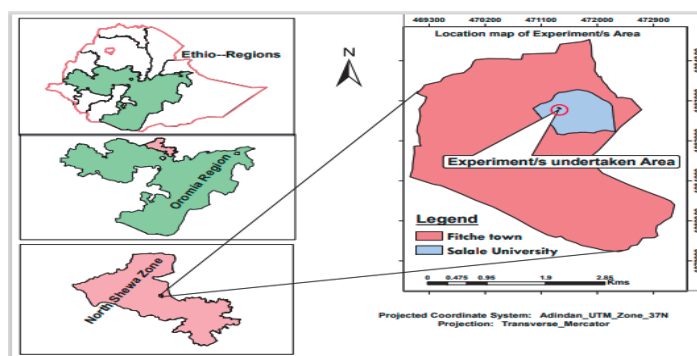


Figure 4. Map of the study area

The field experiment was implemented at the experimental site of General Tadesse Biru Campus, Salale University, during the main cropping season of 2022 under rain-fed conditions. Average landholding per household is approximately 3.2 ha. Mixed farming is the major livelihood activity, with crops such as barley, wheat, maize, teff, sorghum, pulses (beans, peas, lentils), and horticultural crops (apple, cabbage, kale, onion, potato). The soils are clay (Vertisols) with a pH of 6.4 (Abera *et al.*, 2013). According to soil laboratory analysis results at Ethiopian Agricultural Research Institute, Fitcha Agricultural Research Center (EARI FARC (2022), the soil has a sandy clay texture (50.36% sand, 42.36% clay, 7.28% silt), pH 6.60, EC 0.01 dS m⁻¹, organic carbon 9.71%, total nitrogen 0.49%, and C: N ratio 11.48.

Experimental Treatments and Design

The experiment was designed to evaluate the effect of tuber size and inflorescence removal on potato (*Solanum tuberosum* L.) growth and yield. The improved potato variety *Gudenie* (released by Holeta Agricultural Research Center in 2006) was used due to its high yield potential, adaptability, and tolerance to late blight. Treatments consisted of three tuber size categories (25–34 mm, 35–45 mm, and 46–55 mm) Ebrahim *et al.*, 2018 and three inflorescence management methods: Normal growth – plants allowed to flower and set fruits, Flower bud removal – buds removed before opening, Flower removal – flowers removed at full bloom (Gebregwergis *et al.*, 2019). The experiment followed a Randomized Complete Block Design (RCBD) with a factorial arrangement (3×3) and three replications. Each plot measured 3×3 m (9 m^2), consisting of four rows with 10 plants per row. Row and plant spacing were 75 cm and 30 cm, respectively; 1 m between blocks and 0.5 m between plots. Data were collected from the middle two rows (five sample plants). Plots were manually plowed and leveled to 15–20 cm. Uniformly sprouted tubers were selected and treated before planting. Fertilizer was applied at 195 kg ha^{-1} NPS and 111 kg ha^{-1} urea (Fayera, 2017; Alemayehu *et al.*, 2018). Routine field operations (weeding, earthing-up, ridging) followed standard agronomic practices (Desalegn, 2016). Mancozeb fungicide was applied at 3 kg ha^{-1} to control late blight (EARO, 2004). Flower and bud clusters were removed at 3-day intervals for the respective treatments (Bartholdi, 1942).

Data Collection. Data were collected from five representative plants per plot. Phenological traits included days to 50% emergence (DE) and days to 90% maturity (DM). Growth parameters recorded were plant height (cm), number of main stems per hill, and leaf number per plant. Yield and yield components measured included number of tubers per hill, average tuber weight (g), tuber yield per hill (kg), total tuber yield (t ha^{-1}), tuber length and diameter (cm), underground fresh and dry biomass (t ha^{-1}), marketable yield (t ha^{-1}), and unmarketable yield (t ha^{-1}). Experimental data were subjected to analysis of variance (ANOVA) using the R software version 4.03. The mean separations were tested using the LSD test at the 5% level of probability. When there was a significant ANOVA effect and Pearson correlation coefficients were used to determine the relationship of parameters.

RESULTS AND DISCUSSION

Impacts of Inflorescence Removal and Seed Tuber Size on Potato Phenological Parameters

Days to 50% emergence.

The number of days before 50% emergence was significantly influenced by the main effect of seed tuber size (Table 1). The average number of days to reach 50% emergence for all of the studied sizes ranged from 10.33 to 16.89 days. As a result, the large seed tuber emerged from the soil faster (10.33 days) than the other two seed tuber sizes, small seed (16.89 days) and medium seed (12.33 days), (Table 1).

This might be because large tubers can store significantly more food than small seed tubers, which enables them to give the growing seedling the proper quantity of glucose. Similar findings were reported by Ebrahim *et al.* (2018), where larger seed tuber sizes had significantly fewer days to 50% to emergence.

Doboch *et al.* (2022) and Asnake *et al.* (2023) also indicated that smaller seed tubers required the longest days to emerge, whereas larger seed tubers gave early emergence and maximum crop coverage and growth.

Days to 90% maturity

The seed tuber size and the removal of the inflorescence significantly ($p < 0.001$) impacted the days to maturity. The interaction between seed tuber size and inflorescence removal had

no impact on the days to maturity. The average number of days to 90% maturity for flower removal ranged from 92.33 to 105.67 days for all sizes that were tested.

The results showed that the shortest days to 90% maturity (96.89 days) were recorded at medium Size (35-45 mm), which was statistically similar to large seed tuber size (97.11 days), while the longest days (103.56 days) were recorded at small size (25-34 mm). The ANOVA of the results shown in Table 1 revealed that the use of medium seed tubers resulted in a shorter maturation time. Smaller seed tuber diameters (25-34 mm) required the longest maturation period. The results of this study indicated that plants with early sprouting and flowering were more developed than those with later sprouting and flowering. According to Masarirambi *et al.* (2012), larger seed potato tubers showed improved overall resource usage efficiency and allocation of a metabolite, which caused sprouts to emerge earlier, grow and develop more quickly, and reach maturity earlier than small-sized seeds.

The removal of an inflorescence after a bud had been removed was reported to have taken 105.67 days to reach physiological maturity, while normal growth required 92.33 days. According to this study, cutting off flower buds may help to extend canopy life and enable the plant to continue making photo assimilates for a longer period of time. The results supported Gebregwergis *et al.* (2019) evidence that flowerless potato plants matured later than flowering potato plants. In addition to this, the results are in line with Moges (2022), who indicated that very large seed tuber size required the quickest maturity period, whilst smaller seed tubers required the longest maturation period.

Table 4. Interaction effect of tuber size and inflorescence removal on days to emergence and days to maturity of potato (*Solanum tuberosum* L.)

Tuber size (mm)	Inflorescence removal	Days to emergence (days)	Days to maturity (days)
Large (46–55)	Bud removal	10.11 ^d	100.22 ^c
	Flower removal	10.33 ^d	96.55 ^d
	Control (flowers intact)	10.56 ^d	94.55 ^d
Medium (35–45)	Bud removal	12.22 ^c	102.33 ^b
	Flower removal	12.33 ^c	97.89 ^c
	Control	12.45 ^c	95.45 ^d
Small (25–34)	Bud removal	16.45 ^a	108.56 ^a
	Flower removal	16.67 ^a	104.33 ^b
	Control	17.00 ^a	101.11 ^b
Least significant difference (0.05)		1.64	3.12
Coefficient of variation (%)		7.88	2.95

Means within a column that are followed by the same letter do not differ significantly at the 5% level of significance

The Interaction Effects of Seed Tuber Size and Inflorescence Removal on Yield and Yield Components of Potato

Tuber length (cm)

The results of the analysis of variance revealed that the tuber length was affected by the two components' interaction ($p < 0.05$), Table 2. The average tuber length varied from 8.00 cm to 12.06 cm for all of the tuber sizes and inflorescence removal.

The analysis showed that the largest tuber length (12.60 cm) was obtained from the interaction effect of large size (45–55 mm) and flower removal, while the smallest tuber length (8.00 cm) was obtained from the interaction effect of small size (25–34 mm) and flower unremoval (normal growth). The results indicated that using large seed tuber sizes and flower removals resulted in the greatest tuber length. This occurs as a result of the large seed tubers, which are stuffed with food that has been stored, as well as the potato plants' lack of competition for nutrient intake. This result was supported by observations made by Gebregwergis *et al.* (2019),

who noted that the lowest tuber length (7.18 cm) was recorded during normal potato growth and the largest tuber length (17.48 cm) was recorded after the flower bud was removed.

Average tuber weight (gm)

According to the analysis of variance, the interaction of the two components had a substantial effect on the tuber weight ($p < 0.001$). For all of the studied tuber sizes and inflorescence removal, the average tuber weight ranged from 41.63 to 59.00 gm. According to the analysis, the interaction effect of large size (46–55 mm) and bud removal produced the highest tuber weight (59.67 g) statistically similar with interaction of large tuber size with flower removal (58.00 g), while the interaction effect of small size (25–34 mm) and flower removal (normal growth) produced the lowest tuber weight (41.63 g), as shown in Table 2. The results showed that using large seed tuber sizes and blossom bud removals led to the highest average tuber weight. This result is align with Moges (2022), who reported that seed tuber sizes can give the necessary nutrients for growth and developments at the beginning of the growth phase which increase tuber weight.

Total tuber yield ($t\ ha^{-1}$)

According to the analysis of variance, the total tuber production per hectare was also affected by the interaction effects of the two variables. For all of the studied plant sizes and inflorescence removal, the typical quantity of tubers per plant ranged from 29.46 to 34.78 tons ha^{-1} . According to the analysis of the variance, the interaction effect of large size (46–55 mm) and flower removal resulted in the highest total tuber yield per hectare (34.63 tons ha^{-1}) statistically similar with large size with flower bud removal (33.78 tons ha^{-1} , while the interaction effect of small size (25–34 mm) and flower unremoval (normal growth) resulted in the lowest total tuber yield (29.46 tons ha^{-1}) that was statistically no difference interaction of small size flower removal (29.54 tons ha^{-1}) Table 2. The outcome showed that employing large seed tuber size and blossom bud removal led to the maximum overall tuber yield. This may be because, in contrast to small and medium seed tuber sizes in normal growth, an ideal number of stems are produced, leading to less resource rivalry amongst assimilation organs (Tubers and inflorescences). This is align with Sorsa *et al.* (2025) reports indicated that the combination of flower bud removal and larger seed tuber size significantly increased total tuber yield. Ebrahim *et al.* (2018) indicated that larger seed tuber sizes (46–55 mm) produced higher total tuber yields compared with smaller seed sizes (25–34 mm). this result also consistent with findings of Moges (2022) and Zebeay *et al.* (2018), who reported that larger seed tuber sizes produced higher total tuber yields compared to smaller seed tubers.

Marketable tuber yield ($t\ ha^{-1}$)

The analysis of variance revealed that marketable tuber yields (ton ha^{-1}) were significantly affected by the combined effects of seed tuber size and inflorescence removal ($P < 0.01$). According to the analysis of variance, the interaction between large size (46–55 mm) and flower bud removal produced the highest marketable tuber yield (28.59 tons ha^{-1}) statistically no difference between interaction of large tuber size with flower removal (28.47), while the interaction between small size (25–34 mm) and flower removal produced the lowest marketable tuber yield (20.84 tons ha^{-1}), which was statistically similar to the interaction between small seed size (25–34 mm) and normal growth (21.00), as shown in Table 2. The results demonstrated that the largest commercial tuber yield was achieved by using large seed tuber sizes and removing flower buds.

Unmarketable tuber yield (t ha⁻¹)

According to the analysis of variance, the interaction of the two variables significantly affected the yield of unmarketable tubers ($P<0.01$). For all of the tested sizes and Inflorescence removal, the average unmarketable tuber yield ranged from 6.10 to 8.47.

Accordingly, the results of analysis of variance showed that the interaction effect of small tuber Size (25-34 mm) and flower unremove were recorded as the highest unmarketable tuber yield (8.47 tons ha⁻¹), while the interaction effect of medium Size (35-45 mm) and flower bud remove was obtained with the lowest unmarketable tuber yield (6.10 tons ha⁻¹) Table 2. This consequence could be the result of the small seed tubers' low food storage capacity, which causes plant growth failure and flower retention and puts growing tubers and flowers in competition for scarce resources. Hanász *et al.* (2024) also demonstrate that small seed tubers may have lower food storage capacity and are more sensitive to stresses, which supports your reasoning about small seed tuber size leading to greater competition and poorer bulking.

Table 5. Interaction effects of seed tuber size and inflorescence removal on tuber length, average tuber weight, total tuber, marketable yield and unmarketable yield

Seed tuber size	Inflorescence removal	Tuber length	Average tuber weight	Total tuber yield	Marketable yield	Unmarketable yield
Large size (45-55)	Bud removal	9.40 ^{cd}	59.67 ^a	33.78 ^a	28.59 ^a	6.19 ^c
	Flower Remove	12.60 ^a	58.00 ^a	34.63 ^a	28.47 ^a	6.16 ^c
	Flower Unremove	11.18 ^{ab}	54.00 ^a	31.07 ^{cde}	24.00 ^{cd}	7.07 ^b
Medium size (35-45)	Bud removal	9.82 ^{bc}	58.13 ^a	32.91 ^{abc}	26.80 ^{ab}	6.10 ^c
	Flower Remove	9.77 ^{bc}	56.63 ^a	33.18 ^{ab}	26.43 ^b	6.76 ^{bc}
	Flower Unremove	9.79 ^{bc}	46.00 ^b	30.09 ^{de}	23.43 ^d	6.68 ^{bc}
Small Size (25-34)	Bud removal	9.83 ^{bc}	57.47 ^a	31.64 ^{bcd}	25.52 ^{bc}	6.11 ^c
	Flower Remove	9.81 ^{bc}	59.00 ^a	29.54 ^e	20.84 ^e	6.37 ^{bc}
	Flower Unremove	8.00 ^d	41.63 ^b	29.46 ^e	21.00 ^e	8.47 ^a
Least significant difference (0.05)		0.99	3.4	1.13	1.12	0.42
Coefficient of variation (%)		9.94	6.2	3.57	2.12	6.33

Means followed by the same letter within a column are not significantly different at the 5% level of significance

The Relationship between Parameters Influenced by Tuber Size and Inflorescence Removal

The results of the correlation study revealed that the total tuber yield was significantly and positively correlated with the tuber length and marketable tuber yield, but considerably and negatively correlated with the number of days to an emergency (Table 3). The outcome showed that parameters with favorable correlations aid in increasing potato tuber production overall. The total tuber yield was reduced as a result of the potato's days to emergence being prolonged. This showed that the small seed tuber size, which is smaller in stored food and tends to emerge after a specific number of days, produces fewer stems and tubers, which leads to a lower overall tuber production. The Marketable tuber yield had positive ($r=0.493^{**}$, 0.438^{*} and 0.956^{**}) correlations with the following variables: tuber weight, tuber length and total tuber. However, it was significantly and negatively correlated ($r=-0.550^{**}$) with days to emergency. This showed that when the positively linked factors increased, the marketable tuber yield declined while the negatively correlated parameters grew. Gebreselassie and Ajema (2022), negatively correlated parameters revealed that yield increased as parameters declined, whereas positively correlated parameters suggested that yield increased as yield-related parameters increased.

Table 6. Correlation between phenology, growth, and yield parameters of potato, which are affected by seed tuber size and Inflorescence removal

	Days to Emergency	Days to maturity	Tuber weight	Tuber length	Total tuber yield	Marketable tuber yield	Unmarketable tuber yield
Days to Emergency	1	0.419*	-0.21 ^{ns}	-0.458**	-0.543**	-0.550**	0.182 ^{ns}
Days to maturity		1	0.451**	-0.101 ^{ns}	0.117 ^{ns}	0.245	-0.423*
Tuber weight			1	0.443*	0.31 ^{ns}	0.493**	-0.646**
Tuber length				1	0.378*	0.438*	-0.370*
Total tuber yield					1	.956**	-0.115 ^{ns}
Marketable tuber yield						1	-0.367*
Unmarketable tuber yield							1

ns= non-significant, *= significant ($p<0.05$), **= Highly significant ($p<0.01$)

CONCLUSIONS

Seed tuber size and inflorescence removal were found to significantly influence potato yield and yield components. Large seed tuber size (46-55 mm) combined with flower removal produced the highest total yield (34.63 t. ha⁻¹) and marketable yield (28.59 t. ha⁻¹), while smaller seed tuber size (25-34 mm) and without flower removal resulted in the lowest yields. Marketable tuber yield showed a negative correlation with days to emergence but a positive correlation with tuber weight, tuber length, and total tuber yield. Overall, large tuber size with flower removal proved superior in all yield contributing traits, while medium tuber size (34-45 mm) with flower removal was recommended as the most economically viable option for the study area and similar agro-ecological conditions.

ACKNOWLEDGEMENT

First and foremost, we gratefully acknowledge the Ethiopian Biodiversity Institute and Salale University for providing technical support for this study.

REFERENCE

1. Abera, W., Hussein, S., Derera, J., Worku, M. and Laing, M.D. (2013). Preferences and constraints of maize farmers in the development and adoption of improved varieties in the mid-altitude, sub-humid agro-ecology of western Ethiopia.
2. Adem, S.A. (2021). Impacts of rainfall variability on potato productivity in Haramaya district, eastern Hararge zone, Ethiopia. *Journal of Chemical, Environmental and Biological Engineering*, 5(1), pp.9-22.
3. Alemayehu, M. and Jemberie, M. (2018). Optimum rates of NPS fertilizer application for economically profitable production of potato varieties at Koga Irrigation Scheme, Northwestern Ethiopia. *Cogent Food & Agriculture*, 4(1), p.1439663.
4. Ali Faramarzi, Shahram Shahrokhi, Hadi Mehrpouya, Davood Hasan Panah (2011), Evaluation of inflorescence removing and salinity anti-stress matter application effects on potato, *Solanum tuberosum* (cv. Agria) yield, 2011 International Conference on Biology, Environment and Chemistry IPCBEE vol.24
5. Asalfew, G. K. (2016). Review on the effect of gibberellic acid on potato (*Solanum tuberosum* L.) tuber dormancy breaking and sprouting. *Journal of Biology, Agriculture and Healthcare*, 6(7), 68-79.
6. Asnake, D., Alemayehu, M., & Asredie, S. (2023). Growth and tuber yield responses of potato (*Solanum tuberosum* L.) varieties to seed tuber size in northwest highlands of Ethiopia. *Heliyon*, 9(3).
7. Bartholdi, W.L. (1942). Influence of flowering and fruiting upon vegetative growth and tuber yield in the potato.
8. Central Statistical Agency (CSA) .(2020/21). Agricultural sample survey, Report on area and production of crops. Vol. 1 Addis Ababa, Ethiopia 14-26.
9. Central Statistical Agency (CSA).(2016). Agricultural sample survey Report on area and production of crops, Addis Ababa, Ethiopia.

10. Central Statistical Agency (CSA).(2020). Agricultural sample survey, Report on area and production of crops. Vol.1 Addis Ababa, Ethiopia 14-26.
11. Desalegn, G., Turetschek, R., Kaul, H.P. and Wienkoop, S. (2016). Microbial symbionts affect *Pisum sativum* proteome and metabolome under *Didymella pinodes* infection. *Journal of proteomics*, 143, pp.173-187.
12. Doboch, M., Gedebo, A., Haile, A., & Beshir, H. M. (2022). Improving potato productivity through optimum agronomic management to ensure food security of smallholder farmers. *Cogent Food & Agriculture*, 8(1), 2131995.
13. Ebrahim, S., Mohammed, H., & Ayalew, T. (2018). Effects of seed tuber size on growth and yield performance of potato (*Solanum tuberosum* L.) varieties under field conditions. *African Journal of Agricultural Research*, 13(39), 2077-2086.
14. Eid, M.A., Abdel-Salam, A.A., Salem, H.M., Mahrous, S.E., Seleiman, M.F., Alsadon, A.A., Solieman, T.H. and Ibrahim, A.A. (2020), Interaction Effects of Nitrogen Source and Irrigation Regime on Tuber Quality, Yield, and Water Use Efficiency of *Solanum tuberosum* L. *Plants*, 9(1): 110
15. Engels, C., Kirkby, E. and White, P. (2012). Mineral nutrition, yield and source-sink relationships. In Marschner's mineral nutrition of higher plants (pp. 85-133). Academic Press.
16. Ethiopian Agricultural Research Organization (EARO).(2004). Directory of released crop varieties and their recommended cultural practices. Ethiopian Agricultural Research Organization, Addis Ababa, Ethiopia pp. 1-36.
17. Fayera, W. N. (2017). Yield And Yield Components Of Potato (*Solanum Tuberosum* L.) As Influenced By Planting Density And Rate Of Nitrogen Application At Holeta, West Oromia Region Of Ethiopia. *African Journal Of Agricultural Research*, 12, 2242–2254. Doi:10.5897
18. Food and Agricultural Organization Corporate Statistical Database (FAOSTAT). (2019). Food and Agriculture Organization of the United Nations Statistics Division. Rome.
19. Fuentes, S., Gibbs, A. J., Adams, I. P., Hajizadeh, M., Kreuze, J., Fox, A., ... & Jones, R. A. (2022). Phylogenetics and evolution of potato virus V: Another potyvirus that originated in the Andes. *Plant Disease*, 106(2), 691-700.
20. Gebregwergis Fitsum, Mehari Gebremichael, and Hailay Gebremedhin (2019), Flower Bud Removal and Earthing Up Time Increased Growth and Tuber Yield of Potato at Eastern Tigray, Ethiopia. *Indonesian Journal of Agricultural Science* Vol. 20 No. 2 December 2019: 77–85
21. Gebreselassie, H., & Ajema, L. (2022). Correlation, Path Coefficient and Multivariate Analysis for Yield and Yieldassociated Traits among Potato (*Solanum tuberosum* L.) Genotypes Grown in Eastern Ethiopia. *World J. Agric. Soil Sci*, 8(2), 1-7.
22. Hailu Gebru, Ali Mohammed, Nigussie Dechassa and Derbew Belew (2017), Assessment of production practices of smallholder potato (*Solanum tuberosum* L.) farmers in Wolaita zone, southern Ethiopia. *Agric & Food Secururity* 6:31
23. Hanász, A., Zsombik, L., Magyar-Tábori, K., & Mandler-Drienyovszki, N. (2024). Effect of drought and seed tuber size on agronomical traits of potato (*Solanum tuberosum* L.) under in vivo conditions. *Agronomy*, 14(6), 1131.
24. Hasani, R., Lechner, M., Wang, T.H., Chahine, M., Amini, A. and Rus, D. (2022). Liquid structural state-space models. arXiv preprint arXiv:2209.12951.
25. Hassen Yassin, A.M., Fekadu, D. and Hussien, S. (2013). Effect of flower bud removal on growth and yield of anchote root (*Coccinia abyssinica* (Lam.) Cogn.) accessions at Bishoftu. *Advanced Research Journal of Plant and Animal Sciences*, 1(1), pp.7-13.
26. Isaacs, K. B., Snapp, S. S., Chung, K., & Waldman, K. B.(2016). Assessing the value of diverse cropping systems under a new agricultural policy environment in Rwanda. *Food Security*, 8, 491-506.
27. Masarirambi, M.T., Mandisodza, F.C., Mashigaidze, A.B. and Bhebhe, E. (2012). Influence of plant population and seed tuber size on growth and yield components of potato (*Solanum tuberosum*). *Int. J. Agric. Biol*, 14(4), pp.545-549.
28. Moges, H. (2022). Effects of Seed Tuber Size and Intra Row Spacing on Growth And Tuber Yield of Potato (*Solanum Tuberosum* L.) At Koga Irrigation Scheme, North Mecha District, Amhara Region, Ethiopia
29. Salim, M. and Akte, M.A.R. (2023). Effect of different natural storage conditions on keeping quality of potato at Munshiganj Region of Bangladesh.
30. Soresa, D. N. (2025). Effect of flower bud on tuber yield and quality of potato (*Solanum tuberosum* L.) varieties in Horo District, Western Ethiopia.
31. Wudu, D. M., Kassa, M. W., & Demissie, B. N. (2024). Tuber yield components of potato (*Solanum tuberosum* L.) as influenced by earthing up and inflorescence removal. *Romanian Journal Of Horticulture Урeдume.nu: Academia de Stiinte Agricole si Silvice'Gheorghe Ionescu-Sisesti'*, 5, 61-68.
33. Yazie Chanie, Y.C., Akalu Teshome, A.T., Yalfal Temesgen, Y.T. and Baye Berihun, B.B. (2017). Characterization of potato production, marketing, and utilization in North Western Amhara Region, Ethiopia.
34. Zebenay, D., Dechassa, N. and Mohammed, W. (2018). Influence of Plant Spacing and Seed Tuber Size on Yield and Quality of Potato (*Solanum tuberosum* L.) in Central Ethiopia. *Journal of Advances in Crop Science and Technology*, 6:6. DOI: 10.4172/2329-8863.1000406

Total phenolic content in ten potato cultivars with different resistance to potato virus S(PVS)

C.L. Bădăraș^{1,2}, M. Cioloca², N. Bărașcu^{1,2}, A.I. Moja¹, and R.A. Munteanu Ichim¹

¹Faculty of Food and Tourism, Transilvania University, Brasov, Romania

²National Institute of Research and Development for Potato and Sugar Beet
Brasov, Romania

*Corresponding author email: carmen.badarau@unitbv.ro

ABSTRACT

Being one of the most consumed vegetables across the world, potato is a highly nutritious crop, constituting an important source of essential compounds for human health. The present study evaluated the total content of phenolic compounds (TPC) in the skin and flesh of several Romanian potato cultivars with different resistance to potato virus S (PVS) grown in Brasov, over two years. Significant differences were observed across the cultivars. Lower levels of TPC were found in the flesh than in the skin of the tubers. Blue genotype “Blue Purple of Galanesti” and Romanian cultivar ‘Foresta’ had the highest values for TPC in skin and flesh. This study contains information regarding the content of valuable micronutrients of several new Romanian potato cultivars with good nutritional quality and different resistance to PVS.

Keywords: potato, total phenolic content, potato virus S.

INTRODUCTION

Potatoes make a significant contribution to the dietary intake of some functional compounds and valuable nutrients. So many articles on potato composition revealed that there is much more in potatoes than starch (Ezekiel *et al.*, 2013). Phenolic compounds have a large array of health-beneficial characteristics. Based on their concentration and frequency of consumption, potato is considered the third most important source of phenols after apples and oranges (Ezekiel *et al.*, 2013). It is a valuable source of important nutrients (starch, fibers, amino acids, vitamins, minerals), potato tubers representing a complex matrix in the human diet, having potential health benefits.

Some of the reasons that led the choice of this subject (study polyphenol content of potato tubers) were: the special bioavailability of this plant, economic considerations (lower price compared to other sources of antioxidants), the content of anthocyanin and the presence of valuable polyphenols (chlorogenic acid, quercetin and kaempferol glycosylated with rutinose significant amounts of catechin, usually acylated glycosides of rutinose and glucose) (Andre *et al.*, 2006; Ezekiel *et al.*, 2013). In particular, potato antioxidants have been shown to have favorable impacts on several measures of cardio-metabolic health, including lowering blood pressure, improving lipid profiles and decreasing markers of inflammation (Pryia *et al.*, 2023; Salma *et al.*, 2020; Valcarcel *et al.*, 2015; Wang *et al.*, 1999). Tubers with increased levels of polyphenols could have an impact on human health, because these compounds possess antioxidant, antiglycemic, antiviral and anti-inflammatory activities (Duthie *et al.*, 2000; Wang *et al.*, 1999). This impact could be strong, especially for

people where potato is the most important food crop and therefore would be of interest to consumers and producers (Valcarcel *et al.*, 2015).

Potato virus S (PVS) is transmitted mechanically and by aphids in a non-persistent manner (Cojocaru N., 1987, Loebenstein G., 2008). The damage caused by these pathogens is qualitative (commercial depreciation of tubers) and quantitative (reduction of production). Under favorable conditions, in case of sensitive potato cultivars, the financial losses can be important, for consumption (production unmarketable) and for seed potatoes (possibly of downgrading) (Bădărău *et al.* 2012).

The main objective of this work was to evaluate the amount of total polyphenols content of ten Romanian potato cultivars with different resistance to potato virus S grown in Brasov over two years with different climatic conditions.

MATERIALS AND METHODS

The experiment was conducted during 2023–2024 at the National Institute of Research and Development for Potato and Sugar Beet, Braşov, Romania, using ten Romanian potato cultivars with different resistance to Potato Virus S (PVS): ‘Darilena’, ‘Foresta’, ‘Christian’, ‘Cezarina’, ‘Sevastia’, ‘Marvis’, ‘Castrum’, ‘Brasovia’, ‘Cosiana’ (new Romanian cultivars) and ‘Albastru Violet de Galanesti’ (‘Blue Purple of Galanesti’)(with strong pigmentation in the flesh).

Seed tubers were planted in May in three biological replicates under uniform fertilization and crop management practices. Mature tubers were harvested 148–160 days after planting, and medium-sized, healthy tubers were selected, washed and stored at 4°C until analysis.

Phenolic compounds were extracted using 80% (v/v) ethanol according to Valcarcel *et al.* (2015) with minor adjustments, and total phenolic content (TPC) was determined using the Folin–Ciocâlţeu method (Singleton *et al.*, 1999), with absorbance recorded at 725 nm. Results were expressed as mg gallic acid equivalents (GAE) per gram dry weight (DW).

The PVS infection status of the cultivars was verified using ELISA according to Clark and Adams (1977). Statistical analyses were performed using ANOVA followed by Duncan’s multiple range test, with significance set at $p < 0.05$.

RESULTS AND DISCUSSIONS

The climatic conditions (mean temperature and rainfall) in the growing season of the experimental years 2023 and 2024 are presented in Table 1. As can be seen in this table, compared with 2023, the year 2024 was slightly warmer, especially during June–August (mean/sum 17.7°C/106.2°C in the vegetation months of the year 2024, mean/sum 16.2 °C/ 97°C in the same period of the year 2023, respectively).

Also, the level of rainfall in May and June was lower in 2024 (42.7 mm in May, 54.5 mm in June, compared with 77.5 mm, 111.1 mm in the same months of the year 2023). The temperature and precipitation regime for the year 2024 indicated a year with poor precipitations distributions in May and June, compared with 2023.

During these months the potato plants are susceptible to the pathogens attack (Hu *et al.* 2012). At the same time, in this period, the accumulation of biological compounds in the plants is important for their metabolic activities (Hu *et al.* 2012).

Results showed that cultivar ‘Blue Purple of Galanesti’ recorded the highest mean TPC in both skin and flesh in both experimental years, while ‘Darilena’ presented the lowest values (Table 2). The TPC ranged from 0.62 to 3.39 mg GAE g⁻¹ DW in the flesh and from 2.76 to 10.43 mg GAE g⁻¹ DW in the skin, respectively, statistically significant difference among the cultivars as revealed by ANOVA followed by Duncan’s multiple range test ($p < 0.05$) (Table 2). Overall, skin samples contained on average 4.51-fold higher TPC than flesh samples in

2023, and 4.05-fold higher in 2024 (Table 2). Differences between cultivars were also influenced by tuber color, with intensely pigmented cultivars showing higher TPC values.

Table 1. Climatic conditions in the experimental years

Month	Year	Mean temperature (° C)	Rainfall (mm)
April	2023	7.3	83.1
	2024	12.2	43.5
May	2023	13.8	77.5
	2024	13.6	42.7
June	2023	17.6	111.1
	2024	21.1	54.5
July	2023	20.5	59.1
	2024	22.2	79.4
August	2023	20.9	77.5
	2024	21.2	65.0
September	2023	16.9	11.0
	2024	15.9	48.7
Average / Sum	2023	16.2/ 97.0	69.9/ 419.3
	2024	17.7/ 106.2	55.6/ 333.8

Table 2. Total polyphenol content (mg GAE/g DW) of potato samples (Brasov, 2023-2024)

Cultivars /resistance to PVS	Flesh / Skin colour	Year 2023		Year 2024	
		Flesh	Skin	Flesh	Skin
Darilena/r	W / LY	0.62±0.152 (g)*	2.88±0.121(h)	0.84±0.121(f)	2.76±0.168(i)
Foresta/mr	DY / R	0.75±0.089(ef)	7.80±0.057(b)	0.90±0.057(ef)	8.26±0.203(b)
AVG/ nr	B/B	3.17±0.309(a)	9.48±0.090(a)	3.39±0.090(a)	10.43±0.101(a)
Brasovia/r	WY / Y	0.90±0.068(d)	3.34±0.079±(f)	1.07±0.079(e)	3.94±0.139(gh)
Castrum/r	LY / Y	1.14±0.110(c)	4.03±0.185(e)	1.42±0.185(c)	4.47±0.208(f)
Azaria/mr	Y / R	1.33±0.178(c)	6.57±0.090(cd)	1.53±0.090(c)	6.88±0.239(d)
Cosiana/mr	WY / R	0.77±0.139(ef)	7.14±0.121(bc)	1.07±0.121(e)	7.61±0.262(c)
Marvis/r	WY / Y	0.73±0.101(ef)	4.45±0.125(de)	0.91±0.125(ef)	4.63±0.271(f)
Cezarina/r	Y / Y	0.87±0.080(de)	3.18±0.146(g)	1.22±0.146(d)	3.56±0.060(g)
Sevastia/r	DY / Y	1.67±0.255(b)	5.02±0.121(d)	1.96±0.121(b)	5.70±0.297(e)
Mean		1.191	5.370	1.439	5.827

**Means with different letters are significantly different at p<0.05 in each column. Values reported are the mean of three filed replicates. Abbreviations: GAE Gallic Acid Equivalents; DW dry weight; AVG Albastru Violet de Galanesti (Blue Purple of Galanesti). Abbreviations for the type of PVS resistance of the cultivars: r resistant; mr medium resistant; nr no resistant. Abbreviations for the colours:W white; WY white yellow; C cream; LY light yellow; Y yellow; DY dark yellow; R red; B blue.*

The results obtained are comparable to those previously in the literature, where TPC ranged between 1.56 to 12.9 and 0.54 to 3.59 mg GAE g-1 DW for skin and flesh respectively (Valcarcel *et al.*, 2015), 1.4 to 2.4 mg GAE g-1 DW for flesh (Cornacchia *et al.*, 2011),0.92 to

12.37mg GAE g⁻¹ DW for whole tubers (Andre et al., 2006; Xu *et al.*, 2009) and 1.00 to 4.3 mg GAE g⁻¹ DW for skin (Kahkonen *et al.*, 1999; Makris *et al.*, 2007). Although some other vegetables have higher TPC than potatoes, in many countries the potatoes are consumed in higher quantities making them an important contributor to daily dietary intake of phenolic compounds. A study in USA reported that potatoes the third major source of phenolics in the diet, after oranges and apples, with an average consumption of 171 g day⁻¹ Chun *et al.*, 2005). Results concerning the influence of tuber pigmentation on TPC are presented in Figure 1.

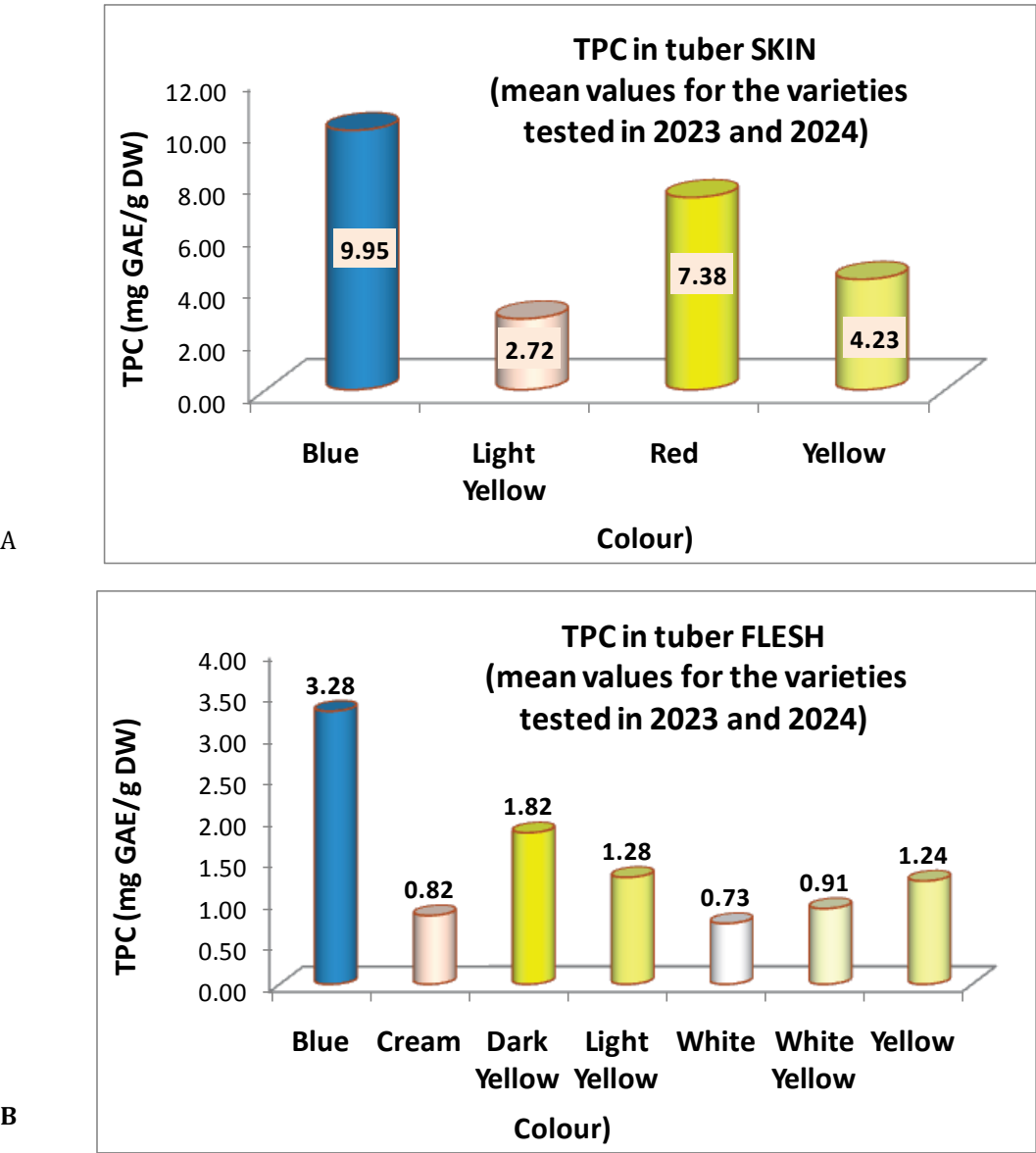


Figure 1. Mean total phenolic content (TPC) in relation to tuber skin colour (A) and flesh colour (B), based on mean values of samples collected in 2023 and 2024.

**Cultivars included resistant, medium-resistant and non-resistant variants to Potato Virus S (PVS). Abbreviations: TPC = total phenolic content; DW = dry weight.*

The tubers with blue skin had the highest TPC values. These values were significantly different compared to all other colors except red-skinned cultivars. Similarly, cultivars with blue flesh recorded the highest TPC values, significantly higher than those of all other flesh

color groups. The increased TPC values in blue and red tissues can be attributed to the presence of anthocyanins (Andre *et al.*, 2008), pigments known for their strong antioxidant potential. Previous studies also reported strong associations between phenolic composition and antioxidant activity in coloured potato cultivars (Andre *et al.*, 2006; Hu *et al.*, 2012; Reyes *et al.*, 2005; Valcarcel *et al.*, 2015), highlighting phenolic and flavonoid compounds as major contributors to antioxidant capacity.

Various reserchers have reported variation in total phenolic content in 297 potato cultivars ranging from 0.2 to 580 mg/FW, wich provides breeders a huge opportunity to incorporate this beneficial treit into popularly consumed cultivars (Pryia *et al.*, 2023). In the same time, enhancing the concentration of chlorogenic acid (the major phenolic compound in potatoes) becomes important in lowering the glicemic index value of potatoes (Salma *et al.*, 2020) wich is a major concern in their consumption.

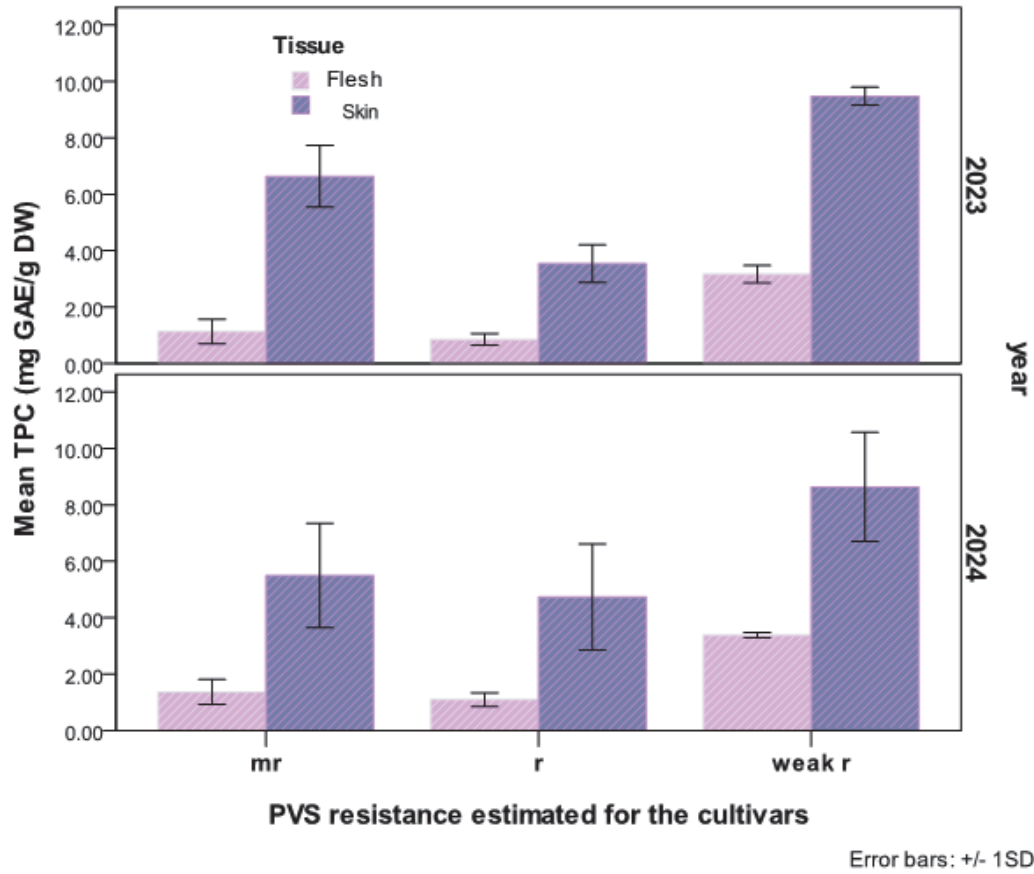


Figure 2. Mean total polyphenols content (TPC) according to the PVS resistance estimated for the cultivars tested. Abbreviations: PVS = Potato Virus S; mr = medium-resistant; r = resistant; weak r = non-resistant.

The nutritional relevance of potatoes could be further enhanced if cultivars with naturally higher. TPC level become more widely adopted by consumers. However, the cv. ‘Blue Purple of Galanesti’-which reported the higher TPC value in both skin and flesh in the present study, is less preferred due to its small, elongated tubers with deep eye. Future breeding programs may address these morphological limitations to combine improved nutritional potential with desirable consumer traits.

Tubers grown in Brasov in 2024 showed higher average TPC than those grown in 2023. Similar to previous reports, potato skin proved to be a rich source of polyphenol, as nearly

50% of phenolic these compounds are located in the peel and adjacent tissues (Albishi *et al.*, 2013; Al-Weshahy *et al.*, 2009).

The mean TPC was generally higher in tubers collected in 2024 compared to 2023. Based on climatic conditions recorded during the growing seasons (Table 1), 2024 was slightly warmer, particularly in June–August, which may have influenced phenolic accumulation, as early tuber development stages are considered critical for biosynthesis. In the present study, deeply pigmented cultivars displayed high TPC values; however, most of these were classified as medium-resistant to PVS (Foresta, Azaria, Cosiana) or non-resistant (AVG), as shown in Table 2 and Figure 2.

Significant interactions between year and cultivar and tissue were also found (Table 3).

Table 3. Effects of the tested factors and their interactions on TPC

Variables /Interactions between variables	ANOVA p values
Year	< 0.01
Cultivar	0.169
Tissue	0.205
Replicate (year)	0.269
<i>Year x cultivar</i>	< 0.01
Year x tissue	0.504
<i>Cultivar x tissue</i>	< 0.01

This suggests that evaluation of TPC across years may be more important than evaluation across PVS resistance of the cultivar, which should be confirmed by further field trials considering a wider cultivar of cultivars and over more than 2 years. A cultivar of factors can influence variation of TPC between the structure to climatic conditions or pressure from pests or pathogens.

CONCLUSIONS

Ten Romanian potato cultivars with different resistance to potato virus S, cultivars with different color of the skin and flesh tissue (grown in Brasov two years) were analyzed for estimation their total polyphenols content. The results obtained were in the range of values reported in the literature.

Significant differences between the different cultivars tested were observed for TPC. Higher contents of antioxidants (polyphenols) were found in intense colored fleshed tubers than in white counterparts, which should allow visual selection of cultivars with enhanced levels of these compounds.

So, the genotype “Blue Purple of Galanesti” with blue skin and flesh had higher values of total polyphenols content, but this cultivar is non-resistant to PVS.

Potato consumption has a great importance in population’s food and this study offers preliminary information to researchers and producers on the level of some phytochemicals such polyphenols, antioxidants with functional properties.

ACKNOWLEDGEMENTS

This work was supported by the project PN 23 19 01 02 (Research on the resistance and tolerance of some Romanian potato cultivars to the main viruses with high harmful potential in the conditions of climate change, project number 404/2023).

REFERENCES

1. Albishi, T., John, J., Al-Khalifa, A., Shahidi, F., (2013). Phenolic content and antioxidant activities of selected potato cultivars and their processing by-products. *Journal of Functional Food* 5: 590-600.
2. Al-Weshahy, A., Venket Rao, A., (2009). Isolation and characterisation of functional components from peel samples of six potatoes cultivars growing in Ontario. *Food Research International* 41: 1062-1066.
3. Andre, C.M., Ghislain, M., Bertin, P., Oufir, M., Herrera Mdel, R., Hoffmann, L., Hausman, J.F., Larondelle, Y., Evers, D., (2006). Andean potato cultivars (*Solanum tuberosum* L.), as a source of antioxidant and mineral micronutrients. *Journal of Agriculture and Food Biochemistry* 55: 366-378.
4. Andre, C.M., Schaffleitner, R., Guignard, C., Oufir, M., Aliaga, C.A., Nombert, G., Hoffmann, L., Hausman, J.F., Evers, D., Larondelle Y., (2008). Modification of the health promoting value of potato tubers field grown under drought stress: emphasis on dietary antioxidant and glycolalcaloid contents in five Andean cultivars (*Solanum tuberosum* L.). *Journal of Agriculture and Food Biochemistry* 57: 599-609.
5. Bădăraș, C. L., Chiru, N., Damșa, F., & Nistor, A. (2012). Effects of Saturejahortensis oil treatments and exogenous H₂O₂ on potato virus Y (PVY) infected *Solanum tuberosum* L. plants under drought conditions. *Annals of Oradea University, Biology Fascicle*, 19(2), 141–145.
6. Burgos, G., Salas, E., Amoros, W., Auqui M., Munoa, L., Kimura, M., Bonierbale, M., (2009). Total and individual profiles in *Solanum phureia* of cultivated potatoes. I. Concentration and relationships as determined by spectrophotometry and HPLC. *Journal of Food Composition and Analysis* 22(6): 503-508.
7. Chun, O.K., Kim, D.O., Smith, N., Schroeder, D., Han, J.T., Lee, C.Y., (2005). Daily consumption of phenolic and total antioxidant capacity from fruit and vegetables in the American diet. *Journal of Sciences and Food Agriculture* 85: 1715-1724.
8. Cojocaru, N. (1978). Viroze. In B. Plămădeală (Ed.), *Protecțiacartofului: boli, dăunători, buruieni* (pp. 60–84). Editura Ceres.
9. Cornacchia, R., Cabezas-Serrano, A.B., Amodi, M.L., Colleli, G., (2011). Suitability of 4 different potato cultivars (*Solanum tuberosum* L.) to be processed as fresh-cut products. Early cultivars. *American Journal for Potato Research* 88: 403-412.
10. Diganta, K. and Sastry, J., (2014). Comparison of polyphenol content and antioxidant capacity of colored potato tubers, pomegranate and blueberries. *Journal of Food Processing Technology* 5(8): 1-7.
11. Duthie G.G., Duthie S.J., Kyle J.A., (2000). Plant polyphenols in cancer and heart disease: implications as nutritional antioxidants. *Nutrition Research Reviews* 13:79-106.
12. Ezekiel R., Singh N., Sharma S., Kaur A., (2013). Beneficial phytochemicals in potato – a review. *Food Research International* 50: 487-496.
13. Hu, C., Tsao, R. Liu, R., Alan Sullivan, J., McDonald, M.R., (2012). Influence of cultivar and year on phytochemical and antioxidant activity of potato (*Solanum tuberosum* L.) in Ontario. *Canadian Journal Plant Sciences* 92: 485-493.
14. Kahkonen, M.P., Hopia, A.I., Vuorela, H.J., Rauha, J-P., Pihlaja, K., Kujala, T.S., Heinonen, M., (1999). Antioxidant activity of plant extracts containing phenolic compounds. *Journal of Agriculture and Food Chemistry* 47: 3954-3962.
15. Loebenstein, G. (2001). Potato virus S (PVS). In G. Loebenstein, P. H. Berger, A. A. Brunt, & R. H. Lawson (Eds.), *Virus and virus-like diseases of potatoes and production of seed-potatoes* (pp. 117–119). Springer. https://doi.org/10.1007/978-94-017-2922-0_14
16. Makris, D.P., Boskou G., Andrikopoulos, N.K., (2007). Polyphenolic content and in vitro antioxidant characteristics of wine industry and other food solid waste extracts. *Journal of Food Composition and Analysis* 20: 125-132.
17. Priya BNV, Saiprasad GVS, (2023). Potato- Powerhouse for many nutrients. *Potato Research* 66(3): 563-580
18. Reddivari, L., Hale, A.L., Miller, J.C., (2007). Genotype, location and year influence antioxidant activity, carotenoid content, phenolic content and composition in specialty potatoes. *Journal of Agriculture and Food Chemistry* 55(7): 8073-8079.
19. Reyes, L.F., Miller, J.C., Cisneos-Zevalhos, L., (2005). Antioxidant capacity anthocyanins and total phenolic in purple and red -fleshed potato (*Solanum tuberosum* L.) genotypes. *American Journal for Potato Research* 82: 271-277.

20. Salma, U, Shahjalal, H Md., Ahmmmed, T., Ansary, Md. M U, Khalil, Md. I, Karim, N, (2020). Effect of Boiling on the Nutritional Value, Phytochemical Contents, and Antioxidant Activity of Commonly Consumed Potato (*Solanum tuberosum* L.) Cultivars in Bangladesh. Journal of Food and Nutrition Research. 8(8), 382-391. DOI: 10.12691/jfnr-8-8-1
21. Singleton, V.L., Orthofer, R., Lamuela-Raventos, R.M., (1999). Analysis of total phenols and other oxidant substrates and antioxidants by means of Folin-Ciocalteu reagent. Methods in Enzymology 299: 152-178.
22. Wang, H., Nair, M.G., Strasburg, G.M., Chang, Y.C., Booren, A.M., (1999). Antioxidant and antiinflammatory activities of anthocyanins and their aglicon, cyanidin, from tart cherries. Journal of Natural Products 62: 294-296.
23. Valcarcel, J., Reilly, K., Gaffney, M., O'Brien, N.M., (2015). Antioxidant activity, total phenolic and total flavonoid content in sixty cultivars of potato (*Solanum tuberosum* L.) grown in Ireland. Potato Research 58: 221-141.
24. Xu, X., Li W., Lu, Z., Beta, T., Hydamaka, A., (2009). Phenolic content, composition, antioxidant activity and their changes during domestic cooking of potatoes. Journal of Agricultural and Food Chemistry 57: 10231-10238.



Fruit growing

Phenological dynamics of some fruit species from the Northeastern area of Romania in the context of climate change

I. Mineață, I. Perju*, S. Sîrbu, I.E. Golache, I.V. Ungureanu and E. Iurea

Research Station for Fruit Growing, Iasi, Romania

*Corresponding author email: ionel_perju@yahoo.com

ABSTRACT

Climate change has had crucial impacts on phenological stages, such as the advancement or decline of the vegetation cycle of stone fruit trees. Vegetation phenology is an important biological indicator in understanding and predicting the behavior of ecosystems and how they respond to environmental factors. This work aims to investigate the influence of interannual climate variation (years 2023 and 2024) on the phenology of some stone fruit tree species belonging to the genus *Prunus*. The study was carried out within the fruit experimental plots of the Research Station for Fruit Growing (RSFG) Iași, North-Eastern Romania, analyzing the onset of four phenological stages from bud swelling to the end of flowering at five cultivars belonging to the species: *Prunus avium* L., *Prunus cerasus* L., *Prunus domestica* L. and *Prunus armeniaca* L. The research conducted on the climatic regime in the studied years, in relation to multiannual values (1972-2022), highlighted the variability of phenological behaviors of stone fruit species both in terms of the number of days (according to the Julian date methodology) for the start of vegetation, and in the duration of phenological stages. Thus, of the two years of study, the year 2024 was noted for an extension of the start of vegetation in cherry by 29 days, in plum by 21 days, in apricot 12 days and in sour cherry by only one day compared to 2023. The obtained results highlight the variability of the phenological behaviors of the fruit tree species but also of the cultivars and the length of the vegetation period was directly influenced by annual climatic conditions. Changes in the duration of the vegetative phases were highlighted, which can generate useful information to predict the risk of late frosts in particular, but also countermeasures to combat the effects of climate change.

Keywords: *Prunus* sp., phenology, climate change, stone fruit trees, BBCH.

INTRODUCTION

Climate change has become an observed reality, already causing an increase in average temperatures with particular intensity in certain areas, such as temperate-continental and mediterranean (Milošević and Milošević, 2023). These changes in meteorological factors lead to difficulties in covering the cold necessary to break endodormancy, the occurrence of late frost events or abnormally early maximum temperatures, with profound consequences in particular on the phenology of *Prunus* species, such as stone fruits (Salama *et al.*, 2021; Egea *et al.*, 2022).

The substantial impacts of global warming on temperate fruit crops are manifested on phenological traits, represented by the developmental stages of the species each year (Menzel *et al.*, 2006; Vanalli *et al.*, 2021). Data recorded in numerous studies (Yu *et al.*, 2010; Luedeling

et al., 2013; Egea *et al.*, 2022; Stoenescu *et al.*, 2024) highlight that the most frequent consequence of sudden temperature increases is early bud burst, thus influencing the length of the species vegetation season and advance in flowering, with a high risk of late spring frosts, if cold temperatures return. Another imbalance caused by climate change is the phenophase gap due to much too low temperatures associated with uneven precipitation by prolonging some stages of growth, such as bud breaking, flowering, fruit ripening (Vitasse *et al.*, 2018; Fadón *et al.*, 2020). Regarding stone fruit trees, they have strong environmental requirements, mainly related to temperature, being conditioned to accumulate a specific amount of cooling during winter to pass through vegetative dormancy and then require warm temperatures to finally bloom in the spring (Fadón *et al.*, 2020; López-Morales *et al.*, 2021).

Understanding how species and cultivars interact and drive phenological processes in space is crucial, given the current scenario of global eco-pedoclimatic habitat change (Rumeu *et al.*, 2020; Stoenescu *et al.*, 2024). Thus, this work aims to provide additional information on the influence of interannual climate variations on the phenology of some stone fruit tree species. Knowing the temperature requirements of a cultivar can be useful to anticipate future performances and to design new strategies that take into account the predicted global warming (Luedeling *et al.*, 2009; Campoy *et al.*, 2011).

MATERIALS AND METHODS

The study was conducted in 2023 and 2024 in the experimental field of Research Station for Fruit Growing Iasi, in Northeastern Romania (Northeastern Europe, 47°20' N; 27°60' E), using as biological material five cultivars of four species of stone fruit trees belonging to the genus *Prunus*: *Prunus avium* L., *Prunus cerasus* L., *Prunus domestica* L. and *Prunus armeniaca* L. (Table 1).

Table 1. The biological material used (species and cultivars)

Species	Cultivar	Species	Cultivar
<i>Prunus avium</i> L.	'Bucium'	<i>Prunus domestica</i> L.	'Centenar'
	'Alexus'		'Carpatin'
	'Amaris'		'Prezident'
	'Cociuvaş'		'Silvia'
	'Maria'		'Minerva'
<i>Prunus cerasus</i> L.	'De Botoşani'	<i>Prunus armeniaca</i> L.	'Tudor'
	'Cygany Meggy'		'Goldrich'
	'Pandy 50'		'Fortuna'
	'Erdy Botermo'		'Traian'
	'Montmorency'		'Augustin'

The region is characterized by a temperate-continental climate, located on a microrelief with a gently sloping plateau and predominantly cambic chernozem soils with good natural drainage, with a humus content of 3.7–4.3%.

Regarding climatic data, the characterization of the average monthly temperature and precipitation of the study years was carried out using the *Field Climate* system within the RSFG Iaşi unit, and these values were compared with multiannual averages recorded over 50 years period (1972–2022) as a reference, using a database stored in MS Office Excel and the National Meteorological Administration Catalog.

The monitoring of phenological stages was carried out through periodic visits to the experimental field and identification according to the Biologische Bundesanstalt, Bundessortenamt, and Chemical Industry (BBCH) scale (Meier, 2001): 51 BBCH (bud swelling), 53 BBCH (bud burst), 61 BBCH (beginning of flowering: about 10% of flowers open) and 69 BBCH (end of flowering). The calendar dates corresponding to each

phenophase was recorded and converted into Julian dates for a quantitative analysis of the number of days between January 1 and the evaluated phenophase (Luedeling *et al.*, 2013; Cosmulescu *et al.*, 2022).

The data obtained from the observations, as well as the relationship between the analyzed and monitored indices, were statistically processed using descriptive statistics such as: Pearson correlation coefficient (r^2) at $p \leq 0.05$, standard deviation (STDEV) and percentage coefficient of variation (CoV%).

RESULTS AND DISCUSSIONS

In order to characterize the phenological characteristics of stone fruit trees, a climatic characterization of the study area was necessary based on monitoring the average monthly temperature (Figure 1) and the amount of precipitation (Figure 2) in the studied years. The meteorological data were compared with the daily values of meteorological parameters from the last 50 years, used as multiannual reference values. Thus, the evolution of average monthly temperatures fluctuated seasonally in 2023, ranging from +1.69°C in December to a maximum of +25.5°C in August.

The average annual temperature in 2023 was +12.4°C. In 2024, the average monthly temperature had a minimum in January of +0.2°C and a maximum of +24.2°C in July. The maximum extreme temperature value was reached in August 2024, at +41.6°C. The deviation of the two-year average from the multiannual average in each month was positive, ranging between +0.1°C in May and +4.8°C in January. The deviation from the multiannual average is +2.9°C (Figure 1).

The changes highlighted in the climatic regime in the NE area of Romania fall within the context of global warming, but with particularities specific to the geographical region to which it belongs (Cheval *et al.*, 2022).

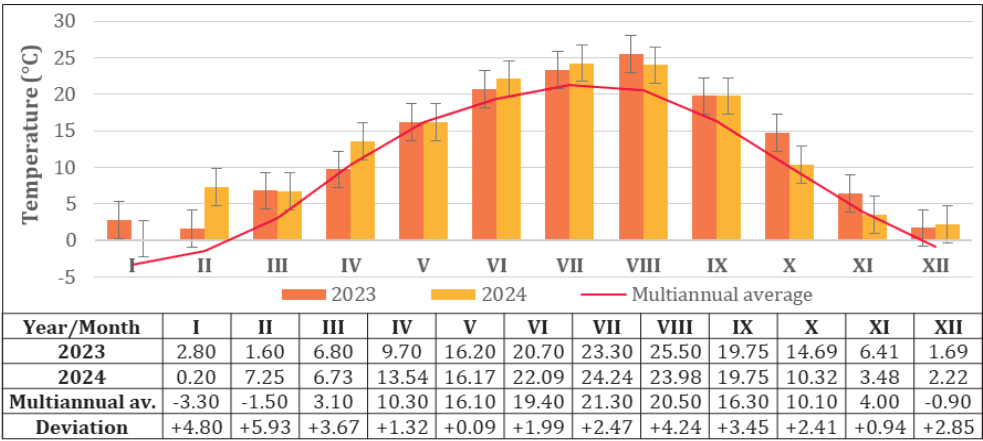


Figure 1. Average monthly temperature during the study years and multiannual reference average

Regarding the precipitation regime (Figure 2), the study years can be characterized as being highly uneven, with periods of excess precipitation followed by very dry intervals. While in 2023 the total precipitation amounted to only 284.5 mm, in 2024, a total of 611.2 mm was recorded. The deviation from the multiannual value ranged between -42.4 mm (August) and + 45.9 mm (July). Tracking phenological milestones is a simple and easy-to-apply method for monitoring flora and provides precise and useful data on how climate affects the development of each organism or species–within the ecosystem. The first phenological stages of the spring season are considered the most prone and affected by temperature

fluctuations, as well as by precipitation (Savchovska and Nesheva, 2021; Stoenescu *et al.*, 2024), with negative effects on the growth and development of fruit species. The progress through the vegetative phases of the monitored species is influenced by both climate and the biological characteristics of each cultivar.

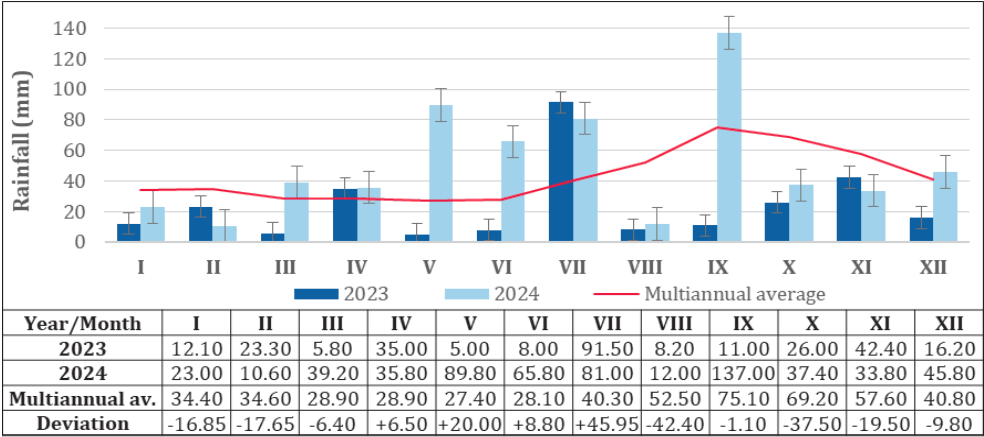


Figure 2. Total monthly precipitation amount in the study years and the multiannual reference average

Thus, following the phenological data obtained and graphically represented in Table 2, the influence of annual climatic conditions on the four stone fruit species was highlighted. The year 2023 was noted as the earliest in terms of bud swelling (51BBCH), with a difference of 29 days in *Prunus avium* L., 21 days in *Prunus domestica* L., 12 days in *Prunus armeniaca* L., with no differences in *Prunus cerasus* L.

Table 2. The phenogram of stone fruit tree species from bud swelling - end of flowering (51-69 BBCH)

Species	Month	February			March						April										May							
	Year/ Day	1	18	27	1	8	9	11	18	20	27	29	2	6	7	9	11	13	14	17	18	21	22	23	26	30	1	4
<i>Prunus avium</i> L.	2023																											
	2024																											
<i>Prunus cerasus</i> L.	2023																											
	2024																											
<i>Prunus domestica</i> L.	2023																											
	2024																											
<i>Prunus armeniaca</i> L.	2023																											
	2024																											
				51BBCH									53BBCH						61-69BBCH									

Regarding the duration of the vegetation period between the start of vegetation through bud swelling (51 BBCH) and the end of flowering (69BBCH), in 2023, although earlier, the periods between phenological stages were much shorter compared to 2024, confirming that the absence of precipitation (in 2023 being a deficit compared to the multiannual average of over 200 mm) and high daytime and nighttime temperatures lead to the occurrence of pedological, atmospheric and physiological drought, thus shortening the phenophases (Jităreanu *et al.*, 2009).To determine the changes that occurred during the phenophases, the number of days from January 1 to the stages of bud swelling, budding, beginning and end of flowering was used. Table 3 shows the statistical processing of the data obtained during the two years of

study. For the 51 BBCH phenophase, based on Julian data, the order in which this phenophase occurred in the identified species was as follows: *Prunus avium* L. (82.5 days), *Prunus domestica* L. (78.5 days), *Prunus cerasus* L. (70.5 days) and *Prunus armeniaca* L. (55.0 days). Stone fruit trees, like other woody species in temperate zones, need to accumulate a cultivar-specific amount of chilling during winter and then experience warm temperatures to overcome vegetative dormancy and trigger vegetative growth (Fadón *et al.*, 2020).

Table 3. Descriptive statistics of the number of days (Julian date) required for the development of the main phenological stages.

Species	Descriptive Statistics	51BBCH	53BBCH	61BBCH	69BBCH
<i>Prunus avium</i> L.	Average	82.50	97.00	104.50	112.50
	Variation range	68-97	92-102	97-112	104-121
	STDEV	20.51	7.07	10.61	12.02
	CoV%	24.86	7.29	10.15	10.69
<i>Prunus cerasus</i> L.	Average	70.50	93.00	109.00	116.50
	Variation range	70-71	88-98	101-117	108-125
	STDEV	0.71	7.07	11.31	12.02
	CoV%	1.00	7.60	10.38	10.32
<i>Prunus domestica</i> L.	Average	78.50	89.50	104.50	107.50
	Variation range	68-89	79-100	95-114	93-122
	STDEV	14.85	14.85	13.44	20.51
	CoV%	18.92	16.59	12.86	19.08
<i>Prunus armeniaca</i> L.	Average	55.00	69.00	88.50	97.00
	Variation range	49-61	67-71	77-100	86-108
	STDEV	8.49	2.83	16.26	15.56
	CoV%	15.43	4.10	18.38	16.04

The correlation matrix between the years of study for the number of days from bud swelling to the end of flowering (51-69 BBCH), (Table 4) revealed a non-significant correlation within the same species between the two years, except for the species *Prunus domestica* L., where a significant positive correlation coefficient was obtained ($r= 0.4486$). A positive correlation indicates that when one variable increases in value, the other variable also increases, following a linear pattern (Ratner, 2009).

Table 4. Correlation matrix between study years for the number of days from bud swelling - end of flowering (51-69 BBCH)

2023 2024	<i>Prunus avium</i> L.	<i>Prunus cerasus</i> L.	<i>Prunus domestica</i> L.	<i>Prunus armeniaca</i> L.
<i>Prunus avium</i> L.	0.1398^{ns}	-0.3028 ^o	-0.0158 ^{ns}	-0.1137 ^{ns}
<i>Prunus cerasus</i> L.	-0.926 ^{ooo}	0.2001^{ns}	0.1893 ^{ns}	0.7120 ^{**}
<i>Prunus domestica</i> L.	0.1414 ^{ns}	0.1893 ^{ns}	0.4486[*]	-0.0943 ^{ns}
<i>Prunus armeniaca</i> L.	-0.807 ^{oo}	0.7120 ^{oo}	0.8087 ^{**}	0.1346^{ns}

CONCLUSIONS

In conclusion, the observations and obtained results confirm the existence of variations in spring phenological stages from one year to another in the studied stone fruit species which are–strongly influenced by variations in climatic factors, including the dormancy period. These data contribute to a deeper understanding of the impact and consequences of current climate change, providing valuable information on the challenges facing the fruit-growing sector.

ACKNOWLEDGEMENTS

This research work was carried out with the support of the Thematic Plan on the Implementation of the 'A.S.A.S. Strategy on Research-Development-Innovation in Fruit Growing' for the period 2021–2027, research topic: ASAS 1/1.1.3. 'Maintaining and exploiting the biodiversity of fruit cultivation in thermophile stone fruit species.

REFERENCES

1. Campoy, J.A., Ruiz, D. and Egea, J. (2011). Dormancy in temperate fruit trees in a global warming context: a review. *Scientia Horticulturae*, 130(2): 357-372.
2. Cheval, S., Bulai, A., Croitoru, A.E., Dorondel, Ș., Micu, D., Mihăilă, D. and Țișcovschi, A. (2022). Climate change perception in Romania. *Theoretical and applied climatology*, 149(1): 253-272.
3. Cosmulescu, S., Ștefănescu, D. and Stoenescu, A.M. (2022). Variability of phenological behaviours of wild fruit tree species based on discriminant analysis. *Plants*, 11(1): 45.
4. Egea, J.A., Caro, M., García-Brunton, J., Gambín, J., Egea, J. and Ruiz, D. (2022). Agroclimatic metrics for the main stone fruit producing areas in Spain in current and future climate change scenarios: Implications from an adaptive point of view. *Frontiers in plant science*, 13: 842628.
5. Fadón, A.E., Herrera, L.S., Guerrero, C.B.I., Guerra, V.M., and Rodrigo, García, F.J. (2020). Chilling and Heat Requirements of Temperate Stone Fruit Trees (*Prunus* sp.). *Agronomy*, 10: 409.
6. Jităreanu, C.D., Toma, L.D., Slabu, C., Marta, A.E. and Radu, M. (2009). Investigations on the development of some physiological processes during apple tree growth and fructification. *Lucrări Științifice, seria Agronomie*, 52: 193-201.
7. López-Morales, J.A., Martínez, J.A., Caro, M., Erena, M. and Skarmeta, A.F. (2021). Climate-aware and IoT-enabled selection of the most suitable stone fruit tree variety. *Sensors*, 21(11): 3867.
8. Luedeling E., Kunz A. and Blanke M.M. (2013). Identification of chilling and heat requirements of cherry trees—a statistical approach. *International Journal of Biometeorology*, 57: 679-689.
9. Luedeling, E., Kunz A. and Blanke, M.M. (2013). Identification of chilling and heat requirements of cherry trees—a statistical approach. *International Journal of Biometeorology*, 57: 679-689.
10. Luedeling, E., Zhang, M. and Girvetz, E.H. (2009). Climatic changes lead to declining winter chill for fruit and nut trees in California during 1950–2009. *PloS one*, 4(7): e6166.
11. Meier, U. (2001) BBCH-Monograph: Growth Stages of Mono-and Dicotyledonous Plants Technical Report, 2nd ed. Federal Biological Research Centre for Agriculture and Forestry: Berlin, Germany. Available online: <https://www.julius-kuehn.de/media/Veroeffentlichungen/bbch%20epaper%20en/page.pdf>.
12. Menzel, A., Sparks, T.H., Estrella, N., Koch, E., Aasa, A., Ahas, R. and Züst, A.N. (2006). European phenological response to climate change matches the warming pattern. *Global change biology*, 12(10): 1969-1976.
13. Milošević, N. and Milošević, T. (2023). Impact of climate change on plum (*Prunus domestica* L.). In *Cultivation for Climate Change Resilience*, Vol. 2. CRC Press, Florida, pp. 257-278.
14. Ratner B. (2009). The correlation coefficient: Its values range between +1/-1, or do they?. *Journal of targeting, measurement and analysis for marketing* 17: 139-142.
15. Rumeu B., Donoso I., Rodríguez-Pérez J. and García D. (2020). Frugivore species maintain their structural role in the trophic and spatial networks of seed dispersal interactions. *Journal of Animal Ecology*, 89(9): 2168-2180.
16. Salama A.M., Ezzat A., El-Ramady H., Alam-Eldein S.M., Okba S.K., Elmenofy H.M. and Holb I.J. (2021). Temperate fruit trees under climate change: Challenges for dormancy and chilling requirements in warm winter regions. *Horticulturae*, 7(4): 86.
17. Savchovska, S. and Nesheva, M. (2021). Fruit quality characteristics of the Bulgarian sweet cherry cultivar 'Rozalina'. *Romania Journal of Horticulture*, 2: 93-98.
18. Stoenescu, A.M., Cosmulescu, S.N. and Gheorghiu, N. (2024). The Influence Of Interannual Climate Variation On The Phenology Of Some Wild Fruit Species And Their Relation With Avifauna. *Scientific Papers. Series B. Horticulture*, 68(2).
19. Vanalli, C., Casagrandi, R., Gatto, M. and Bevacqua, D. (2021). Shifts in the thermal niche of fruit trees under climate change: The case of peach cultivation in France. *Agricultural and Forest Meteorology*, 300:108327.
20. Vitasse, Y., Schneider, L., Rixen C., Christen, D. and Rebetez, M. (2018). Increase in the risk of exposure of forest and fruit trees to spring frosts at higher elevations in Switzerland over the last four decades. *Agricultural and Forest Meteorology*, 248: 60-69.
21. Yu, H., Luedeling, E., and Xu, J. (2010). Winter and spring warming result in delayed spring phenology on the Tibetan Plateau. *Proceedings of the National Academy of Sciences*, 107(51): 22151-22156.

A new Romanian *Prunus domestica* L. cultivar exhibiting high tolerance to natural infections to plum pox virus

L.A. Zagrai and I. Zagrai*

Fruit Research and Development Station Bistrita, Romania

*Corresponding author email: izagrai@yahoo.com

ABSTRACT

This study evaluates the long-term behaviour of plum pox virus (PPV) infections within a genetically diverse plum orchard established in 1998 at the Fruit Research & Development Station Bistrița, Romania. The experimental plot comprises six hybrids, including the recently homologated 'BN 4/1' hybrid as 'Zandra' cultivar, and ten plum cultivars, with a total of 534 trees. Over 20 years (2003–2024), the temporal progression of PPV was monitored through visual inspections and serological tests (DAS-ELISA), revealing a consistent increase in virus incidence from 11.4% to 58.4%. Significant differences in PPV susceptibility were found among the genotypes. Highly susceptible genotypes ('Diana', 'Geta', 'HAS 2G', 'Tuleu timpuriu') exhibited infection rates exceeding 83%, while susceptible genotypes showed incidence levels over 60%. Tolerant cultivars such as 'Stanley' and 'Čačanska Najbolja', displayed moderate infection rates ranging from 40% to 60%. Remarkably, the hybrid 'BN 4/1' ('Zandra' cultivar) displayed the lowest PPV incidence at just 20%. Infected 'Zandra' trees exhibited localized symptoms, confined to one or two shoots per tree, with no evidence of systemic infection throughout the canopy. Detailed serological analyses confirmed the restricted viral movement in symptomatic 'Zandra' trees. Moreover, 80% of the trees remained uninfected despite long-term exposure to high inoculum pressure from surrounding infected trees, indicating a high level of tolerance to natural PPV infection. In addition to its viral tolerance, 'Zandra' exhibits superior organoleptic qualities, highlighting its potential as a valuable cultivar for sustainable plum production systems.

Keywords: plum, sharka, high tolerance, serological diagnosis, visual monitoring

INTRODUCTION

European plum (*Prunus domestica* L.) represents the most important fruit species in Romania, ranking second globally in plum production, after China, and holds the top position in Europe (FAO, 2025). Despite its economic importance, viral infections severely impact both productivity and fruit quality. Sharka disease, caused by the *Potyvirus plumipoxi* (plum pox virus, PPV), is one of the most damaging diseases affecting stone fruit trees worldwide, and has had a significant economic impact for over a century (Barba *et al.*, 2011; Garcia *et al.*, 2024). First identified in Bulgaria at the beginning of the 20th century (Atanasoff, 1932), the virus has since spread across Europe, Asia, and parts of North and South America. It causes severe damage, particularly in susceptible cultivars (Minoiu, 1997), and is ranked among the top ten plant viruses globally due to its broad distribution and serious effects (Scholthof *et al.*, 2011). Cambra *et al.* (2024) estimated the economic losses caused by Sharka at €2.4 billion over the last 28 years (1995–2023); when considering the entire period since its emergence, total losses may exceed €13 billion. PPV drastically reduces the commercial value of stone fruit crops by causing fruit deformation, necrosis,

and premature drop, often rendering the fruit unsuitable for fresh consumption or industrial processing.

Cultivar resistance to viral diseases, particularly to PPV, is recognized as the most efficient strategy for virus control, especially in endemic areas (Ravelonandro *et al.*, 2011; Scorza *et al.*, 2013). Cultivars exhibiting high levels of tolerance can also substantially contribute to mitigating the economic losses associated with viral diseases. Therefore, breeding and promoting the use of such cultivars represent a key strategy for viral disease control, preserving fruit quality, and ensuring stable production.

Conventional breeding efforts aimed at conferring PPV resistance in *Prunus domestica* have achieved limited progress over the past century, primarily constrained by the scarcity of resistance genes within the cultivated germplasm (Zagrai *et al.*, 2021). Nevertheless, breeding for hypersensitivity has led to cultivars like 'Jojo' (Hartmann and Neumüller, 2006, 2013), which respond to PPV infection with a hypersensitive reaction. While such genotypes appeared to have a field resistance, studies indicate this resistance may be strain- or isolate-dependent (Polák and Jarošová, 2011, 2012; Rodamilans *et al.*, 2023).

As an alternative or complement to conventional breeding, pathogen-derived resistance (PDR) led to the development of the RNAi-based transgenic plum clone C5, later named 'HoneySweet', carrying PPV-CP transgenes, which has demonstrated high and durable resistance to PPV (Scorza *et al.*, 1994, 2013; Ravelonandro *et al.*, 1997; Malinowski *et al.*, 2006; Zagrai *et al.*, 2011; Polak *et al.*, 2017). Unfortunately, 'HoneySweet' plum is not available for cultivation in the European Union (EU), where PPV remains a major threat to stone fruit production. Given these considerations, the breeding and use of highly tolerant cultivars continues to be a pertinent and valuable strategy for reducing the economic impact of PPV. Important progress has been made in developing highly tolerant plum cultivars, especially through targeted breeding programs in South-Eastern Europe. In Serbia, results from the breeding program have identified several plum hybrids exhibiting resistance or a high level of tolerance to specific PPV strains, as demonstrated through both artificial inoculation and natural infections (Milošević *et al.*, 2019; Glišić *et al.*, 2021).

Butac *et al.* (2021) evaluated the performance of several Serbian plum cultivars under Romanian ecological conditions, including their response to PPV. The results indicate good adaptability and enhanced tolerance to PPV, highlighting the potential of these cultivars for successful cultivation in regions affected by the virus. A more recent study (Butac *et al.*, 2024) has demonstrated that certain Bulgarian plum cultivars exhibit very low susceptibility to PPV when evaluated under natural infection conditions. This low susceptibility suggests a form of tolerance that enables these cultivars to sustain both productivity and fruit quality in the presence of PPV infection.

In line with regional advancements, the plum breeding program at Bistrița aims not only to develop cultivars resistant to plum pox virus but also to identify and assess genotypes exhibiting a high level of tolerance to the virus. Accordingly, the present study was specifically designed to identify and assess such tolerant cultivars.

MATERIALS AND METHODS

Experimental orchard

An experimental orchard comprising six plum hybrids ('HAS 1 I', 'HAS 2 G', 'HAS 2/5', 'HAS 2/VI', 'HAS 3/4' and BN 4/1 – homologated as 'Zandra' in 2025) and ten plum cultivars ('Diana', 'Geta', 'Tuleu timpuriu', 'Centenar', 'Early Rivers', 'Matilda', 'Piteștean', 'Stanley', 'Čačanska Najbolja' and 'Anna Späth') with a total of 534 trees, was established in 1998 at Fruit Research & Development Station Bistrița (FRDS Bistrița). The cultivars were also planted in variable numbers, ranging from 5 to 188 trees, planted at a spacing of 5/3 meters. Each hybrid was represented by 12 to 60 trees, and interspaced among cultivar rows. The

experimental orchard is located near potential sources of PPV infection, including various *Prunus* species, such as *P. cerasifera*, *P. spinosa* and *P. domestica*, which are commonly found in the surrounding area and may serve as virus reservoirs.

Pest and disease management in an experimental orchard

The maintenance of the experimental orchard involves the application of 8–10 phytosanitary treatments per season to control diseases and pests. These treatments include targeted interventions against aphids, which are known vectors of the PPV, in order to reduce the risk of PPV spread within the orchard.

PPV spreading in the orchard

In addition to assessing the agronomic performance of the hybrids under the local climatic and soil conditions, the trees were also monitored to evaluate their susceptibility, tolerance or resistance to plum viruses, with a focus on natural infections with PPV. Beginning in 2003, systematic monitoring of PPV spread within the orchard was carried out every three years. This included visual inspections during the growing season (minimum twice per year) for characteristic symptoms on leaves and/or fruits, as well as serological testing conducted in 2003 and 2018. It is worth noting that a recent study demonstrated that accurate recognition of PPV-specific symptoms in infected plum trees can serve as a reliable tool for monitoring the presence and spread of the virus within the commercial orchards (Zagrai *et al.*, 2024).

PPV spreading within the canopy

To assess the internal spread of PPV within the tree canopy, selected hybrid trees exhibiting localized PPV infections were sampled and tested in various canopy zones. This approach was applied in the case of the 'BN 4/1' hybrid ('Zandra' cv.). The aim was to determine the extent of virus movement from infected shoots to adjacent branches. The spread of PPV was assessed on three out of twelve trees showing localized PPV symptoms. Both symptomatic and symptomless shoots (leaves) located below, above and beside the infected areas were collected for comparative serological testing. Additionally, symptomless leaves were also sampled from the opposite part of the canopy in order to evaluate the potential latent infections. Where applicable, samples from root suckers emerging near the trunk were also collected and tested serologically. Moreover, all sixty trees of 'BN 4/1' hybrid ('Zandra' cv.) were serologically tested in 2024.

Serological detection

A minimum of ten leaves per sample were collected for serological tests. Fresh leaf samples were homogenized in extraction buffer at a 1:20 (w/v) ratio using a HOMEX 6 homogenizer. Serological testing was conducted using the double antibody sandwich enzyme-linked immunosorbent assay (DAS-ELISA), following the method described by Clark and Adams (1977). A commercial kit from Bioreba (Switzerland), containing polyclonal antiserum specific to PPV, was used in accordance with the manufacturer's instructions. Samples were considered positive if absorbance values exceeded twice those of the negative control. Both positive and negative controls supplied with the kit were included in each assay to ensure reliability.

Statistical analysis

XLSTAT software (Addinsoft, New York, USA) was used to perform statistical interpretation. Analysis of variance (ANOVA) by Duncan's Multiple Range Test was conducted in order to verify the differences between cultivars/hybrids regarding the PPV incidence. The threshold

for statistical significance was set at $p < 0.0001$, indicating a very high level of confidence in the observed differences.

RESULTS AND DISCUSSIONS

Incidence and temporal spread of PPV in the experimental plum orchard

Visual assessments and serological testing conducted over a 20-year monitoring period revealed a progressive and continual temporal spread of PPV within the plum orchard. The overall incidence increased from 11.4% in 2003 to 58.4% (267 infected trees out of 457 total trees) in 2024 (Table 1). Over this timeframe, losses of plum trees due to various causes were recorded; of the 534 trees initially planted in 1998, a total of 457 remained in the orchard by 2024. The annual increase in PPV incidence varied between 3.5% and 9%, highlighting a gradual but consistent expansion of the infection across the orchard.

Table 1. Temporal spread of PPV within the experimental plum orchard during the period 2003-2024 (by visual monitoring and/or serological tests)

Year	PPV infected trees/total trees	Incidence of PPV (%)	The annual increase of PPV incidence (%)
2003*	58/511	11.4	-
2006	75/502	14.9	+3.5
2009	97/491	19.8	+4.9
2012	126/480	26.2	+6.4
2015	155/476	32.5	+6.3
2018*	191/469	40.7	+8.2
2021	229/461	49.7	+9.0
2024	267/457	58.4	+8.7

*Years in which all trees were tested serologically using DAS-ELISA; in the remaining years, PPV incidence was determined only based on visual monitoring.

The temporal spread of PPV within the orchard, assessed at three-year intervals over 20 years, revealed a nearly linear progression in virus incidence. A statistically significant linear relationship was observed between the increase in PPV incidence and passage of time (Figure 1), suggesting a steady and predictable pattern of virus dissemination under the given management conditions.

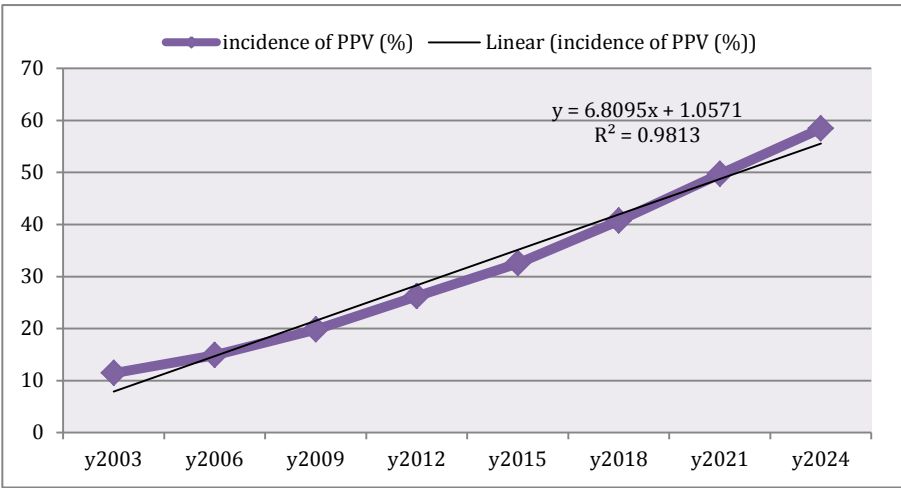


Figure 1. Temporal spread of PPV within the experimental plum orchard during 2003-2024

The continual rise in PPV infection rates reflects sustained and increasing pressure from infections on the remaining uninfected trees, highlighting the persistent potential for virus dissemination within the orchard.

Pattern of PPV symptoms within the canopy of infected plum trees

In 2024, all trees belonging to ten cultivars and six hybrids grown in the orchard were visually inspected to assess the extent of PPV infections within the canopy. Among the 267 PPV-infected trees, 244 (91.4%) exhibited generalized infections affecting the entire canopy, while only 23 trees (8.6%) showed localized PPV infections (Figure 2), typically confined mainly to a single branch or shoot. These results indicate a predominance of systemic virus spread within infected trees. However, the limited infection to particular branches observed in a small subset of trees raised attention due to its potential implications for early-stage infection or partial resistance. Such localized symptoms may reflect initial infection foci before systemic spread or could be associated with host genotype-related responses that restrict viral movement.

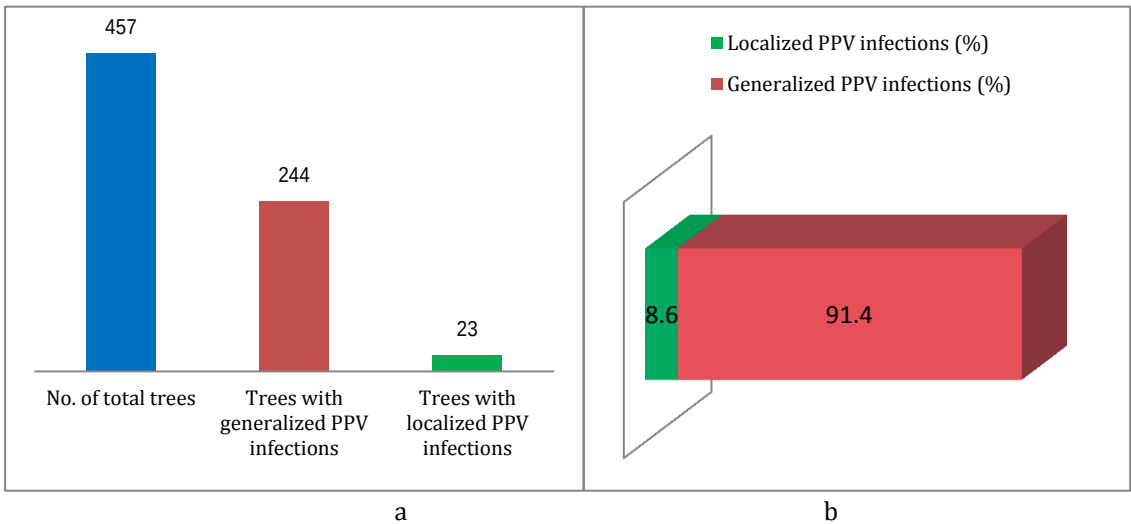


Figure 2. Generalized versus localized PPV infections in trees in 2024 (a. trees; b. percentage)

The results obtained from a long-term study spanning over two decades on the behaviour of various plum cultivars and hybrids to natural PPV infections have enabled the classification of genotypes based on their response (Table 2).

High infection rates, exceeding 83%, were recorded in genotypes such as ‘Diana’, ‘Geta’, ‘HAS 2G’ and ‘Tuleu timpuriu’ with no statistically significant differences among them. These genotypes were thus classified as highly susceptible to natural PPV infection.

Moderate infection levels, ranging between 60% and 80%, were observed in e genotypes including ‘Centenar’, ‘Early Rivers’, ‘Matilda’, ‘HAS 2/VI’, ‘HAS 2/’5 and ‘Piteştean’, which were accordingly classified as susceptible.

In contrast, genotypes such as ‘Stanley’, ‘Čačanska Najbolja’, ‘Anna Späth’, ‘HAS 3/4’ and ‘HAS 1I’, showed lower infection rates, between 40% and 60%, and were therefore designated as tolerant to PPV.

Among all genotypes evaluated only ‘BN 4/1’ (‘Zandra’) consistently exhibited the lowest PPV incidence over the two-decade study period, with just 20% of trees affected, as confirmed by serological tests performed in 2024. Notably, all infections in this hybrid were localized to one or two shoots, and none of the sixty observed trees developed generalized symptoms. This suggests at least a high level of tolerance to natural PPV infection, even under high inoculum pressure within the orchard. Furthermore, statistical analysis revealed

significant differences in PPV incidence between ‘BN 4/1’ hybrid (‘Zandra’ cv.) and all the other genotypes included in the study.

Table 2. PPV incidence and statistical interpretation of the results (2024)

Cultivar/hybrid	PPV-infected trees/no. of trees			PPV incidence (%)
	generalized	localized	total	
‘Diana’	5/5	0/5	5/5	100.0 ± 0.0 a
‘Geta’	4/4	0/4	4/4	100.0 ± 0.0 a
‘HAS 2G’	13/20	3/20	16/20	84.23 ± 5.66 ab
‘Tuleu timpuriu’	35/43	1/43	36/43	83.83 ± 3.32 ab
‘Centenar’	14/17	0/17	14/17	80.23 ± 0.21 b
‘Early Rivers’	6/8	0/8	6/8	77.76 ± 19.25 b
‘Matilda’	7/9	0/9	7/9	77.76 ± 19.25 b
‘HAS 2/VI’	13/19	0/19	13/19	68.20 ± 2.77 bc
‘HAS 2/5’	21/31	0/31	21/31	67.86 ± 3.70 bc
‘Piteștean’	12/18	0/18	12/18	66.6 ± 16.65 bc
‘HAS 3/4’	5/12	2/12	7/12	58.86 ± 8.36 cd
‘Stanley’	87/160	3/160	90/160	56.33 ± 3.18 cd
‘Čačanska Najbolja’	7/16	1/16	8/16	50.00 ± 10.00 cd
‘Anna Späth’	10/23	1/23	11/23	47.63 ± 4.10 d
‘HAS 1I’	5/12	0/12	5/12	41.66 ± 14.43 d
‘BN 4/1’	0/60	12/60	12/60	20.00 ± 5.00 e
Pr>F(Model)				<0.0001
Signification				YES

**Different letters between cultivars/hybrids denote significant differences (Duncan test, $p < 0.0001$). Different letters between PPV incidences of cultivars/hybrids denote significant differences.*

Localized PPV Infections in ‘BN 4/1’ (‘Zandra’)

Out of a total of sixty observed trees of the ‘BN 4/1’ (‘Zandra’) genotype, twelve exhibited localized PPV infections, predominantly confined to the northern part of the canopy. This spatial distribution may be partly attributed to a higher inoculum pressure of PPV in the northern section of the orchard compared to the southern exposure (Figure 3). Additionally, most of the infected shoots originated from dormant buds located near the trunk, suggesting limited virus movement within the canopy. In certain cases, although the suckers arising from the rootstock displayed typical PPV symptoms, the trees of ‘BN 4/1’ scion remained uninfected over time. Notably, despite being surrounded by plenty of PPV-infected trees, 80% of the ‘BN 4/1’ trees remained symptom-free over a period exceeding 25 years. These findings indicate a high degree of field tolerance to natural PPV infections under conditions of permanent and intense inoculum pressure.

Given that ‘BN 4/1’ (‘Zandra’ cv.) exhibited the lowest PPV incidence over 25 years among all studied cultivars and hybrids, shoots displaying localized PPV symptoms were closely monitored, and additional shoots were sampled from adjacent areas surrounding the symptomatic ones. Thus, in the case of some trees exhibiting localized PPV symptoms, four distinct canopy areas were subjected to serological analysis, with the results summarized in Table 3.

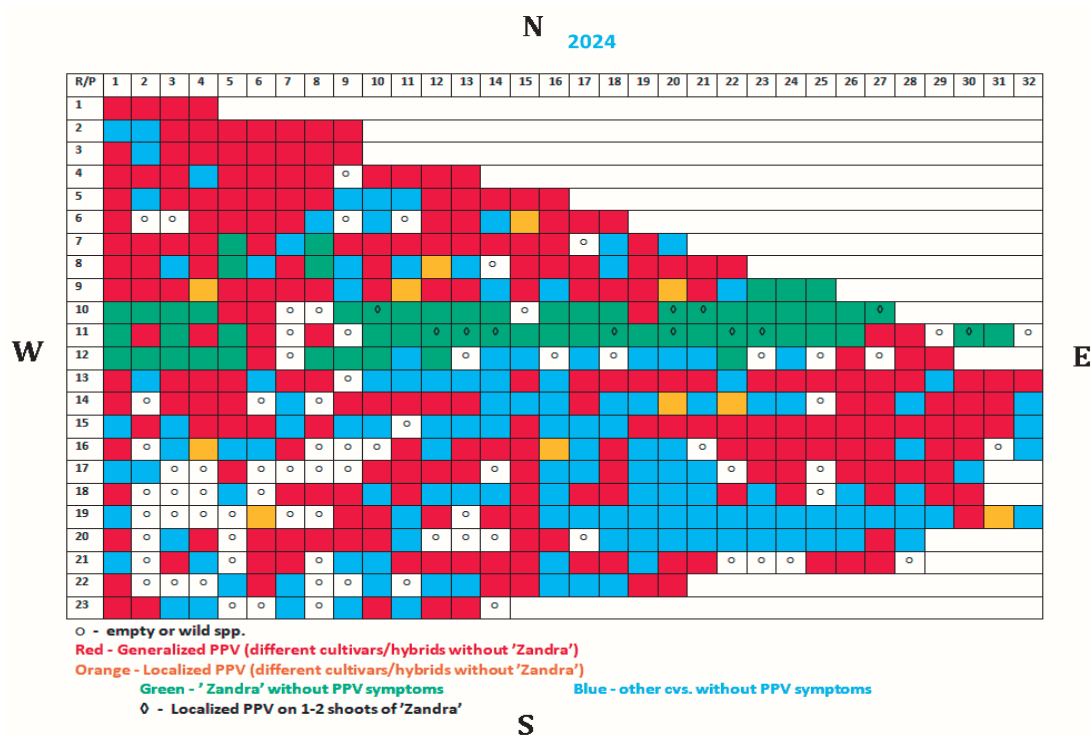


Figure 3. The map of PPV infections in plum trees (2024)

Table 3. The serological results of DAS-ELISA test (2024) of 'Zandra' samples

Part of canopy	Localization of shoots	The visual presence of PPV	DAS-ELISA test results
1	A - symptomatic shoot	Yes	+
	B - above A	No	-
	C - beside A	No	-
	D - below A	No	-
2	E - symptomatic shoot	Yes	+
	F - above E	No	-
	G - above left of E	No	-
	H - above right of E	No	-
	I - below E	No	-
	J - next to E	No	-
3	K - middle of the canopy	No	-
	M - upper part	No	-
	N - lower part	No	-
4	P - symptomatic suckers	Yes	+
	R - lower part of canopy	No	-
	S - average sample from the rest part of canopy	No	-

Figure 4 illustrates the canopy zones of 'BN 4/1' ('Zandra') that were analysed by DAS-ELISA. In the first case, PPV infection was confirmed in the symptomatic shoot (zone A) but was not detected above (zone B), in lateral (zone C) or basal (zone D). This pattern indicates that there was no movement of the virus from the initial infection site to the rest of the canopy.

In the second case, involving another tree of 'BN 4/1' ('Zandra'), PPV was detected exclusively in the symptomatic shoot (zone E), with no detectable virus presence in the surrounding canopy zones (F, G, H, I, and J). In the third case, which examined the opposite side of the canopy in the same tree (case 2), none of the sampled shoots (zones K, M, N) tested positive for PPV. These findings suggest either a true absence of infection in these zones or a viral concentration below the detection threshold of the DAS-ELISA assay.

In the fourth case, involving another 'BN 4/1' ('Zandra') tree, PPV was detected exclusively in the symptomatic suckers (zone P), while no virus was detected in the lower part of the canopy (zone R), or in the rest of the canopy (zone S). This distribution pattern suggests that the infection in the suckers most likely originated from the rootstock which became infected as a result of its susceptibility to the virus, whereas the scion-derived shoots in the canopy remained uninfected.

Overall results indicate that the hybrid 'BN 4/1' ('Zandra' cv.) possesses the ability to restrict PPV infection, keeping the virus localized close to the initial infection site and preventing its systemic spread throughout the tree canopy over time. This behaviour confers it a high level of field of tolerance to natural PPV infections.

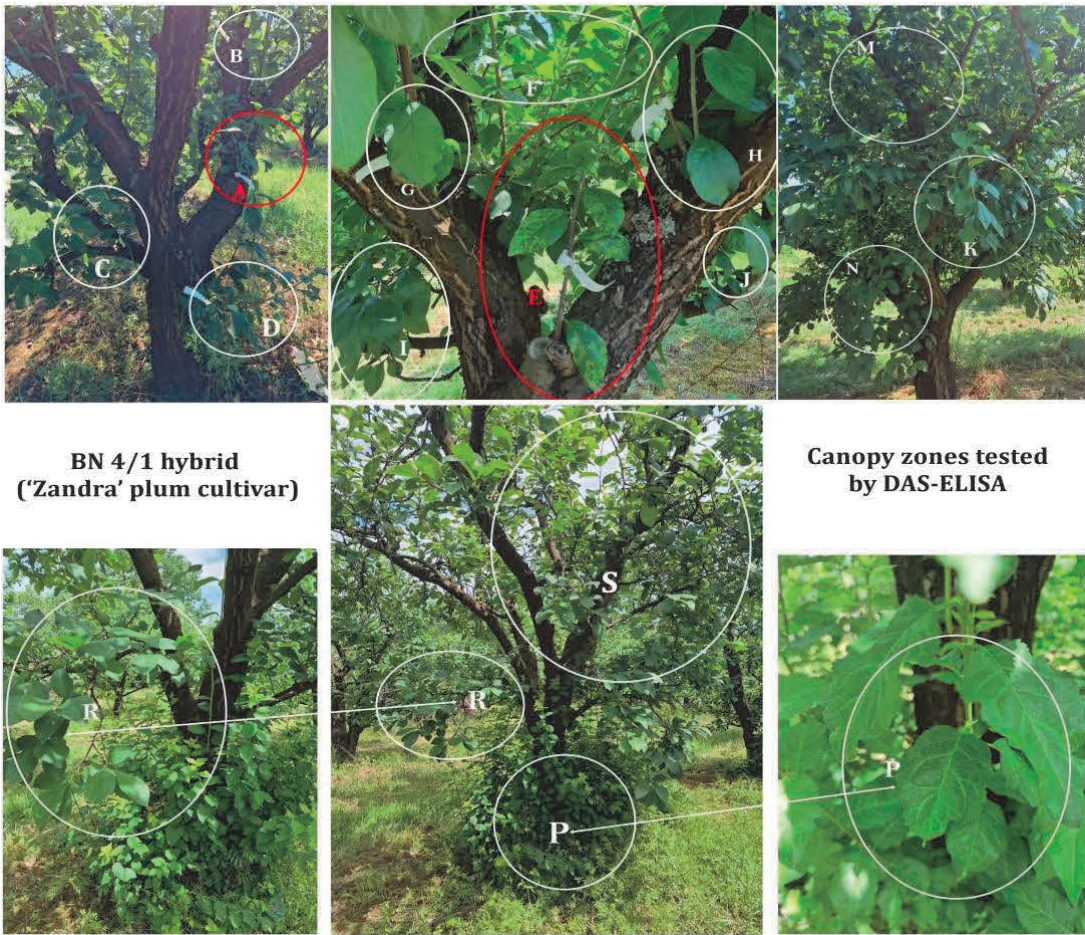


Figure 4. Canopy zones of 'BN 4/1' ('Zandra' cv.) analysed by DAS-ELISA

In addition to its pronounced tolerance to natural PPV infections, the 'Zandra' plum cultivar (Figure 5) exhibits superior organoleptic qualities - including flavour, fruit shape, and desirable size - positioning it as a valuable candidate for integration into the current plum production system.



Figure 5. Fruits of hybrid 'BN 4/1' ('Zandra' cv.)

CONCLUSIONS

The incidence of plum pox virus in the studied plum orchard increased steadily over two decades, reflecting a consistent and progressive spread of the virus under natural conditions. Among the evaluated genotypes, the hybrid 'BN 4/1' ('Zandra' cv.) exhibited the lowest PPV incidence with only 20% of trees affected over the 25 years. Notably, infections in this genotype remain localized to one or two shoots, indicating a high level of tolerance to natural PPV infection, even under conditions of high inoculum pressure. Serological analysis of 'BN 4/1' in different canopy zones confirmed restricted movement of the virus within the tree, further supporting the cultivar's ability to limit systemic viral dissemination.

ACKNOWLEDGEMENTS

We are grateful to the Fruit Research and Development Station Bistrița for the logistical support and administrative assistance.

REFERENCES

1. Atanasoff, D. (1932). Plum pox. A new virus disease. Yearbook University of Sofia, Faculty of Agriculture and Silviculture 11: 49-69.
2. Barba, M., Hadidi, A., Candresse, T. and Cambra, M. (2011). *Plum pox virus*. In Virus-Like Diseases of Pome and Stone Fruits. The American Phytopathological Society, St. Paul, Minnesota. Hadidi A., Barba M., Candresse T. and Jelkmann W. (eds.) pp. 185-197.
3. Butac, M., Plopa, C., Chivu, M. and Mareși, E. (2021). Behaviour of Serbian plum cultivars under Romanian ecological conditions. Acta Hort. 1322: 103-108. DOI: 10.17660/ActaHortic.2021.1322.16 <https://doi.org/10.17660/ActaHortic.2021.1322.16>
4. Butac M., Nesheva M., Chivu M., Stan A., Mareși E., and Nicolae S. (2024). Behaviour of some Bulgarian plum cultivars in the pedo-climatic conditions of Pitesti – Romania area. Romanian Journal of Horticulture, V: 71-76. DOI: <https://doi.org/10.51258/RJH.2024.08>
5. Cambra, M., Madariaga, M., Varveri, C., Caglayan, K., Morca, AF, Chirkov, S, Glasa, M (2024). Estimated costs of plum pox virus and management of sharka, the disease it causes. Phytopathologia Mediterranea, 63(3): 343-365. DOI: <https://doi.org/10.36253/phyto-15581>
6. Clark, M., and Adams, A.N. (1977). Characteristic of the microplate method of enzyme linked immunosorbent assay (ELISA) for detection of plant viruses. J. Gen. Virology, 34: 475-483.

7. Garcia, JA, Rodamilans, B, Martínez-Turino, S, Valli, AA, Simon-Mateo, C, and Cambra, M (2024). Plum pox virus: An overview of the potyvirus behind sharka, a harmful stone fruit disease. *Ann Appl Biol.*, 1-27, DOI:10.1111/aab.12958
8. Glišić, I.S., Paunović, S.A., Milatović, D., Jevremović, D. and Milošević, N. (2021). Evaluation of promising plum (*Prunus domestica* L.) genotypes for resistance to causal agents of the most important diseases. *Acta Hortic.* 1308: 325-332. DOI: 10.17660/ActaHortic.2021.1308.46 <https://doi.org/10.17660/ActaHortic.2021.1308.46>
9. Hartmann, W., and Neumüller, M. (2006). Breeding for resistance: breeding for Plum pox virus resistant plums (*Prunus domestica* L) in Germany. *OEPP/EPPO Bull.* 36: 332-336.
10. Hartmann, W., and Neumüller, M. (2013). The next generation of European plum cultivars resistant to Plum pox virus. *Acta Hortic.*, 985: 149-154.
11. Malinovski, T., Cambra, M., Capote, N., Zawadska, B., Gorris, T., Scorza, R., and Ravelonandro, M. (2006). Field trials of plum clones transformed with plum pox virus coat protein (PPV CP) gene. *Plant Disease*, 90: 1012-1018.
12. Milošević, N., Glišić, I., Lukić M., Popović, B. and Đorđević, M. (2019). Plum breeding in the Fruit Research Institute of Čačak, Serbia – results of the last 15 years. *Acta Hortic.* 1260: 29-34. DOI: 10.17660/ActaHortic.2019.1260.6 <https://doi.org/10.17660/ActaHortic.2019.1260.6>
13. Minoiu, N. (1997). Bolile și dăunătorii prunului (Plum Diseases and Pests). In: *Prunul (The Plum)*, Cociu, I., Botu, I., Minoiu, N., Modoran, I. (eds.) Editura Conphys, Pitesti, Romania. pp. 343-374.
14. Polák, J., and Jarošová, J. (2011). Hypersensitivity of *Prunus domestica* L. “Jojo” was changed by PPV-D strain into very high sensitivity. *Acta Hortic.*, 899: 87-93.
15. Polák, J., and Jarošová, J. (2012). Susceptibility of plum trees cv. ‘Jojo’ to a Czech isolate of Plum pox virus strain D. *Canadian Journal of Plant Pathology.*, 34 (2): 283-287.
16. Polák, J., Kundu, J., Krška, B., Beoni, E., Komínek, P., Pívalova J., and Jarošová, J. (2017). Transgenic plum *Prunus domestica* L. clone C5 (cv. HoneySweet) for protection against sharka disease. *J. Integr. Agric.*, 16: 516-522.
17. Ravelonandro M., Scorza R., Bachelier J.C., Labonne G., Levy L., Damsteegt V., Callahan A.M., and Dunez J. (1997). Resistance of transgenic *Prunus domestica* to Plum pox virus infection. *Plant Disease*. 81, 1231-1235.
18. Ravelonandro, M., Scorza, R. and Hammond, RW. (2011). Biotechnological Approaches for Resistance to Viruses, Viroids and Phytoplasmas. In *Virus-Like Diseases of Pome and Stone Fruits*. The American Phytopathological Society, St. Paul, Minnesota. Hadidi A., Barba M., Candresse T. and Jelkmann W. (eds.) pp. 395-399.
19. Rodamilans, B., Hadersdorfer, J., Berki, Z., García, B., Neumüller, M., and García, J.A. (2023). The mechanism of resistance of European plum to Plum pox virus mediated by hypersensitive response is linked to viral NIa and its protease activity. *Plants*, 12(8), 1609. <https://doi.org/10.3390/plants12081609>
20. Scholthof, KB, Adkins, S, Czosnek, H, Palukaitis, P, Jacquot, E, Hohn, T, Hohn, B, et al. (2011). Top 10 plant viruses in molecular plant pathology. *Molecular Plant Pathology*, 12: 938-954.
21. Scorza R., Ravelonandro M., Callahan A.M., Cordts J.M., Fuchs M., Dunez J., and Gonsalves D. (1994). Transgenic plums (*Prunus domestica*) express the Plum pox virus coat protein gene. *Plant Cell. Repts.* 14: 18-22.
22. Scorza, R., Callahan, A., Dardick, C., Ravelonandro, M., Polak, J., Malinowski, T., Zagrai I., Cambra, M., and Kamenova I. (2013). Genetic engineering of Plum pox virus resistance - ‘HoneySweet’ plum - from concept to product. *Plant Cell, Tissue and Organ Culture*. 115 (1). DOI:10.1007/s11240-013-0339-6
23. Zagrai I., Ravelonandro M., Zagrai L., Scorza R., and Minoiu N. (2011). Overview of the investigations of transgenic plums in Romania. *Acta Hortic.* 899: 153-158.
24. Zagrai, L., Zagrai, I., Scorza, R., Ravelonandro, M., Dardick, C., Guzu, G. (2021). Assessment of agronomic performance of PPV resistant ‘HoneySweet’ transgenic plum in ecological conditions of the Northern Romania. *Romanian Journal of Horticulture*, II, 2021: 49-56. 10.51258/RJH.2021.06.
25. Zagrai, L., Zagrai, I., Guzu, G., Roșu-Mareș, S., Moldovan, C. (2024). Is symptomatology reliable tool for plum pox virus monitoring. *Scientific Papers. Series B, Horticulture*. LXVIII(1), 238-243.
26. Food and Agricultural Organization. (2025). <https://www.fao.org/faostat/en/#data/QCL>.

The influence of soil management practices on biochemical characteristics of soil in plum orchard

M. Butac^{1*}, I. Grafu¹, M. Militaru¹, E. Mareși¹, G. Togan¹ and L. Popa²

¹Research Institute for Fruit Growing, Pitesti, Romania

²National Institute of Research – Development for Machines and Installations
Designed for Agriculture and Food Industry – INMA Bucharest, Romania

Corresponding author email: madalinabutac@yahoo.com

ABSTRACT

Soil management practices such as low or no tillage, mulching and grass cover significantly influence the biochemical characteristics of soil in plum orchards, generally by increasing soil organic carbon, humus and nutrients, enhancing soil aggregation, and improving water infiltration and storage. Research on the application of eco-schemes regarding permanent crops in fruit orchards in the context of ensuring biodiversity and environmental conservation was carried out in a plum field trial located at ICDP Pitesti-Maracineni. The following experimental variants were established: 1. For the interval between the rows of trees, three variants (V1 – clean soil; V2 – spontaneous grass cover; V3 – artificial grass cover (*Lolium perenne*, *Festuca rubra*, *Medicago sativa*, *Lotus corniculatus*); 2. On the row of trees, four variants (V1 – clean soil; V2 – grass cover with clover „Nanissimo”; V3 – herbicide; V4 – mulching). The following biochemical indicators were determined in 2024 and 2025 years: pH, organic carbon, humus, total nitrogen, mobile phosphorus and mobile potassium content. Compared with the results of soil chemical analyses performed before the installation of the experimental variants, it was observed: an increase in pH values, the soil going from an acidic reaction to a neutral one; an increase in soil content in humus, total nitrogen, mobile phosphorus and mobile potassium in the grass cover and mulching variants.

Keywords: soil, tillage, grassing, mulching, herbicides, chemical composition.

INTRODUCTION

In Romania, the plum is the dominant species, the area cultivated with plum is 66,740 ha, which ensures a production of 645,090 t. Romania holds the 2nd place in the world after China and the first one in Europe (FAO data, 2025).

Soil management practices in plum orchards are similar to those applied to other fruit species and are specific to a conventional system. Intensive practices like conventional tillage and monoculture often lead to soil degradation, causing reductions in organic matter and nutrients and stability due to increased erosion and oxidation (Bezabih *et al.*, 2016; Sinore *et al.*, 2022).

Soil is an important resource for sustainable agriculture that influences the ecosystem and orchard productivity (Herencia *et al.*, 2021; Prasad *et al.*, 2021; Rusu *et al.*, 2025). In fruit growing system, including plum orchards, soil quality and soil content in nutrients influence directly growth and yielding capacity (Naraine, 2022; Poonia *et al.*, 2024; Rusu *et al.*, 2025). Soil degradation due to improper practices is a priority goal in agriculture management in Europe and also in Romania (Bezabih *et al.*, 2016; Herencia *et al.*, 2021; Sinore *et al.*, 2022;

Haruna *et al.*, 2025). To avoid soil degradation, many countries have implemented a series of strategies, action plans, programs and rules to improve conservation practices. One of the conservation practices is the application of cover crops in agricultural culture (Haruna *et al.*, 2025).

Cover crops have been used in agriculture for many years ago to improve soil nutrient content. In the mid-twentieth century, these soil maintenance practices were less commonly used due to the excessive use of fertilizers (Haruna *et al.*, 2025). The grass cover (natural or artificial) between the rows in orchards can provide several benefits to the soil: improve the fertility; increase organic matter and the content in nutrients; reduce the erosion; improve the microbial activity (Hoagland *et al.*, 2008; Simoes *et al.*, 2014; Singh *et al.*, 2016; Xiaozhu *et al.*, 2016; Li *et al.*, 2022; Răduțoiu *et al.*, 2023; Ma *et al.*, 2024; Haruna *et al.*, 2025). Some species, such as clover (*Trifolium repens* L.), alfalfa (*Medicago sativa* L.), perennial ryegrass (*Lolium perenne* L.), and tall fescue (*Festuca arundinacea*) are frequently cultivated between the rows in the orchards, depending on the pedo-climatic conditions of the area (Răduțoiu *et al.*, 2023; Ma *et al.*, 2024).

In Romania, the Ministry of Agriculture and Rural Development, within the framework of the National Strategic Plan 2023-2027 (PNS), has implemented the PD-06 eco-scheme – „Grassing the interval between the rows in orchard, vineyard, nursery and hop crops”, which offers viable solutions to active farmers, who practice modern, but at the same time environmentally friendly fruit growing.

Another important conservation practice is mulching, which prevents soil erosion, improves soil structure, fertility and nutrients, controls weeds and microbial activity (Haruna *et al.*, 2025; Guo *et al.*, 2024).

Taking into account the ones presented above, this paper aims to evaluate the effect of different soil management practices on biochemical characteristics of soil in a plum field trial located at ICDP Pitesti-Maracineni.

MATERIALS AND METHODS

Field trial. Research was carried out in a plum field trial established in 2022 in the Genetic and Breeding Department of the Research Institute for Fruit Growing Pitesti, Romania (central part of Romania 44°53'56" Northern latitude, and 24°51'35" Eastern longitude). Soil is medium-textured, heavy-clay, with low humus content.

Experimental variants. In 2024 - 2025, the following experimental variants were applied:

1. On the interval between the rows of trees, three variants: V1 - clean soil; V2 - spontaneous grass cover; V3 - artificial grass cover (*Lolium perenne*, *Festuca rubra*, *Medicago sativa*, *Lotus corniculatus*);
2. On the row of trees, four variants: V1 - clean soil; V2 - grass cover with clover „Nanissimo”; V3 - herbicide; V4 - mulching.

Analysis of biochemical characteristics.

Before applying the experimental variants, soil samples collected at two depths (0 - 20 cm and 20 - 40 cm) were chemically analyzed according to the SR ISO 11464:1998 standard. The standard establishes the pretreatments required for soil samples before being subjected to chemical analyses and describes five types of sample pretreatment: drying, crushing, sieving, separation and grinding. The following analyses were performed on soil samples, both before and after the application of the experimental variants: soil pH was measured by potentiometric method in soil: water solution mixture=1:2.5 according SR ISO 7184-13: 2001; organic carbon according to the Walkley Black method, modified by Gogoasă, respectively humus by calculation using the formula $\text{Humus \%} = C_{\text{org}} \times 1.724$ (STAS 718/21-82); the total nitrogen was determined by the Kjeldahl method (Kjeldahl, 1983); the mobile phosphorus was determined spectrophotometrically, according STAS

7184/19-82; the mobile potassium was determined flamephotometrically from the same extract obtained for phosphorus determination, according STAS 7184/18-80.

Statistical analysis. The data were included in an Excel database and statistically interpreted with the SPSS 14.0 program, which uses the Duncan test (multiple t-tests) at a 0.05 level of probability.

RESULTS AND DISCUSSIONS

Chemical analyses before the installation of the experimental variants

Chemical analyses carried out before the experiments were set up revealed that the soil reaction was slightly acidic and the humus content was relatively low: 1.50% at the surface, decreasing to 1.27% at 40 cm depth. The soil had a low content of total nitrogen (0.180% at the surface and 0.153% at 40 cm depth). Also, the mobile phosphorus was low, 18.10 mg/100 g soil at the surface, decreasing to 4.10 mg/100 g soil at 40 cm depth. The content of mobile potassium was 33.2 mg/100 g soil in the superficial horizon and 16.60 mg/100 g soil at 40 cm depth (Table 1).

Table 1. The main chemical components of the soil before the installation of the experimental variants

No.	Horizon	Depth (cm)	pH	N (%)	P ₂ O ₅ mg/kg soil	K ₂ O mg/kg soil	Humus %
1	Aa	0-20	5.5	0.180	18,10	33,20	1,50
2	A1	20-40	6.0	0.153	4,10	16,60	1,27

Chemical analyses after the installation of the experimental variants

After the installation of the experimental variants, soil chemical analyses were performed to determine the influence of these soil maintenance variants on the agrochemical indicators of the soil. Soil samples were collected from the plum field trial at a depth of 20 cm, on each experimental variant.

Soil pH. Fruit trees grow and develop properly on neutral soils, with a pH between 6.3 and 7.5. The consumption of nutrients by fruit trees is influenced by the pH of the soil. Thus, at a pH between 7 and 8.5, fruit trees easily assimilate calcium and magnesium; at a pH between 6.0 and 6.8, nitrogen is assimilated; at a pH of 6.5 to 7.5, phosphorus is assimilated; at a pH higher than 6, potassium is easily assimilated (Nagy *et al.*, 2022).

In the plum field trial, the soil pH showed a significant difference between the experimental variants, both on the row and on the interval. On the row, pH average values ranged between 7.04 in variant 2 – grass cover with clover „Nanissimo” and 6.28 in variant 1 – clean field, with an average value of 6.57 (Table 2). On the interval between rows, pH had values ranging between 6.27 in variant 1 – clean soil and 6.53 in variant 3 – artificial grass cover, with an average value of 6.44 (Table 3).

Compared with the results of soil chemical analyses performed before the installation of the experimental variants, it is observed an increase in pH values of the soil going from an acidic reaction to a neutral one. The best results were obtained in the case of variant 2 - grassing with dwarf clover on the row and in the case of variants 2 - spontaneous grassing and 3 - artificial grassing on the interval between the rows.

It can be observed that in the two clean soil variants, both in the row direction and in the interval, the pH values were lower. It is known that the lowest pH values on the surfaces maintained as clean soil are due to soil erosion and the washing out of soluble basic cations, while the concentration of hydrogen ions increases in the soil solution, which leads to a decrease in pH. Our results are similar to those obtained by other researchers (Bezabih *et al.* 2016; Sinore *et al.*, 2022; Poonia *et al.*, 2024).

Humus and organic carbon. Humus is a fundamental constituent of soil that contributes to the formation of structure, water absorption, adsorption and exchange of cations, and the

provision of nutrients resulting from the mineralization of organic matter (Dumitru *et al.*, 2011).

The organic carbon and humus content of soil were significantly affected by soil management practices. On the row, the humus content of soil ranged between 2.64% in variant 2 – grass cover with clover „Nanissimo” and 1.88% in variant 3 – herbicide; the soil organic carbon ranged between 1.53% in variant 2 – grass cover with clover „Nanissimo” and 1.09% in variant 3 – herbicide (Table 2). On the interval between rows, the humus content ranging between 2.05% in variant 1 – clean soil and 2.43% in variant 3 – artificial grass cover; the soil organic carbon ranged between 1.19% in variant 1 – clean soil and 1.41% in variant 3 – artificial grass cover (Table 3).

Compared with the results of soil chemical analyses performed before the installation of the experimental variants, it is observed an increase of humus content of soil, from 1.50% to 2.14% on the row and 2.23% on the interval.

Both on the row direction and on the interval, the soil organic carbon and humus content were lower in the variants maintained as clean soil and herbicide and higher in the grassing and mulching variants.

The low values of humus and organic carbon in the herbicide variant can be explained by the fact that herbicides can hurt the soil. Thus, they can negatively influence the activity of soil microorganisms responsible for the decomposition of organic matter. They can also block the release of carbon, and in some cases, contribute to the accumulation of carbon by inhibiting mineralization or by incomplete decomposition of organic matter. All of this prevents the formation of humus on the long term with consequences on soil fertility. The same results regarding the influence of herbicides on soil fertility were also reported by Meena *et al.* in 2023 and by Ikioukenigha *et al.* in 2024.

The same explanation can be given in the case of variant 1 – clean soil, where the values of humus and organic carbon were low. Maintaining the soil as clean of weeds in orchards, although reducing competition for water and nutrients, can decrease humus content on the long term, as it removes organic matter from the surface and destroys the microorganisms that contribute to humus formation and affecting soil fertility (Bezabih *et al.* 2016; Sinore *et al.*, 2022).

On the other hand, the results regarding the high soil content in humus and organic carbon in the grassing and mulching variants can be explained by good interaction between biomass and grassing, respectively the cover crop can lead to an increase in the organic carbon content and implicitly in humus (Herencia *et al.*, 2021; McClelland *et al.*, 2021; Guo *et al.*, 2024; Haruna *et al.*, 2025; Rusu *et al.*, 2025).

Macronutrients (nitrogen, phosphorus, and potassium).

The content of soil in *total nitrogen* is generally low, being linked to soil organic matter and is important in plant nutrition (Dumitru *et al.*, 2011).

In both situations, in row and in interval, the content of soil in total nitrogen was low. On the row, the total nitrogen content ranged between 0.208% in variant 2 – grass cover with clover „Nanissimo” and 0.146% in variant 1 – clean soil (Table 2). On the interval between rows, the total nitrogen content ranged between 0.158% in variant 1 – clean soil and 0.213% in variant 3 – artificial grass cover (Table 3).

Compared with the results of soil chemical analyses performed before the installation of the experimental variants, it is observed a very small increase, from 0.180% to 0.185% on the row and 0.193% on the interval.

It is also noted that in the variants where grassing and mulching were practiced, the content of soil in total nitrogen was somewhat higher than in the clean soil and herbicide variants due to the poor nutrient management in the last two variants. The presence of vegetation accords soil adequate cover, thereby reducing soil loss and enhancing soil water storage would contribute to soil organic matter and nitrogen input in the soil.

Phosphorus is important in plant growth and fruiting. The solubility of phosphorus differs depending on the soil reaction and the chemical combinations in which it is found (Dumitru *et al.*, 2011).

The mobile phosphorus was significantly affected by practice management. On the row, the mobile phosphorus content ranged between 30.81 mg/kg in variant 2 – grass cover with clover „Nanissimo” and 21.19 mg/kg in variant 1 – clean soil (Table 2). On the interval between tree rows, the mobile phosphorus content ranged between 16.21 mg/kg in variant 1 – clean soil and 19.70 mg/kg in variant 3 – artificial grass cover (Table 3).

As in the case of the other chemical indicators analyzed, a slight increase in the soil content of mobile phosphorus is observed compared to the initial chemical analyses, from 18.10 mg/kg to 25.41 mg/kg on the row and 18.28 mg/kg on the interval.

In the variants where grassing and mulching were applied, the mobile phosphorus content was higher than in the clean soil variant, because these soil maintenance practices add minerals and organic matter to the soil and better phosphorus fixation.

Table 2. Influence of experimental variants (on tree row) on the main soil components in the plum field trial

Year	Variant	pH	Humus (%)	Organic carbon (%)	Total nitrogen (%)	Mobile phosphorus (mg/kg)	Mobile potassium (mg/kg)
2024	V1 – clean soil	6.24 c	1.86 c	1.08 c	0.135 c	20.56 c	50.20 c
	V2 – grass cover with clover	6.96 a	2.48 a	1.44 a	0.200 a	29.25 a	76.35 b
	V3 - herbicide	6.24 c	1.75 d	1.02 d	0.172 b	23.47 b	38.45 d
	V4 - mulching	6.42 b	2.03 b	1.18 b	0.198 a	23.82 b	105.84 a
2025	V1 – clean soil	6.32 d	1.95 c	1.13 c	0.157 d	21.83 c	70.49 c
	V2 – grass cover with clover	7.13 a	2.79 a	1.62 a	0.215 a	32.36 a	95.83 b
	V3 - herbicide	6.45 c	2.00 c	1.16 c	0.189 c	25.48 b	67.96 d
	V4 - mulching	6.76 b	2.27 b	1.32 b	0.209 b	26.51 b	117.34 a
2024-2025	V1 – clean soil	6.28 c	1.90 c	1.11 c	0.146 c	21.19 c	60.34 c
	V2 – grass cover with clover	7.04 a	2.64 a	1.53 a	0.208 a	30.81 a	86.09 b
	V3 - herbicide	6.35 c	1.88 c	1.09 c	0.181 b	24.48 b	53.07 c
	V4 - mulching	6.59 b	2.15 b	1.25 b	0.204 a	25.16 b	111.59 a
Average		6.57	2.14	1.25	0.184	25.41	77.77

*Duncan multiple ranges test. Numbers followed by the same letter within a column are not significantly different ($P \leq 0.05$).

Potassium, along with nitrogen and phosphorus, is one of the macroelements with an important role in plant nutrition. The part of potassium that becomes accessible to plants depends on the soil clay content, organic matter, soil reaction and soil water regime (Dumitru *et al.*, 2011).

In our experience, on the row, the mobile potassium content varied between 111.59 mg/kg in variant 4 – mulching and 53.07 mg/kg in variant 3 – herbicide (Table 2). On the interval between rows, the mobile potassium content varied from 71.49 mg/kg in variant 1 – clean soil to 84.95 mg/kg in variant 3 – artificial grass cover (Table 3). The addition of potassium by the cover crops was higher compared to the initial analyses carried out before the installation of the experiment and compared to the other chemical parameters analysed, from 33.20 mg/kg to 77.77 mg/kg on the row and 78.08 mg/kg on the interval.

Similar results regarding the content of soil in total nitrogen, mobile phosphorus and mobile potassium were found by Bezabih *et al.* (2016), Herencia *et al.* (2021), Sinore *et al.* (2022), Poonia *et al.* (2024), and Haruna *et al.* (2025).

From one year to the next (2025 compared to 2024), an increase in soil organic carbon, humus, total nitrogen, mobile phosphorus and mobile potassium content can be observed, which confirms that the continuous addition of biomass can improve the content of soil in biochemical indicators by prolonged practice.

Table 3. Influence of experimental variants (on the interval between the rows) on the main soil components in the plum field trial

Year	Variant	pH	Humus (%)	Organic carbon (%)	Total nitrogen (%)	Mobile phosphorus (mg/kg)	Mobile potassium (mg/kg)
2024	V1-clean soil	6.21 c	1.91 c	1.11 c	0.139 c	15.47 b	62.35 c
	V2-spontaneous grass cover	6.46 b	2.10 b	1.22 b	0.195 b	18.23 a	67.52 b
	V3-artificial grass cover	6.48 a	2.29 a	1.33 a	0.198 a	19.15 a	72.46 a
2025	V1-clean soil	6.33 b	2.19 c	1.27 c	0.176 c	16.95 b	80.63 c
	V2-spontaneous grass cover	6.56 a	2.34 b	1.36 b	0.220 b	19.61 a	88.09 b
	V3-artificial grass cover	6.58 a	2.57 a	1.49 a	0.227 a	20.24 a	97.44 a
2024-2025	V1-clean soil	6.27 b	2.05 b	1.19 b	0.158 b	16.21 b	71.49 a
	V2-spontaneous grass cover	6.51 a	2.22 b	1.29 b	0.208 a	18.92 a	77.81 a
	V3-artificial grass cover	6.53 a	2.43 a	1.41 a	0.213 a	19.70 a	84.95 a
	Average	6.44	2.23	1.30	0.193	18.28	78.08

*Duncan multiple ranges test. Numbers followed by the same letter within a column are not significantly different ($P \leq 0.05$).

However, taking into account the classification scale of the Research Institute for Pedology and Agrochemistry Bucharest (1976), the soil on which the experiments are located falls into class 2 – 3 in terms of humus and carbon content, respectively, low to medium supply (Dumitru *et al.*, 2011). According to the classification scale of Bogaci (1981), the soil is poorly supplied with total nitrogen and mobile phosphorus and medium supplied with mobile potassium (Dumitru *et al.*, 2011)..

Correlations between the main traits

The statistical analyses revealed significant correlations between pH, content of soil in humus, organic carbon and nutrients under varying management practices, which demonstrated that different maintenance practices play an important role in improving soil fertility and optimizing nutrient cycling (Tables 4 and 5). Similar trends are observed by Rusu *et al.* in 2025 in a plum orchard from the Moldavian Plateau.

Table 4. The Pearson Correlations between the characteristics studied (on the row)

Indicator	pH	Humus	Organic carbon	Total nitrogen	Mobile phosphorus	Mobile potassium
pH	1	0.976**	0.977**	0.786**	0.927**	0.581**
Humus	0.976**	1	1.000**	0.728**	0.903**	0.588**
Organic carbon	0.977**	1.000**	1	0.730**	0.903**	0.589**
Total nitrogen	0.786**	0.728**	0.730**	1	0.837**	0.738**
Mobile phosphorus	0.927**	0.903**	0.903**	0.837**	1	0.465*
Mobile potassium	0.581**	0.588**	0.589**	0.738**	0.465*	1

* Correlation is significant at the 0.05 level (2-tailed); ** Correlation is significant at the 0.01 level (2-tailed);

Significant correlations were observed between: pH and nitrogen ($r=0.786$ on the row and $r=984$ on the interval); pH and phosphorus ($r=0.927$ on the row and $r=875$ on the interval); pH and potassium ($r=0.581$ on the row and $r=724$ on the interval), the consumption of nutrients by fruit trees being influenced by the pH of the soil. It is known that at a pH above 6, nitrogen, phosphorus and potassium are easily assimilated. Significant correlations were also recorded between humus and organic carbon content, given that humus is a fraction of

organic carbon ($r=1.000$ both on the row and on the interval). Correlations between total nitrogen and mobile phosphorus ($r=0.837$ on the row and $r=0.896$ on the interval) and mobile potassium ($r=0.738$ on the row and $r=0.796$ on the interval) indicate a strong nutrient interdependence within the soil management practices. Recent studies by Rusu *et al.* (2025) further highlight that nitrogen availability supports the efficient utilization of phosphorus and potassium.

Table 5. The Pearson Correlations between the characteristics studied (on the interval)

Indicator	pH	Humus	Organic carbon	Total nitrogen	Mobile phosphorus	Mobile potassium
pH	1	0.867**	0.867**	0.984**	0.875**	0.724**
Humus	0.867**	1	1.000**	0.905**	0.832**	0.911**
Organic carbon	0.867**	1.000**	1	0.905**	0.835**	0.911**
Total nitrogen	0.984**	0.905**	0.905**	1	0.896**	0.796**
Mobile phosphorus	0.875**	0.832**	0.835**	0.896**	1	0.672**
Mobile potassium	0.724**	0.911**	0.911**	0.796**	0.672**	1

** Correlation is significant at the 0.01 level (2-tailed).

CONCLUSIONS

This study underscores the importance of different management practices in improving the soil fertility and the content in nutrients within the plum orchard from ICDP Pitesti-Maracineni.

Compared with the results of soil chemical analyses performed before the installation of the experimental variants, the following results were obtained: an increase in pH values (from 5.5 to 6.59), the soil going from an acidic reaction to a neutral one; an increase in soil content in humus (from 1.5% to 2.80%, respectively 2.57%), total nitrogen (from 0.180% to 0.215%, respectively 0.227%), mobile phosphorus (from 18.10 mg/kg soil to 32.36 mg/kg soil, respectively 20.25 mg/kg soil) and mobile potassium (from 33.20 mg/kg soil to 95.83 mg/kg, respectively 97.44 mg/kg) in the clover grassing variant on the row and respectively in the artificial grassing variant on the interval. Also, in the mulch variant, good results were recorded regarding soil pH (6.59), humus content (2.15%), organic carbon (1.25%), total nitrogen (0.204%), mobile phosphorus (25.16 mg/kg) and potassium (111.19 mg/kg).

The statistical analyses revealed significant correlations between pH, content of soil in humus, organic carbon and nutrients under varying management practices, which demonstrated that different maintenance practices play an important role in improving soil fertility and optimizing nutrient cycling.

ACKNOWLEDGEMENTS

This research was supported by a sectoral project ADER 6.1.9/18.07.2023 - The influence of the application of eco-schemes regarding permanent crops in fruit orchards in the context of ensuring biodiversity and environmental impact, supported by the Ministry of Agriculture and Rural Development.

REFERENCES

1. Bezabih, B., Aticho, A., Mossisa, T., Dume, B. (2016). The effect of land management practices on soil physical and chemical properties in Gojeb Sub-river Basin of Dedo District, Southwest Ethiopia. *Journal of soil science and environmental management*, vol. 7(10): 154-165.
2. Dumitru, M., Dumitru, S., Tănase, V., Mocanu, V., Manea, A., Vrînceanu, N., Preda, M., Eftene, M., Ciobanu, C., Calciu, I., Rîșnoveanu, I. (2011). *Monitoringu stării de calitate a solurilor din România*. Ed. Sitech, Craiova (in Romanian).

3. Guo, L., Liu, S., Zhang, P., Hakeem, A., Song, H., Yu, M., Wang, F. (2024). Effects of different mulching practices on soil environment and fruit quality in peach orchards. *Plants*, 13, 827: 1-20. <https://doi.org/10.3390/plants13060827>.
4. Ikioukenigha, M., Abamba, E., Chikukula, A., Dugeri, D.R., Ibeneme, U.J., Olukayode, O.J., Okpro, O., Akpome, A. (2024). Herbicides effects on soil functions: A review. *ASIAN Soil Research Journal*, vol. 8(4): 34-41.
5. Kjeldahl Johan, Z. (1983). A new method or the determination of nitrogen in organic odies. *Analytical Chemistry*, 22: 366.
6. Haruna, S., Eke, D., Ariful-Haque, Md., Ku, S. (2025). Cover crop effects on select soil physicochemical and biological properties. *Elsevier - Agriculture, Ecosystems and Environment*: 1-15. <https://doi.org/10.1016/j.agree.2025.109817>.
7. Herencia, J.F., Perez-Romero, L.F., Daza, A., Arroyo, F.T. (2021). Chemical and biological indicators of soil quality in organic and conventional Japanese plum orchards. *Biological agriculture and horticulture*, vol. 37(2): 71-90. <https://doi.org/10.1080/01448765.2020.1842243>.
8. Hoagland, L., Carpenter-Boggs, L., Granatstein, D., Mazzola, M., Smith, J., Peryea, F. (2008). Orchard floor management effects on nitrogen fertility and soil biological activity in a newly established organic apple orchard. *Biol. Fertil. Soils* no. 45: 11-18.
9. Li, T., Wang, Y., Kamran, M., Chen, X., Tan, H., Long, M. (2022). Effects of grass inter-planting on soil nutrients, enzyme activity, and bacterial community diversity in an apple orchard. *Front Plant Sci*. 13: 901143.
10. Ma, J., Li, G., Tian, J., Jia, Y., Chu, Y., Yue, H., Wang, H., Li, X. (2024). Assessing and quantifying the carbon, nitrogen, and enzyme dynamics effects of inter-row cover cropping on soils and apple tree development in orchards. *HortScience* 59(8): 1088-1096. <https://doi.org/10.21273/HORTSCI17779-24>.
11. McClelland, S.c., Paustian,K., Skipanski, M.F. (2021). Management of cover crops in temperate climates influences soil organic carbon stocks: a meta-analysis. *Ecol. Appl.* 31 (3), e02278. <https://doi.org/10.1002/eap.2278>.
12. Meena, M., Singh, R.K., Dhayal, S., Meena, P.K. (2023). Effect of different dose of herbicide on soil physico-chemical and biological properties after harvest of wheat. *International Journal of Plant and Soil Science*, vol. 35(5): 12-17.
13. Nagy, E.M., Nicola, C., Paraschiv, M., Mihalache, M.A. (2022). Properties of granular organo-mineral fertilizer and the influence of its application on some chemical properties of the soil in an apple plantation, Jonathan variety. *Scientific papers, Series B, Horticulture*, vol. LXVI, no. 1: 162-167.
14. Naraine, L. (2022). Diversified integrated farm model: Case study-plum tree farms, St. Kitts. *Research anthology on strategies for achieving agricultural sustainability*: 353-377.
15. Poonia, T., Choudhary, M., Kakraliya M., Dixit, B., Sahay-Jat, H. (2024). The influence of soil types and agricultural management practices on soil chemical properties and microbial dynamics. *Frontiers in Sustainable Food Systems*: 1-11.
16. Prasad, S., Malav, L.C., Choudhary, J., Kannojiya, S., Kundu, M., Kumar, S., Yadav, A.N. (2021). Soil microbiomesfor healthy nutrientrecycling. *Curent trends in microbial biotechnologyfor sustainable agriculture*. Springer: 1-21.
17. Răduțoiu, D., Cosmulescu, S.N., Georghiu, N., Stoenescu, A.M. (2023). Considerations regarding the vascular flora of some fruit orchards in Dolj county, Romania. *Annals of the University Craiova*, vol. XXVIII (LXIV): 107-114.
18. Rusu, M., Filip, M., Cara, I.G., Topa, D., Jităreanu, G. (2025). Soil nutrient dynamics and farming sustainability under different plum orchard management practices in the pedoclimatical conditions of Moldavian plateau. *Agriculture*, 15, 509: 1-20.
19. Simoes, M.P., Belo, A.F., Pinto-Cruz, C., Pinheiro, A.C. (2014). Natural vegetation management to conserve biodiversity and soil water in olive orchards. *Spanish Journal of Agricultural Research*, 12(3): 633-643.
20. Singh, S.K., Sharma, M., Singh, P.K. (2016). Combined approach of intercropping and INM to improve availability of soil and leaf nutrients in fruit trees. *Journal of Chemical and Pharmaceutical sciences*, 9(2): 823-829.
21. Sinore, T., Chernet, M., Detamo, K., Yohannes, M. (2022). Effect of soil management practices on soil physic-chemical properties: a case of Wera Sub-Watershed, Southern Ethiopia. *Hindawi – Applied and environmental soil science*, vol. 2022: 1-9. <https://doi.org/10.1155/2022/5370477>.
22. Xiaozhu, Y., Zhuang, L.I., Cheng, C. (2016). Effect of conservation tillage practices on soil phosphorus nutrition in an apple orchard. *Horticultural Plant Journal* 2(6): 331-337.
23. SR ISO 11464:1998. Soil quality – Pretreatment of samples for physic-chemical analyses.
24. SR 7184-13:2001. Soils – determination of pH in aqueous and saline suspensions and in paste at saturation.
25. STAS 7184/18-80. Soils – determination of available and potentially available potassium content for plants.
26. STAS 7184/19-82. Soils – determination of extractable phosphorus in ammonium acetate-lactate.
27. STAS 7184/21-82. Soils – determination of humus content.
28. Food and Agricultural Organization (2025). www.fao.org/statistics/yearbook.

Transnational Evolution of *Myristica fragrans* from the Banda Islands to Global Spice Markets

B. Damoah*

University of Southern Mississippi, Hattiesburg, School of Biological, Environmental,
and Earth Sciences
Department of Geography and Geology, USA

*Corresponding author email: Benjamin.Damoah@usm.edu

ABSTRACT

This study investigates the transnational evolution of *Myristica fragrans* (nutmeg and mace) from its origin in the Banda Islands of Indonesia's Maluku to its establishment as a globally cultivated commodity. For centuries, Indigenous Bandanese farmers managed nutmeg through ecologically sustainable and spiritually rooted practices that sustained biodiversity and cultural identity. European imperial expansion disrupted these systems. The Portuguese initiated overseas trade, while the Dutch East India Company (VOC) intensified imperial control by deploying military campaigns, forcing labor, and sterilizing seeds. Using a comparative historical-ecological approach, the study integrates archival documents and peer-reviewed literature (1970–2024), and GIS mapping of VOC maritime routes and present-day production regions. Thematic analysis focused on five dimensions: Indigenous agroecology, VOC monopoly mechanisms, British and French botanical transfers, postcolonial production landscapes, and ecological impacts. Findings reveal that the VOC embedded nutmeg within global commodity chains linking the Banda Islands to Amsterdam. Later British and French interventions, particularly seed transfers to Grenada, Mauritius, and Zanzibar, broke Dutch dominance and diffused nutmeg across tropical regions. Today, nutmeg cultivation supports livelihoods in Asia, Africa, and the Caribbean but faces ecological threats from soil depletion, monoculture dependency, and climate change. The study concludes that imperial ambition and ecological exploitation profoundly restructured tropical agriculture. Understanding this intertwined history highlights the enduring legacies of colonial trade and underscores the need for sustainable, equitable, and historically informed agricultural systems.

Keywords: Banda Islands, transnational commodity chains, *Nutmeg trade*, Maluku province

INTRODUCTION

Myristica fragrans, commonly known as nutmeg, belongs to the family Myristicaceae (Ashokkumar *et al.*, 2022). This botanical family comprises 19 genera and includes over 400 species, with a rich presence in Indonesia and Papua New Guinea. Nutmeg, the seed of *Myristica fragrans*, originates from the evergreen spice tree native to the Moluccas, or Maluku Islands, in Indonesia (Kusuma *et al.*, 2023). This perennial tree yields two highly valued spices, nutmeg and mace, from a single fruit (Sujianto *et al.*, 2024). The yellow fruit, measuring approximately 5 centimeters in diameter, splits upon ripening to reveal a crimson aril known as mace that encases a brown kernel identified as nutmeg (Moripi and Peki, 2015).

The spice tree matures after seven years and can reach heights up to 65 feet. It continues to bear fruit for as long as ninety years (Ducène, 2024; Ravindran, 2017). The simultaneous production of two distinct spices, mace, and nutmeg, makes *Myristica fragrans* unique among tropical spice trees (Sasikumar, 2021; Nair and Nair, 2021). Originally confined to the Banda Islands, nutmeg eventually reached diverse tropical and subtropical regions, including India, Grenada, Sri Lanka, Réunion, Guatemala, the Solomon Islands, Mauritius, Malaysia, Seychelles, Tanzania (Zanzibar), and Vietnam (Gordon, 2020).

Nutmeg's transnational journey reflects centuries of maritime navigation, mercantile ambition, and colonial conquest. The spice's high market value made it a focal point of early modern imperial economies. Portuguese, Dutch, British, and French traders competed to control nutmeg production and exportation. These rivalries shaped the political and economic architecture of the colonial spice trade (Sasikumar, 2021; Kusuma *et al.*, 2023). Pirates, chartered companies, and monarchs all contributed to moving nutmeg from the Banda Islands to plantations across continents. The Dutch East India Company (VOC) implemented ruthless measures to secure monopoly control, including the violent suppression of local populations and the forced relocation of nutmeg trees.

The economic value of *Myristica fragrans* extends beyond culinary use. Nutmeg supports multiple industries, including perfumery, timber, medicine, beverages, oils, and cosmetics (Moripi and Peki, 2015; Ulfah, 2020; Sujianto *et al.*, 2024). This versatility accounts for its reputation as green gold. It remains a staple in households worldwide, enhancing both sweet and savory dishes and serving as a component in traditional remedies and contemporary health products.

Human agency has played a central role in the dispersal and domestication of nutmeg. Species do not migrate uniformly across forests or bioregions. Instead, environmental forces such as wind and water, combined with human cultivation and commerce, drive the spread of plant life. The movement of *Myristica fragrans* involved several such forces, including ecological adaptation, political ambition, and economic interest (Schluter and Pennell, 2017). As a result, nutmeg established itself in soils and climates far removed from its place of origin.

This paper investigates the historical drivers behind nutmeg's geographic dispersion and commercial prominence. The research focuses on the political economies and ecological consequences of colonial spice networks that altered the botanical and human landscape of entire regions. As colonial powers moved nutmeg beyond the Banda Islands, they restructured agricultural systems, transformed land tenure, and introduced monoculture plantations. These changes generated long-term environmental pressures and social dislocations still observable in modern spice economies.

Despite nutmeg's cultural and commercial relevance, much of the existing literature remains fragmented across disciplines such as botany, history, and economics. Few studies adopt an integrative lens that connects plant distribution, imperial politics, labor exploitation, and ecological adaptation. This paper addresses that gap by synthesizing insights from historical and scientific sources to present a comprehensive account of *Myristica fragrans* as both a botanical subject and a geopolitical asset.

The relevance of this study lies in its interdisciplinary approach to understanding how a single plant species became embedded in the global economy through systems of exploitation and resilience. In tracing nutmeg's trajectory from the Banda Islands to distant plantations, this paper offers a lens into how natural resources become contested commodities. It also invites reflection on the colonial legacies embedded in contemporary agricultural practices and global supply chains. By uncovering the complex history of nutmeg's movement, cultivation, and commodification, the study advances conversations about historical accountability, environmental justice, and the socioecological consequences of colonial trade systems.

Methodological Framework

This study employed a comparative historical–ecological research design to trace the transnational diffusion and ecological transformation of *Myristica fragrans* from the Banda Islands into a globally cultivated commodity. The methodology combined archival research, bibliographic review, and spatial analysis to reconstruct the historical, economic, and ecological trajectory of nutmeg cultivation.

Sources of Data

The study reviewed literature published between 1970 and 2024 to establish a robust historical and ecological foundation. The research team collected peer-reviewed journal articles, archival documents, VOC shipping logs, colonial administrative records, and historical monographs from Google Scholar, Web of Science, JSTOR, EBSCOhost, and ERIC. The reviewed works spanned from seventeenth-century accounts to contemporary analyses, focusing on sources addressing the Banda Islands, the Dutch East India Company (VOC), British and French colonial expansion, and postcolonial agricultural systems. The literature search employed targeted keywords, such as *Myristica fragrans*, VOC spice monopoly, Banda Islands agriculture, Dutch colonial plantations, British and French botanical transfers, nutmeg biogeography, and postcolonial spice economies.

Spatial and Visual Analysis

The study reconstructed historical trade routes and mapped present-day nutmeg production using GIS tools. The research team digitized VOC maritime routes linking the Banda Islands to Amsterdam and overlaid them with port locations and resupply points such as Batavia and Cape Town. The mapping process used coastline shapefiles and integrated FAOSTAT (2023) production data to highlight current nutmeg-producing regions. Figures 6 and 7 visualize these historical circuits and contemporary geographies.

All materials were selected based on their relevance to the study objectives and their capacity to provide historical and ecological evidence that contributes to reconstructing the global diffusion of nutmeg.

Analytical Method

A qualitative analytical framework was used to organize and interpret the data. The information extracted from the reviewed materials was grouped thematically to highlight key aspects such as: Indigenous cultivation and ecological practices in the Banda Islands; The Dutch East India Company's monopoly mechanisms and colonial strategies; British and French interventions and botanical transfers; The postcolonial redistribution of nutmeg production; The ecological and socio-economic impacts of these transitions.

The analysis compared historical and modern contexts to identify continuities and transformations in nutmeg cultivation and trade. This thematic organization allowed the study to trace both spatial and temporal dimensions of nutmeg's evolution as a global commodity.

Limitations

The study relied on digitized archives and published literature that often amplify colonial perspectives and minimize Indigenous voices. The English-language focus limited engagement with Dutch-, French-, and Malay-language research that might offer additional nuance. The study did not include field surveys, soil testing, or genetic sequencing, which could further verify patterns of diffusion and ecological adaptation.

Even with these constraints, the research team combined documentary evidence, thematic coding, and GIS mapping to produce a rigorous, interdisciplinary methodology. This approach illuminates both the historical depth and geographic reach of nutmeg's global

trajectory and situates *Myristica fragrans* within the broader context of imperial trade, colonial extraction, and postcolonial agricultural economies.

FINDINGS

Traits of *Myristica Fragrans*

The *Myristica fragrans* tree grows up to 65 feet or 20 m in height. The tree matures to bear fruit at the prime age of 25 years and continues to yield fruit for a further 60 years in a favorable condition (Swaminathan *et al.*, 2019). Nutmegs have evolved in a transnational pendulum that has classified *Myristica fragrans* into a diverse family of fruits and seeds. Table 1 illustrates the scientific classification of *Myristica fragrans*.

Table 1. *Myristica fragrans* taxonomy

Kingdom	Phylum	Class	Order	Family	Genus	Species
Plantae	Tracheophyta	Magnoliopsida	Magnoliales	Myristicaceae	Myristica	Myristica Fragrans

Note: Priyanka *et al.* (2016)

The *Myristica fragrans* grows successfully in a humid tropical area (Adharsh, 2021). The tree thrives in volcanic soils with rich nutrient deposition, which made the Banda Islands fertile for nutmeg cultivation (Wattimena *et al.*, 2021). In general, flowers are dioecious and unisexual. However, there are also bisexual flowers with different sexual compositions. The plantations also occasionally have trees with both male and female blooms (Kharde, 2022). The plant grows in equatorial belts with stable temperatures between 25 and 30 degrees Celsius. Figures 2 and 3 illustrate the various stages of nutmeg before and after harvest. The evergreen plant fruit matures and splits into two, revealing the mace (aril) that covers the kernel (Figure 2). The indigenes consumed the pulp of the fruit (pericarp) locally.



Figure 2. Stages of Nutmeg Harvesting

Note: Gordon (2020)



Figure 3. Production stages of Nutmeg

Note: Gordon (2020). The harvester removes the mace (C) from the kernel (A). The mace (D) dries separately from the seed for up to eight weeks in the sun. It will require patience to harvest quality nutmeg because the kernel demands turning under the sun approximately twice daily (Figure 3). The kernel shell cracks to get the nutmeg seed, which makes the seed ready for consumption and exportation (F).

***Myristica fragrans* Indigenous Cultivation.**

The Banda Islands within the enclave of Maluku archipelago in the 15th Century represented an iconic source of nutmeg (*Myristica fragrans*). The indigenes from Southeast Asia, Indonesia's spice island, cultivated nutmeg for economic value, cultural, culinary, and medicinal purposes (Dhont, 2022). The tree grows well in Humid climatic zones in volcanic-induced soils under a tropical canopy. Birds' seed dispersal has played a significant role in the sustainability and spread of the *Myristica fragrans* on Banda Island. The Banda Islanders' ancestral connection to nutmeg goes beyond economic and medicinal (Kuete, 2017) utilization boundaries. The natives of the Island held nutmeg in high esteem with strong ritual and spiritual value, which deepened their cultural identity and spirituality (Welly *et al.*, 2012; Neswati *et al.*, 2019). Figure 4 illustrates the Moluccas (Maluku province) or Banda Islands.



Figure 4. Topographic map of the Banda Islands, showing the major islands (Neira, Banda Besar, Ai, Run, Hatta) and their location in the Banda Sea.
Note: Adapted from Welly et al. (2012)

The Banda Islands are a volcanic archipelago located in the Banda Sea, comprising 11 small islands, seven of which are inhabited. The principal islands include Banda Besar (Lonthoir), Banda Neira, Banda Api, and Pulau Run.

Historically significant, Run was ceded by the British to the Dutch in exchange for Manhattan Island in the 17th century. Banda Api, an active volcano, has erupted 20 times in the past 400 years, most recently on May 9, 1988 (Neswati *et al.*, 2019). Its volcanic soils are highly fertile and ideal for cultivating *Myristica fragrans* (nutmeg) (Wattimena *et al.*, 2021).

The convergence of the Eurasian, Pacific, and Indo-Australian tectonic plates makes the Banda Sea prone to frequent seismic activity (Hengesh, 2019).

According to the 2023 population census, the islands cover approximately 172 km² and are home to about 21,902 inhabitants, primarily Bandanese and Javanese immigrants (Ali and Heaney, 2021).

Despite the colonial dominance, brutality, and massacres of the 17th century, the Banda Islands remain the world's leading producers and exporters of nutmeg (Mini Raj *et al.*, 2023).

Today, the islands account for approximately 85% of global nutmeg production, with net exports estimated at around 142,000 tons (Figure 5).

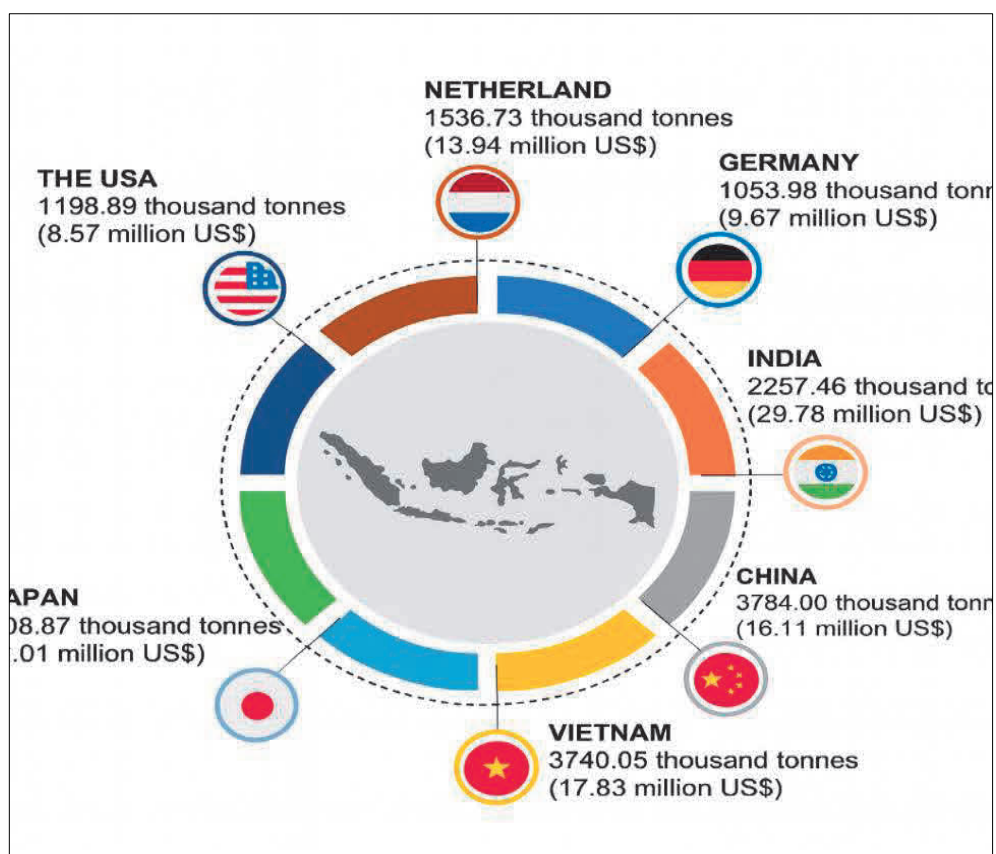


Figure 5. Economic Implications of Nutmeg Exportation from the Banda Islands

Note: Adapted from Statistik Perkebunan Indonesia: Pala 2018–2020 (Directorate General of Estate Crops, 2020) and Statistik Perkebunan Unggulan Nasional 2019–2021 (Directorate General of Estate Crops, 2021).

The Banda Islands continue to maintain global dominance of the spice trade, running into millions of US dollars. The Indonesian government has kept an upbeat global outlook, boosting trade partnerships with seven major economies (the USA, Germany, India, China, Japan, Vietnam, and the Netherlands). In 2019, the Island recorded a net export of 1536.73 thousand tons of nutmeg to the Netherlands (figure 4).

***Myristica fragrans* Colonial Monopolies and Distribution**

Historical evidence shows that the spice trade began centuries ago, predating organized civilization. During the early stages of global exchange, only a limited number of goods traversed long distances. Among the most widely traded spices in the ancient world were cinnamon, clove, pepper, nutmeg, and mace. The acquisition and control of these spices became a marker of political power and global influence.

The intercontinental spice trade originated with the movement of cinnamon and pepper from India and Indonesia to Egypt. Historical accounts suggest that Arab merchants served as the central intermediaries connecting Southeast Asia and the Red Sea port. Functioning as the primary conveyor of spices, Arab traders delivered goods at exorbitant prices to their trade partners. They transported nutmeg to the European market through Mediterranean routes while deliberately concealing the spice's true geographic origin (Hancock, 2021; Micklos Jr., 2017).

Hancock (2021) asserts that nutmeg from Southeast Asia entered the global market through multiple waves of colonial and imperialist intervention. Arab and Indian traders dominated the broader Moluccan spice trade until European interests intensified. Nutmeg's unique

aroma and preservative properties enticed European traders to pursue the plant's origin directly. The collapse of the Arab spice monopoly occurred around 120 BC, when Egyptian Greeks failed to reach the Banda Islands. However, in 30 BC, the Romans assumed control of Egypt and began shipping 120 consignments of Indian spices annually from the Red Sea to various parts of the world (Micklos Jr., 2017).

In 1512, the Portuguese reached the Banda Islands to establish direct trading partnerships. Their fleets, led by Vasco da Gama and Alfonso de Albuquerque, aggressively pursued nutmeg for export to the European market. The Portuguese negotiated unfair deals, acquiring nutmeg, mace, and cloves from the Bandanese at suppressed prices. This development disrupted the existing Arab trade dominance and reduced the market value of their spices. To protect their economic interests, the Portuguese constructed forts across the Banda Islands (Thomaz, 2020). Their dominance persisted until the local population, supported by Dutch conspirators, staged a revolt. Following the uprising, the Portuguese relied on Moluccan intermediaries to access spices, but their influence declined sharply after the Dutch East India Company (VOC) invalidated their trade agreements in the seventeenth century (Landwehr, 2024).

Ghosh (2021) documents that the Dutch invaded and seized control of the Banda Islands, employing brutal tactics against the indigenous population. In a violent assertion of control, they massacred over 15,000 Bandanese people and decimated the local community. The Dutch enslaved survivors and imported laborers from other parts of Asia to cultivate nutmeg plantations. Their strategy included manipulating global market prices and controlling supply by sterilizing seeds. To prevent rival nations from growing nutmeg, the Dutch treated the seeds with lime. They transported the spices through Cape Town enroute to the Netherlands, as illustrated in Figure 6.

The Dutch monopoly exemplified a calculated and violent attempt to dominate global spice routes. Their systematic control over cultivation, labor, pricing, and trade routes elevated nutmeg into a high-value commodity while transforming its native Banda Islands into a site of suffering and resistance. The enduring impact of these colonial monopolies continues to shape the global distribution and economic significance of *Myristica fragrans*.



Figure 6. Nutmeg and Mace Colonial spice trade route connecting Amsterdam, Cape Town, Jakarta, and the Banda Islands.

Note: Adapted from Mappingrecipes (n.d.)

Dutch and British Imperial Rivalries and the Global Spread of *Myristica fragrans*

For over a century, the Dutch East India Company (VOC) maintained a dominant position in the global spice trade. As illustrated in Figure 6, Dutch spice fleets transported substantial quantities of nutmeg and other high-value commodities to Amsterdam via long-distance maritime routes spanning approximately 25,481 kilometers (15,833 miles). The Dutch partitioned the Banda Islands into plantation plots known as *perken*, placing them under the management of VOC-appointed planters to ensure complete control of spice production.

In 1621, the Dutch invaded and destroyed the city of Jakarta.

They established a new administrative and commercial hub, which they named Batavia, now Jakarta. Batavia became the central point for stocking spices from the Banda Islands for onward shipment to the European market. To support their extensive maritime voyages, the Dutch established a strategic resupply station in Cape Town in 1652.

Cape Town provided fresh water, a favorable climate, and a midway point for VOC vessels enroute to the Netherlands. Over the centuries, Amsterdam emerged as a major commercial hub, receiving goods from Asia, Africa, and the Americas for redistribution across Europe (Van der Linde, 2020; Landwehr, 2024).

British imperial forces challenged Dutch dominance in the early 19th century. In 1810, the British navy captured the Moluccas and gained control of nutmeg cultivation and trade. British administrators transported nutmeg trees from the Banda Islands to colonies with similar tropical climates, including Zanzibar and several Caribbean territories. Grenada became a significant producer of nutmeg, a position it retains in today's global market. This strategic transplantation of nutmeg trees undermined the Dutch monopoly and contributed to the worldwide diffusion of *Myristica fragrans* (Callister, 2017; Moore and Van Nierop, 2017; Mini Raj *et al.*, 2023).

Sasikumar (2021) argues that British control of the Moluccas ended with the Treaty of Paris in 1814, which restored the islands to Dutch rule. However, the Dutch could not reestablish their former monopoly. A combination of colonial rivalry, international redistribution, and seed smuggling permanently disrupted their dominance.

French naturalist Pierre Poivre played a critical role in this transformation. In 1770, Poivre smuggled 32 fertile nutmeg seeds and established successful plantations on the island of Mauritius. The success of these plantations led to the spread of nutmeg cultivation to the Maldives, Seychelles, and Madagascar, further diluting Dutch control over the global spice trade (Spence, 2024).

These overlapping imperial interventions, including Dutch colonization, British transplantation, and French botanical smuggling, collectively transformed nutmeg from a locally grown commodity into a transnational crop embedded within global economic systems. Each colonial power exploited the spice's high market value while contributing to the redistribution of nutmeg beyond its native habitat.

Figure 7 presents a contemporary map of nutmeg production zones, illustrating the enduring legacy of colonial redistribution and commercial expansion.

This map highlights the principal nutmeg-producing countries and major tropical regions in the Americas, Asia, and Africa where *Myristica fragrans* is cultivated. Native to the Moluccas of Indonesia, nutmeg spread globally through colonial trade networks and is now commercially grown in Guatemala, India, Indonesia, Laos, Nepal, Sri Lanka, Bhutan, Tanzania, Honduras, and Grenada.

The historical trade of *Myristica fragrans* (nutmeg and mace) demonstrates how imperial ambition, ecological exploitation, and maritime logistics shaped global commodity networks. Archival records and spatial analyses of maritime routes reveal that the Dutch East India Company (VOC) engineered an efficient yet extractive monopoly. As Figure 7 illustrates, VOC ships traveled approximately 25,500 km (15,833 mi) from the Banda Islands to Amsterdam, embedding nutmeg within a worldwide network of supply and demand (Landwehr, 2024;

Van der Linde, 2020; Knaap and Firman, 2017). This transformation elevated nutmeg from a locally cultivated seed to a globally traded commodity and highlighted the VOC's mastery over geography, logistics, and coerced labor.

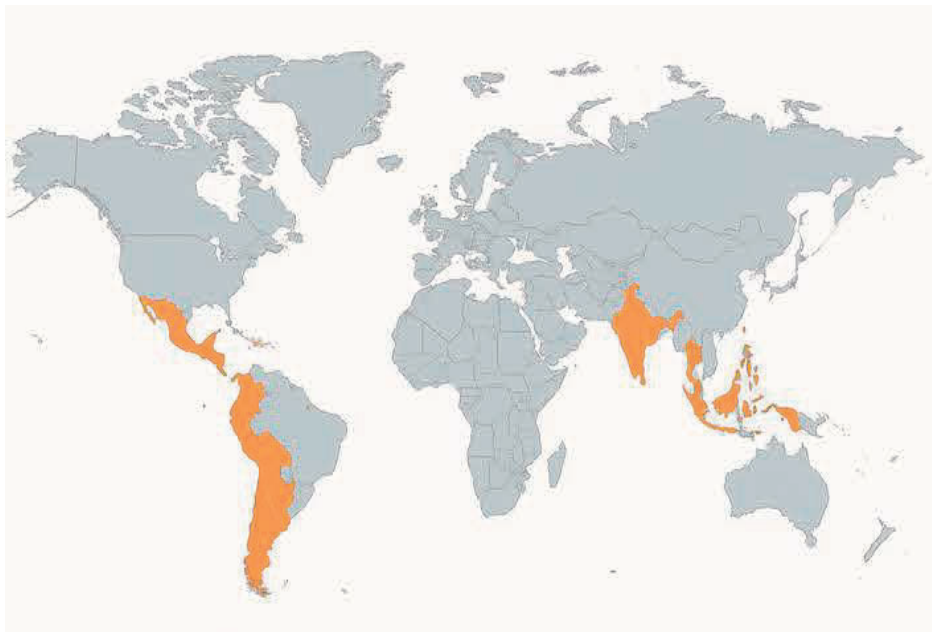


Figure 7. Global distribution of highlighted countries across Latin America, South Asia, and Southeast Asia.

Note: Map created by the author

Nutmeg's historical exclusivity has diminished as its cultivation expanded across multiple biogeographical realms, including the Indo-Malay, Afrotropical, and Neotropical zones. Production has also taken root in Malaysia, the Pacific Islands, and several Caribbean nations. This global distribution has increased supply, lowered prices, and transformed nutmeg from a rare luxury commodity into a widely accessible spice—reshaping its economic and cultural significance.

DISCUSSION

Colonial Expansion and Globalization of *Myristica fragrans*

Monopoly, Violence, and Ecological Control

To consolidate its control, the VOC divided the Banda Islands into *perken* (spice plantations) and installed Dutch planters who depended on enslaved labor imported from across Asia. The founding of Batavia (modern-day Jakarta) in 1621 created a central hub for processing and shipping spices, connecting Southeast Asian production zones to European markets. The 1652 establishment of Cape Town as a resupply port further optimized shipping, reduced voyage times, and increased volumes reaching Amsterdam, soon Europe's primary spice redistribution center (Van der Linde, 2020).

This logistical success carried a human and ecological cost. More than 15,000 Bandanese islanders were massacred for resisting Dutch rule (Ghosh, 2021). The VOC also sterilized nutmeg seeds with lime to prevent rival cultivation, creating a vertically integrated system of monoculture and extraction that set a precedent for later colonial agricultural regimes (Ricklefs, 2020).

Post-Monopoly Diffusion and British Expansion

VOC dominance fractured under geopolitical pressure. British forces seized the Moluccas in 1810, breaking the monopoly and transplanting nutmeg seedlings to colonies with similar climates, including Zanzibar, Sri Lanka, and Grenada (Callister, 2017). Grenada's volcanic soils and rainfall patterns proved ideal for nutmeg cultivation, and its production soon rivaled that of the Moluccas. By diversifying supply, the British diluted Dutch price control and democratized access to nutmeg (Moore & Van Nierop, 2017; Mini Raj *et al.*, 2023).

French naturalist Pierre Poivre further undermined monopolistic control by smuggling 32 nutmeg seeds to Mauritius in 1770, establishing plantations that thrived in the Indian Ocean basin. His efforts catalyzed additional plantings in Madagascar, the Maldives, and Seychelles, embedding *Myristica fragrans* in new agroecological zones and decentralizing global production (Spence, 2024; Cribb and Kahin, 2019). Although the Treaty of Paris (1814) restored Dutch sovereignty over the Moluccas, colonial rivalries and ecological diffusion had permanently transformed nutmeg into a multipolar commodity (Sasikumar, 2021).

Contemporary Production and Global Distribution

The present-day geography of nutmeg production, reflecting the enduring ecological legacy of colonial diffusion (Figure 7). Commercial cultivation now concentrates in Guatemala, India, Indonesia, Laos, Nepal, Sri Lanka, Bhutan, Tanzania, Honduras, and Grenada (FAO, 2023). Smaller but significant production also persists in Malaysia, parts of the Pacific Islands, and select Caribbean nations. This global spread increased supply, lowered prices, and shifted nutmeg from an elite luxury to an accessible household spice, altering centuries-old consumption patterns.

Current Challenges and Sustainability Implications

Modern nutmeg production continues to support livelihoods and regional economies, but also faces ecological and economic vulnerabilities. Monoculture cultivation reduces biodiversity, depletes soil nutrients, and leaves production systems susceptible to pests, diseases, and extreme climate events. These risks mirror the overexploitation concerns of the colonial era and emphasize the need for sustainable agricultural models. Agroforestry integration, crop diversification, and climate-resilient practices can mitigate these threats and ensure long-term production viability (Patra *et al.*, 2022).

Contribution

The historical and contemporary trajectories of *Myristica fragrans* reveal how human agency extends plant dispersal beyond natural boundaries, reshaping ecological and economic landscapes. Nutmeg's journey from a closely guarded island resource to a widely available commodity underscores the intertwined roles of imperialism, forced labor, scientific transfer, and global trade in shaping agricultural systems. This discussion provides a critical framework for understanding the colonial roots of today's spice economy and invites further inquiry into the socio-political histories embedded within everyday agricultural goods.

CONCLUSIONS

This study traced the evolution of *Myristica fragrans* from its origins in the ecological traditions of the Banda Islands to its emergence as a globally cultivated commodity embedded in colonial and postcolonial trade systems. The analysis shows that colonial powers, particularly the Dutch East India Company, used military power, economic leverage, and botanical manipulation to monopolize the nutmeg trade. Dutch forces replaced

biodiverse, community-based cultivation with forced labor regimes and export-oriented plantations, severing the ecological and cultural ties that once defined nutmeg production. Indigenous farmers cultivated nutmeg in ways that preserved biodiversity and sustained ecosystems. Dutch colonizers disrupted these systems, sterilized seeds, fortified trade posts, and suppressed local resistance to secure their monopoly. These actions turned nutmeg into a symbol of imperial ambition and economic dominance while reshaping tropical landscapes.

British and French actors disrupted Dutch control through seed transfers and deliberate introductions of nutmeg to Grenada, Mauritius, and Zanzibar. These interventions broke the monopoly, spread cultivation to new ecological zones, and increased global supply. While these moves widened access to nutmeg and diversified production, they also commodified traditional knowledge and placed new pressures on local environments.

These findings highlight the importance of examining globally traded crops to understand colonial disruption, environmental exploitation, and cross-continental economic entanglement. Nutmeg's transformation from an island-bound spice to a global staple shows how imperial strategies continue to shape agricultural systems, trade flows, and biodiversity today.

The transnational story of *Myristica fragrans* urges researchers, policymakers, and growers to build food systems rooted in equity, sustainability, and cultural preservation. Stakeholders must integrate social and ecological histories into trade and agricultural policy, reclaim indigenous knowledge, protect biodiversity, and redress historical injustice to cultivate inclusive, resilient, and ethically grounded futures.

Future Directions

Researchers should analyze the genetic diversity of *Myristica fragrans* cultivars across producing regions to strengthen resilience against pests, disease, and climate change. Scholars can compare postcolonial nutmeg economies to reveal how plantation-era structures influence today's labor practices and land governance. Interdisciplinary studies that link environmental history, agroecology, and trade policy can generate practical strategies for fairer and more sustainable spice supply chains

Data Availability Statement

This study is based entirely on publicly available secondary data from previously published sources. No new datasets were generated or analyzed during the current study. All data referenced are cited appropriately within the manuscript, and no data are deposited in a public repository.

Declaration of conflicting interest

There is no conflict of interest.

Funding: No funding was received.

REFERENCES

1. Adharsh, C. J. (2021). *Weather extremes preparedness of nutmeg (Myristica fragrans) farmers in Kerala* (Doctoral dissertation, Academy of Climate Change Education and Research, Vellanikkara).
2. Ali, J. R., & Heaney, L. R. (2021). Wallace's line, Wallacea, and associated divides and areas: history of a tortuous tangle of ideas and labels. *Biological Reviews*, 96(3), 922–942.
3. Ali, T. M., Butt, N. A., & Shaikh, M. (2022). Characterization and Extraction Techniques of Nutmeg Oleoresin. In *Handbook of Oleoresins* (pp. 187-233). CRC Press.
4. Allen, C. D., Diller, S. L., & Zabaras, T. (2017). Grenada: the Spice Isle. *Landscapes and Landforms of the Lesser Antilles*, 243-265.

5. Arsenijević, J., Sisto, F., Drobac, M., Šoštarić, I., Maksimović, Z., & Kovačević, N. (2024). In vitro anti-Helicobacter pylori activity of extracts and essential oil of Thymus pannonicus All.(Lamiaceae).
6. Ashokkumar, K., Simal-Gandara, J., Murugan, M., Dhanya, M. K., & Pandian, A. (2022). Nutmeg (Myristica fragrans Houtt.) essential oil: A review on its composition, biological, and pharmacological activities. *Phytotherapy Research*, 36(7), 2839–2851.
7. Borelli, T., Keim, A., Sujarwo, W., Koostanto, H., Pawera, L., Gullotta, G., ... & Hunter, D. (2024). Invisible Treasures: Assessing Indonesia's Unique Agrobiodiversity for Food and Nutrition Security. *Sustainability*, 16(22), 9824.
8. Callister, G. (2017). *War, public opinion and policy in Britain, France and the Netherlands, 1785-1815*. Basingstoke: Palgrave Macmillan.
9. Cribb, R., and Kahin, A. (2019). *Historical dictionary of Indonesia* (3rd ed.). Rowman & Littlefield.
10. Damoah, B., and Khalo, X. (2024). Extreme Temperature Variability in the Southeastern United States: Trends in Mississippi State. *International Journal of Environmental, Sustainability, and Social Science*, 5(6), 1991-2002.
11. Damoah, B., Adu, E., Ofori, E., & Apraku, A. (2024). Aggravation of climate change crisis in Gulf Regions of United States: A Review of Anthropogenic Factors. *Journal of Ecohumanism*, 3(4), 438–449.
12. Dhont, F. (2022). Of Nutmeg and Forts: Indonesian Pride in the Banda Islands' Unique Natural and Cultural Landscape. *ETropic: Electronic Journal of Studies in the Tropics*, 21 (1), 83–98. <https://doi.org/10.25120/etropic.21.1.2022.3864>
13. Directorate General of Estate Crops. (2021). *Statistik Perkebunan Unggulan Nasional 2019–2021* [PDF]. Ministry of Agriculture, Republic of Indonesia. <https://ditjenbun.pertanian.go.id/template/uploads/2021/04/BUKU-STATISTIK-PERKEBUNAN-2019-2021-OK.pdf> DOI: 10.13140/RG.2.1.3543.9201
14. Ducène, J. C. (2024). Medieval Spice Trade in the Arabic World. In *Oxford Research Encyclopedia of Food Studies*.
15. FAO. (2023). *FAOSTAT: Nutmeg production data*. Food and Agriculture Organization of the United Nations. Retrieved from <https://www.fao.org/faostat>
16. Frost, L., Santamaría-Aguilar, D. A., Singletary, D., & Lagomarsino, L. P. (2022). Neotropical niche evolution of Otoba trees in the context of global biogeography of the nutmeg family. *Journal of Biogeography*, 49(1), 156–170.
17. Ghosh, A. (2021). The nutmeg's curse: Parables for a planet in crisis. In *The Nutmeg's Curse*. University of Chicago Press. doi.org/10.7208/chicago/9780226815466
18. Gordon, A. (2020). Market & technical considerations for spices: Nutmeg & Mace case study. In *Food safety and quality systems in developing countries* (pp. 367–414). Academic Press.
19. Hancock, J. F. (2021). *Spices, Scents and Silk: Catalysts of World Trade*. CABI.
20. Hengesh, J. (2019). Neotectonics, seismic hazards, and seafloor stability on Australia's Northwest Shelf.
21. Kharde, S.S. (2022). Overview of Nutmeg. *World Journal of Pharmaceutical Research*, 11(6), 83–89. DOI: 10.20959/wjpr20226-23730.
22. Kuete, V. (2017). Myristica fragrans: A review. *Medicinal spices and vegetables from Africa*, 497–512.
23. Landwehr, J. (2024). *VOC: a bibliography of publications relating to the Dutch East India Company, 1602-1800*. Brill. mappingrecipes. (n.d.). *Nutmeg and Mace Trade Route* [Map]. MapHub. <https://maphub.net/mappingrecipes/map>
24. McCallum, S. (2021). *The Secret Life of an Arable Field: Plants, Animals and the Ecosystem*. Micklos Jr, J. (2017). *Mediterranean trade routes*. Cavendish Square Publishing, LLC.
25. Mini Raj, N., Vikram, H. C., Muhammed Nissar, V. A., & Nybe, E. V. (2023). Nutmeg. In *Handbook of Spices in India: 75 Years of Research and Development* (pp. 2739–2786). Singapore: Springer Nature Singapore
26. Moore, B., & Van Nierop, H. (2017). *Colonial Empires Compared: Britain and the Netherlands, 1750–1850*. Routledge.
27. Moripi, L., & Peki, M. (2015). Analyzing the Distribution Patterns of Nutmeg (Myristica spp.) in Busama Forest Area Using Quadrat Size Technique.
28. Nair, K. P., & Nair, K. P. (2021). Mace and Nutmeg. *Minor Spices and Condiments: Global Economic Potential*, 143–155.
29. Neswati, R., Lopulisa, C., & Basir, A. (2019, June). Characteristics and Classification of Soil Formed from Banda Recent Volcanic Ash on Various Topographic Positions. In *IOP Conference Series: Earth and Environmental Science* (Vol. 280, No. 1, p. 012017). IOP Publishing.
30. Priyanka, S. C., & Miniraj, N. (2016). Morphological characterization of unique genotypes of nutmeg (Myristica fragrans Houtt.). *Journal of Tropical Agriculture*, 54(2), 120–120.
31. Ravindran, P. N. (2017). *The encyclopedia of herbs and spices*. CABI.
32. Sasikumar, B. (2021). Nutmeg-Origin, diversity, distribution and history. *Journal of Spices and Aromatic Crops*, 30(2), 131–141.
33. Schluter, D., & Pennell, M. W. (2017). Speciation gradients and the distribution of biodiversity. *Nature*, 546(7656), 48–55.
34. Spence, C. (2024). Nutmeg and mace: The sweet and savoury spices. *International Journal of Gastronomy and Food Science*, 100936.

35. Sujianto, S., Pribadi, E. R., Saptati, R. A., Mahendri, I., Santoso, A. B., Sondakh, J. O. M., ... & da Silva, H. B. (2024). Assessing Indonesian Nutmeg Commodity Trade Competitiveness and Developing Sustainable Strategies in the Global Market. *Agris On-Line Papers in Economics & Informatics*, 16(3).
36. Swaminathan, M. S., & Kochhar, S. L. (2019). *Major flowering trees of tropical gardens*. Cambridge University Press.
37. Thomaz, L. F. F. (2020). The Portuguese in the Seas of the Archipelago during the Sixteenth Century. In *European Commercial Expansion in Early Modern Asia* (pp. 25–42). Routledge.
38. Ulfah, T., Hardjomidjodjo, H. H., & Anggraeni, E. (2020, April). Nutmeg determination as the main commodity in South Aceh: a literature review. In *IOP Conference Series: Earth and Environmental Science* (Vol. 472, No. 1, p. 012040). IOP Publishing.
39. Van der Linde, H. (2020). *Jakarta: History of a misunderstood city*. Marshall Cavendish International Asia Pte Ltd.
40. Wattimena, A. Y., Makaruku, M. H., Kembauw, E., & Mahulette, A. S. (2021, October). Nutmeg Cultivation Intensity (*Myristica fragrans* Houtt) in Banda District. In *IOP Conference Series: Earth and Environmental Science* (Vol. 883, No. 1, p. 012046). IOP Publishing.
41. Welly, M., Djohani, R., Suharsono, A. G., Muljadi, A., Korebima, M., Hehuat, Y., ... & Rijoli, N. (2012). MARINE RAPID ASSESSMENT.
42. Whiteman, N. (2023). *Most delicious poison: the story of nature's toxins—from spices to vices*. Little, Brown Spark.



Viticulture and Oenology

Confirmed vocations towards high quality oenological achieved standards ascertained in regard to some vine kinds created at the Dragasani vineyard

A. Popa and F. Tuțulescu*

**University of Craiova, Horticulture Faculty,
Romania**

*Corresponding author e-mail: felicia.tutulescu@edu.ucv.ro

ABSTRACT

At the Research Station for Grape Growing and Winemaking in Dragasani, there have been and continue to be concerns related to improving grapevine genotypes as well as creating new genotypes for both table grapes and wine grapes. This study presents the oenological characteristics of the 'Victoria', 'Novac', and "Negru de Drăgășani" genotypes, obtained and cultivated at the Dragasani Station. Comparing the biological and technological characteristics of the progenitors of the 'Victoria' genotype, it is observed that it surpasses its progenitors and shows the greatest technological potential in Dragasani, compared to other vineyards where it is cultivated. Wines obtained from the 'Novac' and "Negru de Drăgășani" grapes can be compared to those obtained from the 'Cabernet Sauvignon' genotype, which they can surpass in certain oenological indicators.

Keywords: grapevine genotypes, improving grapevines, genotypes for the table, wine genotypes

INTRODUCTION

Research conducted over time in the field of grapevine cultivation in Romania has allowed the achievement of remarkable levels of scientific accuracy, gradually accumulated and reinforced through practical observations. These results enabled Romanian practitioners, especially after 1970, to develop new grapevine genotypes and clones adapted to the specific Romanian ecological conditions, all characterized by efficient productive capacities and clear potential for achieving the highest oenological standards.

The genuine oenological value respectively held by all of these creations has been consequently pointed out through many scientific publications (Teodorescu 1928; Popescu, Basamac 1960; Constantinescu et al. 1968; Neagu *et al.* 1968; Lepădatu, Condei 1991; Condei *et al.* 2004; Oprea, Moldovan 2007; Teodorescu *et al.* 2021; Popa *et al.* 2023; 2024).

As direct consequences of our investigations aimed at precisely defining the agricultural, biological and technological advantages empirically observed in the newly developed grapevine genotypes ('Victoria', 'Negru de Drăgășani' and 'Novac') this study presents a set of scientific arguments confirming the evident potential of these genotypes to achieve high levels of oenological quality.

MATERIALS AND METHODS

The study material consisted of the newly developed grapevine genotypes from the Dragasani vineyard, including 'Victoria', "Negru de Drăgășani", and 'Novac'. To accurately assess the agricultural, biological, and technological characteristics of the newly developed grapevine

genotypes, research methods recognized by the International Organization of Vine and Wine were applied. Observations and empirical evaluations were conducted directly in the vineyards and auxiliary processing facilities of the selected production units. Laboratory analyses were carried out at the Research Station for Vine Culture and Winemaking in Dragasani, the Faculty of Horticulture at the University of Craiova, and the Regional Laboratory for Wine Control and Analysis in the commune of Banu Maracine, Dolj County. Table 1 illustrates the vine genotypes obtained over time at Dragasani Research Station. In this article, scientific arguments are presented to confirm the vocation of these genotypes for producing high-quality wines, hypotheses that can be validated through their progressive expansion in current vineyards.

Table 1. New valuable vine kinds and clones created from the currently cultivated kinds and obtained by the use of the *Vine Germs&Plasma Collection* from Dragasani

Vine kind/Genitors	Homologation year	Authors
1	2	3
I. Vine kinds destined to produce table grapes and sultanas		
'Azur' (Coarnă Neagră X 'Cardinal')	1984	'Victoria' Lepădatu, Gheorghe Condei
'Victoria' ('Cardinal' X Afuz Ali)	1978	'Victoria' Lepădatu, Gheorghe Condei
'Călina' (Braghină X Sultanină)	1993	M. Mărculescu, M. Vlădăşel
II. Vine kinds destined to produce white wines		
Selected Crâmpoşie (naturally fecundated Crâmpoşie)	1972	Em. Popescu, M. Neagu, P. Baniţă, I. Dobrescu, M. Mărculescu
Vilarom (Hamburg Muscat X Crâmpoşie X Ottonel Muscat 436)	2003	M. Mărculescu
'Aromatic Crâmpoşie' (Selected Crâmpoşie X Hamburg Muscat)	2023	M. Mărculescu, I.B. Truşcă, S.Şt. Gorjan, I.R. Popescu, M. Şeiculescu
III. Vine kinds destined to produce red and rosé wines		
Alutus (Băbească Neagră X 'Saperavi')	2003	M. Mărculescu
'Negru de Drăgăşani' ('Negru Vârtos' X 'Saperavi')	1993	M. Mărculescu, M. Vlădăşel
'Novac' ('Negru Vârtos' X 'Saperavi')	1987	M. Mărculescu
IV. Clones obtained from the vine kinds destined to produce white and red wines		
'Petit Sauvignon' 62 Dg	1984	M. Mărculescu
'Tămâioasă Românească' 104 Dg	1982	M. Neagu, M. Mărculescu
'Cabernet Sauvignon' 7 Dg	1976	P. Baniţă, D. Basamac
V. Vine kinds and clones destined to produce engraft-bearing plants		
'Drăgăşani M 70'	2003	M. Mărculescu, M. Baniţă, M. Vlădăşel, M. Ciolacu, M. Şeiculescu
'Teleki 8 B' selected from Dragasani 57	1987	P. Baniţă

RESULTS AND DISCUSSIONS

The "Victoria" grapevine genotype was created through a deliberate interbreeding process between the "Cardinal" and 'Afuz Ali' genotypes.



Figure. 1. 'Victoria' vine kind'

Table 2. The 'Victoria' vine kind's main agricultural, biological and technological features hereby compared to the ones held by its genitors

Size parameter	"Cardinal"	'Victoria'	'Afuz Ali'
Cluster weight (g)	342.3	594.7	488.1
100 grapes weight (g)	596.8	860.7	628.3
Pedicle length (mm)	9.0	11.5	9.8
Grape length (mm)	23.4	30.4	24.1
Grape width (mm)	20.6	21.8	18.5
Seeds number in grape	2.5	1.9	1.6
Mean harvest (t/ha)	14.5	21.2	13.7
Commodity output (t/ha)	10.5	18.3	11.9
Sugar (g/l)	141.2	144.3	127.1
Tartaric acid amount (g/l)	5.27	5.63	6.51
Ascorbic acid amount (mg/100 g)	8.8	11.3	11.2
Transportation safety index	25	38	36
Resilience degree (points)	50	80	67
Dry substance biological mass (g/log)	2320	2910	2818

From the data presented in Table 2, we are therefore entitled to ascertain the respective facts that this vine kind's main agricultural, biological and technological features do prove to be by far superior in regard to the ones respectively held by its parental kinds. When being cultivated within three plots from Oltenia of which the local micro-climates and soil specific circumstances are for each of them different from the ones held by the other two (Table 3) the 'Victoria' kind does manifest a most suitable offshoots productive output while in the meantime its harvest's both quantitative dimensions and attained to quality levels do indeed overcome the ones respectively held by all of the other vine kinds destined to produce table grapes which are also cultivated in our country (Table 4).

Table 3. Offshoots productive output of the 'Victoria' vine kind when cultivated within some plots from Oltenia

Vine cultivated area	Year	Mean cluster weight (g)	Productivity Indices	
			Absolute	Relative
Dragasani	2020	291	456	331
	2021	294	441	358
	2022	293	501	381
	<i>Mean (2020-2022)</i>	292	484	347
Ramnicu Valcea - Jiblea	2020	285	456	324
	2021	290	435	353
	2022	293	501	351
	<i>Mean (2020-2022)</i>	286	474	340
Bechet - Dabuleni	2020	289	410	179
	2021	290	397	185
	2022	290	411	159
	<i>Mean (2020-2022)</i>	283	396	169

Yet as a matter of fact should the same vine kind be cultivated within the Dealul Olt – Dragasani area along its naturally shaped slope (Table 5) we could therefore ascertain the facts that its respectively obtained harvest's quantitative amounts and their attained to quality levels would be quite different in respect to the above presented case since upon it

only the grapes harvested from its upside could effectively reach for their highest possible quality level.

Table 4. Grape harvests and respective quality levels held by the 'Victoria' vine kind when cultivated within some plots from Oltenia

Vine cultivated area	Year	Grapes amount for one vine log				Sugar (g/l)	Acidity (g/l H ₂ SO ₄)	Gluco-acidity metric index
		Total amount (kg)	Extra (%)	I - st quality (%)	Commodity output (%)			
Dragasani	2020	3.709	71	11	82	135	4.11	32.84
	2021	6.450	69	12	81	139	4.00	34.75
	2022	7.200	75	10	85	140	4.05	34.56
	Mean	5.786	71.66	11	82.66	138	4.05	34.07
Ramnicu Valcea - Jiblea	2020	5.250	62	16	78	136	4.00	34.00
	2021	7.040	64	17	81	140	3.95	35.44
	2022	6.940	69	18	87	141	3.84	36.71
	Mean	6.410	65	17	82	139	3.93	35.36
Bechet - Dabuleni	2020	4.390	69	21	90	130	3.20	40.62
	2021	5.040	70	22	92	129	3.29	39.28
	2022	5.000	68	23	91	139	3.26	42.63
	Mean	4.810	69	22	91	132.66	3.25	40.81

Table 5. Amounts and quality levels achieved by the 'Victoria' vine kind while cultivated within the Dealul Olt – Dragasani area, throughout various segments of its naturally shaped slope

Log situation upon the slope	Harvest year	Grape amounts for one log				Sugar (g/l)	Acidity (g/l H ₂ SO ₄)	Gluco-acidity metric index
		Total amount (kg)	Extra (%)	I - st quality (%)	Commodity output (%)			
Downside slope's third part	2020	7.440	53	33	86	136	4.35	31.26
	2021	7.140	51	30	81	135	4.40	30.68
	2022	6.295	52	29	81	137	4.52	30.30
	Mean	6.958	52	30.66	82.66	136	4.42	30.76
Amidst slope's third part	2020	3.709	71	11	82	135	4.11	32.84
	2021	6.450	69	12	81	139	4.00	34.75
	2022	7.200	75	10	85	140	4.05	34.56
	Mean	5.786	71.66	11	82.66	138	4.05	34.07
Upside slope's third part	2020	6.480	73	20	93	150	4.95	37.97
	2021	6.497	74	18	92	145	3.88	37.37
	2022	6.590	74	19	93	146	3.92	37.24
	Mean	6.522	73.66	19	92.66	147	3.91	37.59

The grapes maturation process is therefore effectively accomplished during the scientifically speaking acknowledged as the II-nd time period because this vine kind's naturally imparted resilience interval (before falling down from the log) does seem to be rather large (of around 30 days). Its harvests quantity amounts are as well rather large (of about 16 until 18.6 t/ha) from which their respective commodity outputs' amounts may elevate themselves towards 83% or even 85%. At the very moment of their most adequate maturity (in view of consumption) this vine kind's grapes might accumulate from 140 g/l until 150 g/l of sugars while in the meantime their acidity's respective levels do prove to be (at least relatively) some quite low ones such as standing from 3.5 g/l H₂SO₄ until 3.8 g/l H₂SO₄. These do as well present some most adequate resilience capacities during their transportation processes. Their most outstandingly remarkable features respectively are:

earliness in time; average high productive potentialities; an empirically most attractive physical appearance of the grapes. All of these above-mentioned assets do point out this vine kind as being one among the valuable Romanian such kinds (plainly speaking it is therefore seen as the best ever genuine Romanian creation among them) destined to produce table grapes. It is thus most highly recommended for cultivation throughout all of the vineyards and vine cultivating centers which are specialized for the production of table grapes. As a matter of fact we are entitled to state that recently in Romania (more precisely in 2021) the 'Victoria' kind has occupied a total cultivated surface of 154.01 hectares. As it is indeed a most highly praised vine kind its culture has as well been taken into due consideration by some other vine cultivating countries throughout Europe (Italy – with around 7000 hectares; France; Spain; Portugal; Greece). It has also become duly known outside of our continent (as for example in Australia. China. South Africa etc.).



Figure 2. 'Negru de Drăgășani' vine kind's



Figure 3. 'Novac' vine kind's

Those two vine kinds have both been issued from the same genitors (namely from 'Negru Vârtos' and 'Saperavi'). They are also both destined to create some high-quality red and *rosé* wines.

At the 'Novac' kind the grapes maturation process should be accomplished throughout the scientifically speaking acknowledged as the V-th time period; its average fertility ratio is rather a middle-sized one (54% of fertile offshoots); its respectively related coefficients are: - the relative one of 0.84; - the absolute one of 1.48; it does present some most suitable tolerance capacities towards manna. oidium and the grapes rotten grey mildew; the moment of its grape's fully accomplished maturity does occur during the second half of September; its averagely accumulated amounts of sugar do use to stand in-between 200 g/l and 220 g/l; its total acidity amounts could then be situated from 5 g/l until 6 g/l (of H₂SO₄); its averagely ensured harvested quantities might usually stand in-between 19 t/ha and 24 tons/ha. As it is cultivated within the Dealul Olt – Dragasani area (for trading purposes) the oenological potentialities of which it could effectively dispose have been by us most accurately evaluated (Table 6). In the hereby studied case the grapes have been harvested ten days after the moment of having reached to their full oenological maturity.

Table 6. Oenological potentialities held by the 'Novac' vine kind as it is cultivated in the Dealul Olt – Dragasani area (Harvesting the grapes 10 days after full maturity)

Harvest year	Grapes produced amounts (kg/ha)	Glucose s (g/l)	Total acidity (g/l H ₂ SO ₄)	Tartaric acid (g/l)	pH	Total poly-phenols (g/kg of grapes)	Anthocyanin s (mg/kg of grapes)
2006	10150	213	4.36	3.67	3.06	4.11	1395
2007	10080	214	4.23	3.88	3.04	4.31	1402
2008	9960	210	4.05	3.61	3.06	4.10	1411
2009	9855	207	4.09	3.39	3.07	4.25	1401
Mean	10019	211	4.18	3.64	3.06	4.19	1402

Table 7. Evolution throughout time of chromatic features held by wines issued from grapes of the 'Novac' vine kind

Wines chromatic features	At separation moment of must hard-boiling process	Ages of wines expressed in months					
		3	6	9	12	15	18
		Maturation process age gathering process					
Wines colours intensities (CI)	1.675	1.624	1.504	1.436	1.366	1.351	1.324
Wines colors tonalities (CT)	0.542	0.550	0.566	0.567	0.592	0.593	0.596
<i>Flavilium</i> cathode ions (dA%)	60.56	59.89	59.35	59.70	58.10	58.07	58.21

From the above presented Tables 6 and 7 we do estimate ourselves as being scientifically entitled in the case of the 'Novac' vine kind to ascertain the existence of the following active as well as highly significant oenological valuable features: the existence of a great productive potentiality; some quite large quantitative accumulations of sugar while this vine kind is also most magnanimously endowed with some rather considerable respective amounts of poly-phenols and of anthocyanins. By the use made of the classical red-type wine-making technology the wines issued from the grapes of the 'Novac' kind had been therefore respectively submitted to the maturation and then age gathering processes. Table 7 above does present as scientific evidence some experimentally gathered data which do effectively stand as proofs of the existence in the case of the 'Novac' kind's issued wines of a genuine vocation towards some as successful as highly valuable (under oenological terms) age gathering processes. Insofar they could be ever concerned the facts are respectively obvious that the 'Novac' wines chromatic parameters (intensity and tonality) do both evolve in the sense of improving the wines held quality levels since they are not just endowed with some perfectly balanced compositional structures but furthermore (as some most attractive details) their above mentioned chromatic respectively attained to intensities do obviously reach for some quite high values while their corresponding tonalities (nuances of their colors) could indeed acquire some ruby red reflective glimpses which do essentially define the greatest wines that pertain to this type.

'Negru de Drăgășani'

This vine kind could be essentially defined through the following features: a medium-sized stamina growth ratio; a most suitable fertility output (65% to 75% of fertile offshoots); a quite large tolerance capacity concerning the grapes' grey mildew; some rather adequate resilience capacities regarding drought, to manna and to the flour-like disease; a rather developed survival capacity while submitted to extremely low – plainly speaking to stone-freezing - temperatures (even until -22°C); the grapes maturation process does become fully accomplished during the scientifically denominated *V-th time period*; the grapes are averagely accumulating sugars in amounts which do stand in-between 195 g/l and 230 g/l; its total acidity amounts do never fall under the minimum value of 4.7 g/l of H₂SO₄; in quantitative terms its achieved harvests do averagely reach for productions going from 11 tons/hectare until 16 tons/hectare. As for its wines organoleptic (that is to say empirically attractive) features these do as well seem to be quite outstanding: their contained amounts of extract do averagely oscillate from 22 g/l until 26.6 g/l; their alcoholic concentrations do stand in-between 12.5 vol% and 13 vol%; these do always present some averagely quite high color intensities (should this even be after the passed time intervals dedicated to the maturation and next to the age gathering undergone processes when as a matter of fact these do eventually come to acquire some exquisite color nuances which do seem to be most beautifully kindred to the ones commonly held by rubies). As this vine kind is cultivated

within the Dealul Olt – Dragasani area (for trading purposes) the oenological potentialities of which it could effectively dispose have been by us most accurately evaluated (Table 8). In the hereby studied case the grapes have been harvested ten days after the moment of having reached to their full oenological maturity.

Let us therefore enumerate some obvious and through figures above illustrated assets of the 'Negru de Drăgășani' vine kind: at the moment of having achieved their full maturity its grapes are holding some quite suitable levels of total acidity; under these circumstances the average harvests productive potentialities as well as the ones of their average sugars accumulations do prove to hold some rather constant values; the future wines do prove to be endowed with a quite strong vocation for some long-lasting and in a good health preservation time intervals through the ratios respectively held by tartaric acid within the frames of their total acidity amounts; its wines are quite likely to dispose of both some quite high colors intensities and simultaneously of some rather beautiful colors tonalities; these assets are respectively guaranteed through the most suitable levels reached to by their contained amounts of poly-phenols and anthocyanins.

Table 8. Oenological potentialities held by the 'Negru de Drăgășani' vine kind as it is cultivated in the Dealul Olt – Dragasani area

Harvest year	Grapes produced quantities (kg/ha)	Glucides (g/l)	Total acidity (g/l H ₂ SO ₄)	Tartaric acid (g/l)	pH	Total poly-phenols (g/kg grapes)	Anthocyanins (mg/kg grapes)
2006	11105	216	4.29	3.52	3.05	4.09	1389
2007	10600	219	4.17	3.69	3.04	4.38	1398
2008	10050	212	4.11	3.72	3.06	4.19	1465
2009	9988	209	4.12	3.44	3.07	4.21	1422
Mean	10436	214	4.17	3.59	3.06	4.22	1419

Table 9. Anthocyanins held amounts and amounts of respective components of their coloring complex chemical aggregate at wines issued from the 'Novac' and 'Negru de Dragasani' vine kinds while cultivated within the Dealul Olt - Dragasani area
(Mean values for the time interval 2006 - 2009)

Vine kinds	Anthocyanins (mg/l)	Chromatic structure			Chromatic assets		
		Yellow pigments (%)	Red pigments (%)	Blue pigments (%)	Colors intensities (I _c)	Colors tonalities (T _c)	Flavilium cathodeions (dA%)
'Cabernet Sauvignon'	724.3	28.9	60.3	10.8	15.79	0.478	66.74
'Merlot'	662.8	30.0	60.2	9.5	14.44	0.502	66.56
'Fetească Neagră'	662.7	30.2	59.8	10.0	13.94	0.505	65.97
'Novac'	768.5	30.5	57.0	12.5	15.95	0.534	61.83
'Negru de Drăgășani'	776.6	30.3	57.5	12.2	16.06	0.527	62.80
'Pinot Noir'	383.8	34.5	56.6	8.9	9.35	0.609	61.38

To provide the clearest outlines of the above mentioned two vine kinds coloring chemical complex components as well as the exact values of their anthocyanins amounts we are hereby presenting (Table 9) the results obtained through the analyses we have performed upon some wines issued from the vine kinds of 'Negru de Drăgășani' and 'Novac' while these are placed in a comparative situation regarding some other wines respectively created from vine kinds which have longtime since already acquired some at least excellent reputations through undergoing their cultivation processes ("Cabernet Sauvignon", "Merlot", 'Fetească

Neagră’ and ‘Pinot Noir’). We have therefore been able to ascertain the respective facts that the wines created from ‘Novac’ and ‘Negru de Drăgășani’: do enjoy a chromatic structure endowed with an exquisite and most finely balanced equilibrium; do effectively dispose among the hereby compared vine kinds of the greatest amounts of anthocyanins; as the ‘Cabernet Sauvignon’ wines do continue to be most highly praised as being the: "kings of red wines" we are therefore scientifically entitled to state that the respective colors intensities and tonalities held by the wines coming from the kinds of ‘Novac’ and ‘Negru de Drăgășani’ are indeed more obviously outstanding than the ones achieved by the former. As the vine kinds ‘Novac’ and ‘Negru de Drăgășani’ (as we have ourselves demonstrated above) have undoubtedly proven their effective vocations for high quality levels and for highly productive amounts throughout their harvests these have as well of course been quickly adopted by the Romanian practitioners of our domain for current cultivation: until the present day each of them two has come to be cultivated upon total surfaces of around 100 hectares. As a matter of fact these two kinds have been also legally authorized for cultivation throughout all of the Romanian vineyards and vine cultivated centers which are really endowed with the vocation towards creating red high-quality wines (this been said with an outstandingly particular mention addressed to the ones respectively situated in the Romanian historically attested provinces of Oltenia, Wallachia, Banat and Dobrousa).

CONCLUSIONS

The grape genotypes ‘Victoria’, “Negru de Drăgășani”, and ‘Novac’ were developed at the Dragasani Research Station and subsequently introduced into cultivation in many vineyards across the country and abroad, especially the ‘Victoria’ genotype.

A comparative analysis of the agro-biological characteristics of the ‘Victoria’ genotype and its parent genotypes showed that it surpasses the genotypes from which it was created, which supports its introduction into cultivation as a table grape genotype. The “Negru de Drăgășani” and ‘Novac’ genotypes exhibit very good characteristics, particularly regarding color structure, surpassing certain parameters of the ‘Cabernet Sauvignon’, “Merlot”, or ‘Pinot Noir’ genotypes, which are renowned worldwide for producing red wines.

The three analyzed genotypes perform very well in cultivation in the Dragasani vineyard, constituting a very valuable germplasm resource.

REFERENCES

1. Condei, Gh., Lepădatu, V., Popa, D., Popa, A. (2004). La vocation Européenne du cru ‘Victoria’. W.O.V.W.’s Worldwide Congress. Vienna – Austria.
2. Constantinescu, Gh., Olga Alexei, O. (1968). Les cépages roumains. Rédaction des revues agricoles. București.
3. Lepădatu V., Condei Gh. (1991). Quelques aspects de la technologie utilisée au cépage ‘Victoria’ en plein air et sur plastique. Atti del symposium internazionale sulle enze di tavola Bari – Italia.
4. Neagu, I.M., Gorodea, Gr., Negreanu, E., Toader, M., Oprea, Șt., Popa, E., Duschin, V., Popescu, Gh., Mărculescu, M., Toma, O. (1968). Studiul însușirilor agrobiologice ale soiurilor de struguri pentru masă recent introduse în cultură. Analele I.C.V.V. Valea Călugărească vol. I pag. 37 – 54. Centrul de documentare agricolă. București.
5. Oprea, Șt., Moldovan, S.D. (2007). Ameliorarea viței-de-vie în România Editura Poliam. Cluj-Napoca.
6. Popa A., Trușcă I.B., Genoiu C.T., Gorjan S., Popescu I.R. (2023). Colecția de germoplasmă viticolă Dragasani. Editura AIUS Craiova.
7. Popa, A., Trușcă, I.B., Genoiu, C.T., Gorjan, S., Popescu I.R. (2024). Soiuri de viță-de-vie autohtone tezaurizate la Dragasani Editura AIUS Craiova. 2024.
8. Popescu, E., Basamac, D. (1960). Caracteristicile de productivitate ale principalelor soiuri de viță roditoare din podgoria Dragasani. Lucrările științifice ale I.C.H.V. vol. IV 1960/1961. Editura Agrotehnică. București.
9. Teodorescu, C.I. (1928). Valoarea productivă a vițelor românești Revista România Viticolă. București.
10. Teodorescu, Șt., Popa, A., Sandu, Gh. (2021). Oenoclimatul României ediția a II-a. Editura AIUS Craiova.

Agrobiological evaluation of seedless grapevine cultivars for improving the grapevine assortment

S. Tofan* and L. Yuşhan

Department of Genetic Resources, Breeding and Clonal Selection of Horticulture Plants, Public Institution National Institute for Applied Research in Agriculture and Veterinary Medicine, Chisinau, Republic of Moldova

*Corresponding author email: svetatofan79@gmail.com

ABSTRACT

In the paper are presented the results of studying the agrobiological characteristics of some seedless cultivars from the Ampelographic collection of PI NIARAMV: 'Bessemeannyi V-6', 'Călina', 'Centennial seedless', 'Flame seedless', 'Kışmış lucistâi', 'Mecita', 'Otilia', 'Perlette', 'Perlon' – *V. vinifera* L. cultivars and interspecific cultivars 'Himrod', 'Interlaken' and 'Romulus'. The phenological stages, fertility coefficients, productivity indices and winter hardiness were assessed, and the relationships among the main fertility components were examined through linear regression analysis. The investigated cultivars exhibited a wide range of berry maturity periods—from very early to late—together with variable levels of fertility and productivity indices. Several genotypes showed favorable agronomic traits, highlighting their potential value as genetic resources for seedless grape breeding programs.

Keywords: phenophases, fertility, productivity indices, genotypes, *Vitis vinifera*

INTRODUCTION

Worldwide, grapes play a substantial role in food consumption, being one of the most widely used fruits (Iasiello, 2017). According to data provided by FAO and OIV (2016), grapes have the highest value of global production in the world. The use of fresh grapes and raisins in the last 20 years, on a global scale, has increased 1.5 times (Bucur, 2011). The market is in demand for grapes with an attractive external appearance, medium or large berries, of various colors, with an intact layer of bloom, with a crispy flesh consistency, aromatic taste and a low number of seeds. There is an increased demand for cultivars with seedless grapes and those grown with reduced chemical treatments, environmentally friendly. Dgani *et al.* (2014), mention that of the table grapes sold in the world, seedless cultivars account for about 80%, the lack of seeds being a much appreciated and sought-after quality. Seedless grapes have certain characteristics, which make the fruit of superior quality and are used both in fresh form and for technological processing to produce raisins, juices, compote, jam, marinades.

The trend for the consumption of seedless grapes has increased, and is continuously growing, thus, emphasis is placed on the creation and research of seedless cultivars, as well as the expansion of the cultivated areas of seedless cultivars. Due to the specific requirements of the classic seedless cultivars (of oriental origin) in relation to climatic conditions, their distribution area is limited. These cultivars are more widespread in regions with a longer vegetation period, with the high sum of temperatures and with warm and dry autumns. Cultivated in temperate climate areas, they are characterized by low productivity,

explained by the insufficiency of the necessary climatic conditions during the period of flower bud deposition (Constantinescu and Indreăş, 1976).

The need to complete the assortment with seedless cultivars requires the creation of cultivars with a short vegetation period, with an earlier flower bud deposition period, adapted to the climatic conditions of the temperate zone, with high genetic resistance to unfavourable biotic and abiotic environmental factors, high productivity, quality, precocity, large berry, obtaining ecological production, environmental protection, reduction of chemical pressure (Savin, 2012).

The success of breeding programs is determined by the presence and availability of a sufficient number of diverse, valuable genetic resources. The genotypes accumulated in the ampelographic collection of PI NIARAMV, including the seedless ones, represent a vast agrobiodiversity of genetic resources (Savin *et al.*, 2009; Savin, 2012; Tofan, 2023), which allows highlighting potential candidates for their inclusion in breeding programs, as parents, the creation of new seedless cultivars for the continuous improvement of the grapevine assortment in the Republic of Moldova.

MATERIALS AND METHODS

The research was conducted during 2018-2022 within the Ampelographic Collection and adjacent sectors of the Grapevine Gene Fund of the Scientific-Practical Institute of Horticulture and Food Technologies (currently Public Institution National Institute of Applied Research in Agriculture and Veterinary Medicine).

The Ampelographic collection and experimental fields are located in the southern part of the Chisinau municipality (46°58'39.65" N and 28°46'21.68" E, altitude 201 m), on plot with a south-westerly exposure, with a slope inclination of 3-5°. The soil is a weakly eroded ordinary chernozem, clayey, density 1.21-1.38 g/dm³, pH - 7.2-7.3. The study included introduced seedless cultivars (Table 1), diverse by direction of grape use, country of origin and genetic origin (intraspecific cultivars *V. vinifera* L., as well as interspecific cultivars 'Himrod', 'Interlaken' and 'Romulus').

Table 1. List of seedless cultivars included in the study

Cultivar	Country of origin	Genetic origin	Berry color
'Besemeannyi V-6'	Bulgaria	Italia x Kişmiş alb	Green yellow
'Călina'	Romania	Braghină x Sultanină	Rose
'Centennial seedless'	USA	Gold x [Emperor x Pirovano 75]	Green yellow
'Flame seedless'	USA	[Cardinal x Thompson seedless] x [(Red Malaga x Tifafihi Ahmer) x (Muscat de Alexandria x Sultanina)]	Dark red
'Himrod'	USA	Ontario x Kişmiş alb	Green yellow
'Interlaken'	USA	Ontario x Sultanina	Green yellow
'Kismis lucistâi'	R. Moldova	Cardinal x Kişmiş roz	Rose
'Mecita'	Ukraine	Ceauş roz x Kişmiş negru	Rose
'Otilia'	Romania	Alphonse Lavallee x Perlette	Blue black
'Perlette'	USA	Regina viilor x Kişmiş alb	Green yellow
'Perlon'	Argentina	Emperor x Perlette	Dark red violet
'Romulus'	USA	Ontario x Sultanină	Green yellow

Development of the main phenological phases during the vegetation period, Fertility (Fertile shoots and Coefficient of fertility), and the Index of productivity of seedless cultivars were made. Each genotype is represented by 5-10 plants. Planting scheme of 3.00 x 1.50 m, the training system of the bushes is a bilateral cordon on the trunk of 60 cm. The vine culture is

unprotected for winter conditions. Statistical processing of the data (regression analysis, ANOVA and LSD test) and graphical presentation of the results were carried out with the EXCEL and STATISTICA 7.0 program packages.

RESULTS AND DISCUSSIONS

The climate of the Republic of Moldova is temperate-continental and is characterized by relatively warm, short winters with little snow, warm and long summers, a sum of active temperatures of 2750- 2750-3400°C. The weather conditions of the experimental sectors correspond to the conditions of the Codru wine-growing area of the Republic of Moldova and represent the average values for the republic (Savin, 2012).

During the study period, various temperature regimes were observed, including dry summers with record high temperatures, which allowed the study of the behaviour of genotypes in extreme conditions. The average annual air temperature recorded values of +10.6°C °C...+12.7°C, the absolute maximum reaching +33.2°C °C...+35.4°C °C, and the absolute minimum -6.9°C ...-16.7°C (Figure 1, a). The amount of annual precipitation falling in the research years (2018-2022) varied between 403-666 mm, and in the vegetation period (April-October) it amounted to 260-465 mm (Figure 1, b). The year 2019 was recorded as the driest year in the research period.

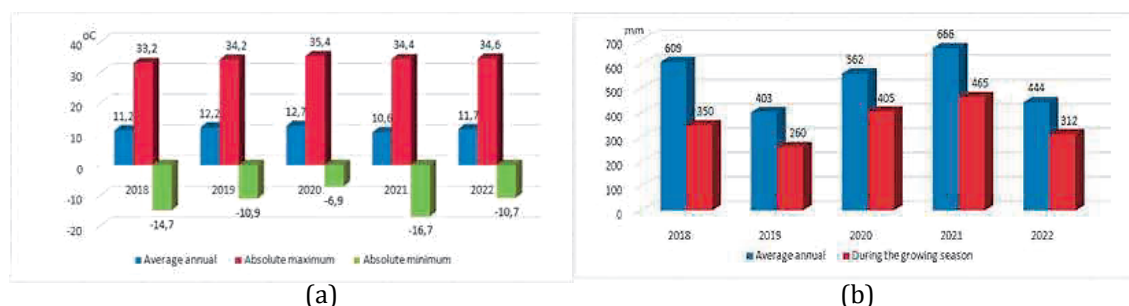


Figure 1. Average annual air temperature, absolute maximum and minimum (a) and amount of atmospheric precipitation (b) (2018-2022)

The length of the vegetation period and the progression of phenological phases depend on the interaction between the hereditary characteristics of the cultivar and the environmental conditions, which may vary from year to year (Perstnirov *et al.*, 2000).

Grapevine bud burst generally occurs in late April to early May, lasting from 3-5 days up to 10-15 days, and the interval between cultivars may reach two weeks. During the study period, bud burst in the analyzed cultivars was recorded between April 20 and May 07 (Table 2). The earliest bud burst was observed in the cultivars 'Interlaken', 'Himrod', and 'Mecita' while 'Perlon', 'Centennial seedless', 'Bessemeannyi V-6' and 'Călina' showed a distinctly later onset.

Blooming in the seedless cultivars occurred from late May to early June.

In 2021, the prolonged period of moderate temperatures slowed down the progression of the blooming stage, resulting in a delay of approximately two weeks (June 10-25) compared with the multiannual average. Based on the onset of blooming, early-flowering cultivars included 'Himrod' and 'Interlaken', medium-flowering cultivars included 'Flame Seedless', 'Perlette', 'Kişmiş Lucistâi', 'Romulus', and 'Centennial Seedless', whereas 'Călina', 'Perlon', and 'Bessemeannyi V-6' were classified as late-flowering.

Table 2. Development of the main phenological phases of the seedless cultivars (average for 2018-2022)

Cultivar	bud burst	blooming	beginning of berry ripening	full maturity of the berry	leaf fall
'Centennial' seedless	24.04-07.05	27.05-10.06	23.07-03.08	25.08-07.09	24.10-29.10
'Flame' seedless	21.04-05.05	27.05-11.06	22.07-30.07	23.08-05.09	26.10-01.11
'Perlette'	24.04-02.05	27.05-11.06	20.07-28.07	22.08-10.09	27.10-02.11
'Perlon'	24.04-03.05	30.05-14.06	28.07-05.08	30.08-14.09	23.10-31.10
'Călina'	23.04-07.05	28.05-12.06	28.07-07.08	03.09-15.09	23.10-01.11
'Otilia'	23.04-02.05	30.05-14.06	16.07-27.07	15.08-06.09	24.10-04.11
'Mecita'	20.04-04.05	27.05-10.06	25.07-05.08	03.09-20.09	24.10-04.11
'Bessemeannyi V-6'	24.04-04.05	01.06-12.06	12.08-22.08	18.09-26.09	24.10-04.11
'Himrod'	20.04-01.05	25.05-07.06	10.07-18.07	08.08-20.08	22.10-29.10
'Interlaken'	21.04-01.05	26.05-08.06	12.07-20.07	13.08-22.08	23.10-28.10
'Romulus'	22.04-03.05	28.05.-12.06	26.07-07.08	02.09-20.09	22.10-29.10

Based on the observational data (Figure 2), the duration of the period from bud burst to blooming in the studied seedless cultivars ranged from 39 days ('Centennial Seedless') to 45 days ('Perlon'). The average length of the ripening-maturity interval varied between 29 days in 'Himrod' and 43 days in 'Romulus'.

During the study years, the period of full maturity occurred between the second decade of August and the second decade of October (Table 2). The earliest ripening cultivars were 'Himrod' and 'Interlaken' (August 14–17 days), followed 15–17 days later by 'Perlette', 'Flame Seedless', 'Otilia' and 'Centennial Seedless'. The latest grape maturity was recorded in the cultivar 'Bessemeannyi V-6' (September 20–25).

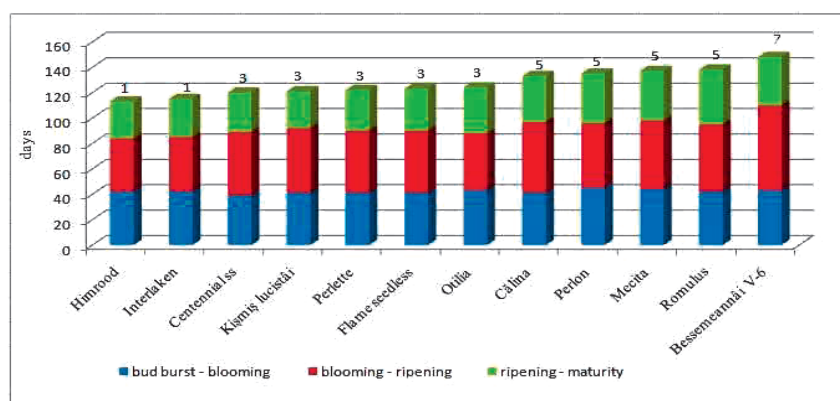


Figure 2. Duration of phenological phases of seedless genotypes

According to the number of days from bud burst to full maturity, the seedless cultivars were classified, following Buletinul I.C.V.V. (1988) and OIV (2001), by time of full maturity of berry as follows: Extra early (1): 'Himrod', 'Interlaken'; Early (2): 'Perlette', 'Flame seedless', 'Otilia', 'Centennial seedless', 'Kişmiş lucistâi'; Medium (5): 'Perlon', 'Călina', 'Mecita', 'Romulus'; Late (7): 'Bessemeannyi V-6'.

Seedless cultivars of the *Vitis vinifera* generally show lowest resistance, tolerating temperatures down to -15°C (Doulati, 2017). The percentage of buds that resumed growth

after pruning reflects their ability to withstand winter conditions (Guzun et al., 1976). Across the research years, this percentage ranged between 60.4% ('Flame seedless') and 73.6% ('Himrod') (Figure 3). Higher percentage of buds started in growth were recorded in 'Himrod', 'Perlon', and 'Perlette' while 'Flame seedless', 'Otilia', and 'Centennial seedless' differed significantly ($LSD_{0.95}=8.56\%$), by showing lower values.

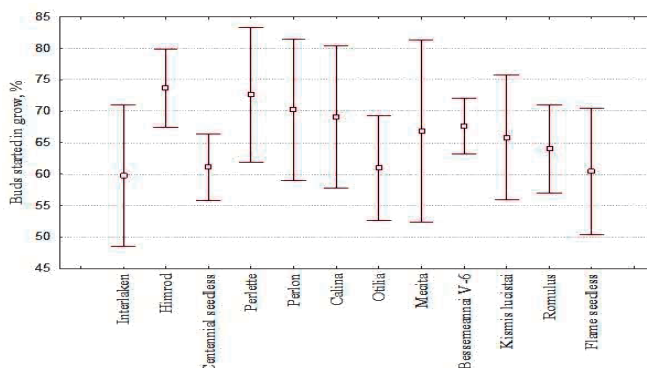


Figure 3. Percentage of buds started in growth
(* mean value, I-95% confidence interval)

Table 3. Fertility and productivity of the seedless cultivars (average for 2018-2022)

Cultivar	Fertile shoots, %	Coefficient of fertility		Index of productivity	
		absolute	relative	absolute	relative
'Centennial seedless'	50,2±1,8	1,1±0,2	0,6±0,2	544,5±4,0	271,0±3,7
'Flame seedless'	50,7±0,9	1,2±0,2	0,6±0,1	263,1±3,8	127,9±3,1
'Perlette'	44,0±1,2	1,06±0,1	0,5±0,1	323,4±5,2	149,5±3,7
'Perlon'	44,6±1,6	1,05±0,1	0,48±0,2	459,4±3,0	194,2±4,1
'Călina'	48,0±1,8	1,2±0,2	0,6±0,2	343,8±3,2	164,9±2,5
'Otilia'	72,6±1,2	1,68±0,2	1,2±0,2	432,8±3,0	302,2±3,5
'Mecita'	50,3±1,3	1,2±0,2	0,6±0,2	411,7±5,7	207,4±4,1
'Bessemeannyi V-6'	47,1±0,9	1,15±0,1	0,52±0,1	477,6±5,2	215,9±3,1
'Kismis lucistai'	43,3±1,1	1,13±0,2	0,5±0,2	368,6±2,3	163,9±3,2
'Himrod'	59,1±1,9	1,16±0,1	0,7±0,02	228,7±2,2	144,7±3,3
'Interlaken'	66,5±1,5	1,26±0,02	0,75±0,2	200,7±3,8	118,5±2,7
'Romulus'	68,3±1,1	1,25±0,1	0,8±0,1	294,2±3,4	189,6±3,3
<i>LSD_{0,95}</i>	12,347	0,153	0,186	98,169	72,208

The percentage of buds that resumed growth showed medium to high values for all studied seedless cultivars. Since the data represent average values over several years, the observed differences reflect mainly the effect of the cultivar, with a smaller contribution of annual climatic variation. The basic characteristics of grape production are fertility and productivity, which largely determine both the quantity and the quality of the yield. Fertility is an important factor for assessing crop profitability. The percentage of fertile shoots is one of the key indices used to evaluate fertility and, according to Mustea (2004), typically ranges from 20–40% in seedless cultivars and 40–60% in table grape cultivars.

The cultivars 'Otilia' and the interspecific hybrids 'Romulus', 'Interlaken' and 'Himrod' recorded the highest average percentages of fertile shoots over the period 2018–2022

(Table 3). Lower values of this indicator were found in 'Perlette' (44.0%) and 'Perlon' (44.6%). These differences are mainly attributed to the genetic potential of the cultivars, while the influence of environmental conditions appears to be secondary.

The percentage of fertile shoots is influenced primarily by the hereditary potential of the genotype, and to a lesser extent by climate, soil, and cultivation practices. The absolute and relative fertility coefficients (CFA and CFR) are indicators that vary mainly with cultivar. Analysis of the average CFA and CFR values for the whole study period (Table 3) shows that the highest values were recorded for the cultivar 'Otilia' (CFA $\approx$ 1.7; CFR $\approx$ 1.2), followed by the interspecific cultivars 'Interlaken', 'Romulus' and 'Himrod', which generally exceeded the *V. vinifera* seedless cultivars in fertility.

Productivity represents the vine's ability to form and develop grape clusters and is closely correlated with fertility (Mustea, 2004). It was assessed by the absolute and relative productivity index, which expresses the average grape yield per shoot. For the entire research period, the highest average productivity indices (IPA and IPR) were recorded in 'Centennial Seedless', 'Bessemeannyi V-6', 'Otilia' and 'Mecita', while the interspecific cultivar 'Interlaken' showed lower average values compared with the other studied genotypes.

The productivity indices of the seedless cultivars varied from year to year. This variation can be explained by unfavourable environmental conditions during the formation and development of fertile buds in some years, which reduced the expression of the productive potential of the cultivars. According to productivity indices and following the criteria of Buletinul I.C.V.V. (1988), the studied genotypes were classified as follows: cultivars with high productivity indices – 'Bessemeannyi V-6', 'Perlon', 'Otilia'; and cultivars with medium productivity indices – 'Centennial Seedless', 'Flame Seedles', 'Perlette', 'Călina', 'Mecita', 'Romulus', 'Himrod' and 'Interlaken'.

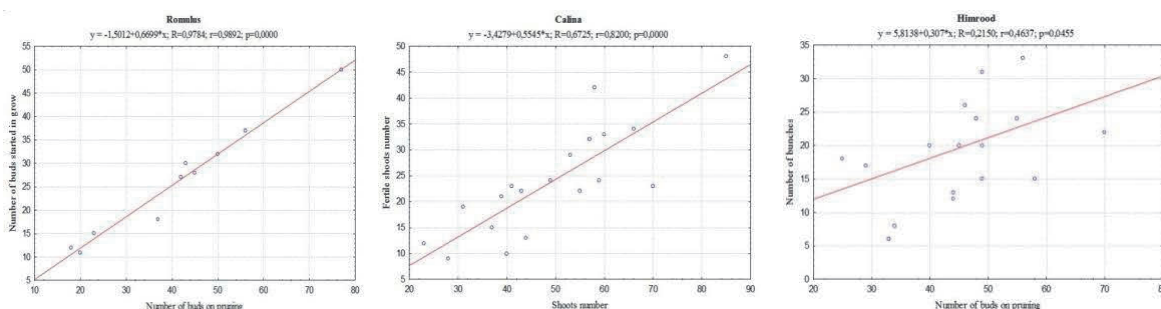
The cultivation of seedless cultivars, which are relatively new in the viticultural sector of the republic, requires an assessment of the relationships between fertility-related traits of the genotypes proposed for planting (Savin, 2012; Tofan, 2023). The fertility elements determined for the cultivars included in the study provide a basis for estimating the relationships among these traits before setting up specific agrotechnical experiments and help to orient further research. In order to highlight, for the studied seedless genotypes, the relationship between the total number of buds left after pruning and the number of buds that resumed growth, linear correlation coefficients were calculated and regression equations were constructed.

The relationship between the total number of buds pruning (x) and the number of buds started in vegetation (y), varies considerably among the studied seedless cultivars (Table 4). Most cultivars show relatively high linear correlation coefficient ($r=0.83-0.99$), indicating a consistent association between initial bud load and the number of buds that entered vegetation. Lower r values were recorded for 'Interlaken' ($r=0.78$), while 'Centennial Seedless' showed only a modest association ($r=0.45$) (Table 4, Figure 4).

The correlation between total shoots and fertile shoots shows notable variation between cultivars. Higher r values ($0.67-0.80$) were obtained for 'Centennial Seedless', 'Bessemeannyi V-6', 'Kişmiş lucistâi', and 'Romulus' genotypes. Several cultivars, including 'Mecita', 'Otilia', 'Călina', and 'Interlaken', exhibited even higher coefficients ($0.81-0.94$), suggesting a more consistent association between shoot development and fertile shoot formation. In contrast, 'Perlon' and 'Perlette' displayed moderate r values ($0.45-0.52$), while 'Flame Seedless' showed a weak association ($r=0.38$).

Table 4. Correlation of fertility elements for seedless cultivars

Cultivar	The regression equation and the linear correlation coefficient between:		
	no. total buds x no. buds started	no. total shoots x no. shoots fertile	no. buds started x no. of bunces
'Centennial seedless'	$y=11,88+0,27x$; $r=0,45$	$y=2,31+58x$; $r=0,80$	$y=3,44+0,22x$; $r=0,40$
'Flame seedless'	$y=1,47+0,59x$; $r=0,89$	$y=8,34+0,22x$; $r=0,38$	$y=16,89+0,005x$; $r=0,08$
'Perlette'	$y=2,77+0,68x$; $r=0,93$	$y=4,27+0,23x$; $r=0,45$	$y=5,92+0,156x$; $r=0,37$
'Perlon'	$y=3,24+0,62x$; $r=0,99$	$y=3,77+0,26x$; $r=0,52$	$y=7,07+0,07x$; $r=0,16$
'Călina'	$y=3,35+0,65x$; $r=0,95$	$y=-3,43+0,55x$; $r=0,82$	$y=0,21+0,42x$; $r=0,67$
'Otilia'	$y=4,42+0,53x$; $r=0,96$	$y=0,93+0,69x$; $r=0,93$	$y=7,77+0,38x$; $r=0,68$
'Mecita'	$y=4,52+0,58x$; $r=0,94$	$y=-7,63+0,71x$; $r=0,94$	$y=-7,98+0,55x$; $r=0,95$
'Bessemeannyi V-6'	$y=18,49+0,47x$; $r=0,83$	$y=-11,04+0,59x$; $r=0,67$	$y=-8,81+0,40x$; $r=0,63$
'Kismis lucistâi'	$y=27,22+0,005x$; $r=0,12$	$y=8,16+0,10x$; $r=0,57$	$y=14,99+0,07x$; $r=0,39$
'Himrod'	$y=1,76+0,70x$; $r=0,87$	$y=5,16+0,36x$; $r=0,52$	$y=5,79+0,31x$; $r=0,46$
'Interlaken'	$y=2,89+0,49x$; $r=0,78$	$y=5,47+0,94x$; $r=0,81$	$y=8,55+0,24x$; $r=0,22$
'Romulus'	$y=-1,50+0,67x$; $r=0,99$	$y=4,62+0,39x$; $r=0,79$	$y=4,62+0,36x$; $r=0,79$

**Figure 4.** Linear regression equation between the main fertility elements for seedless cultivars

The relationship between the number of buds that resumed growth and the number of clusters formed ranged from low to relatively high, depending on cultivar. Most genotypes showed r values between 0.63 and 0.79, with the highest value recorded for 'Mecita' ($r=0.95$), indicating a more proportional relationship between vegetative bud development and cluster formation. Moderate associations were found in 'Centennial Seedless', 'Perlette', and 'Himrod', while 'Flame Seedless', 'Perlon', and 'Interlaken' recorded low r values, indicating greater variability in cluster formation.

CONCLUSIONS

The studied seedless cultivars showed considerable diversity in the timing of full berry maturity, ranging from extra-early ('Himrod', 'Interlaken'), to early ('Otilia', 'Centennial Seedless'), medium ('Mecita', 'Călina') and late ('Bessemeannyi V-6'). This wide maturity span allows for an extended period of fresh grape consumption, from early August to late October, and provides valuable genetic material for breeding new seedless cultivars with targeted ripening periods.

The cultivars 'Otilia', 'Romulus', 'Himrod', and 'Interlaken' displayed higher values of shoot fertility, while 'Otilia', 'Bessemeannyi V-6' and 'Centennial Seedless' also expressed elevated productivity indices. These results indicate the capacity of these genotypes to ensure a

stable grape production potential and highlight their suitability as genetic sources for fertility and productivity in breeding programs.

The linear relationships observed between the total number of buds left after pruning and the number of buds that resumed growth ($r=0.68-0.98$), as well as between the total number of shoots and the number of fertile shoots ($r=0.60-0.97$), indicate consistent associations among vegetative and reproductive traits in several cultivars. These descriptive correlations underline the inherent vegetative and fertility potential of many of the studied genotypes.

ACKNOWLEDGEMENTS

The research was conducted within the framework of the project "State Program" 20.80009.5107.17 "Creation, evaluation and implementation of new grapevine cultivars and clones of homologated cultivars in accordance with climate change and the principles of sustainable agriculture".

REFERENCES

1. Buletinul I.C.V.V. (2/1988). Nr.7. (Valea Călugărească) 98 p.
2. Bucur, G.M. (2011). Viticultură. (București) 381 p.
3. Constantinescu, Gh. and Indreăș, A. (1976). Ampelologia soiurilor apirene. (ed. Academia RS România). 340 p.
4. Clark, J. (2017). Table Grape Breeding at the University of Arkansas USA: progress and challenges. Paper presented at: 8th International Table Grape Symposium. (Apulia&Sicily) pp. 13-14.
5. OIV Descriptor List for grape varieties and *Vitis* species. (2001). <https://www.oiv.int>.
6. Doulati Baneh, H. and Ali Nejatian, M. (2017). Results of comparison of bunch and berry traits of some commercial foreign and Iranian grape cultivars in Urmia, Iran. In: 8th International Table Grape Symposium. (Apulia&Sicily), pp. 18-19
7. Dgani, O. *et al.* (2014). Breeding project in Israel for the improvement of table grapes. Paper presented at: First Israel-Italy Symposium: GRACIOUS symposium. (Sede Boquer, Israel) p.6.
8. Mustea, M. (2004) Viticultură. Bazele biologice, înființarea și întreținerea plantațiilor tinere de vii roditoare. (Iași: ed. Ion Inculescu de la Brad). 308 p.
9. Iasiello, B. (2017). Table and Dried Grapes Non-alcoholic products of the vitivinicultural sector intended for human consumption. Paper presented at: 8th International Table Grape Symposium. (Apulia&Sicily)
10. Perstnirov, N., Surugiu, V., Moroșan, E. and Corobca, V. (2000). Viticultura. (Chișinău) 504 p.
11. Savin, Gh. (2012). Ameliorarea sortimentului viticol al Republicii Moldova. (Chișinău) 260 p.
12. Savin, Gh., Cornea, V., Tofan, S. and Nofit, D. (2009). Elaboration of initial biological material with seedless properties in order to create new grapevine varieties in Republic of Moldova. In: Analele Universității din Craiova. vol. XIV [XLX], pp. 77-80.
13. Table and dried grapes. Focus FAO-OIV. (2016). 64 p. <https://www.oiv.int>.
14. Tofan, S. (2023). Evaluarea unor genotipuri de viță de vie apirene ca material genetic inițial pentru ameliorarea sortimentului viticol în Republica Moldova. Rezumatul tezei de doctor în științe agricole. (Chișinău, ed. Print-Caro). 35 p.
15. Guzun, N., Minakova, R., Legaciova, L., Karaivan, A. (1976). Informații masive pe ampelografie și selecție. In: Sortoizucenie și selecția vinograda. (ed. Știința, Chișinev) p. 67-81.

Impacts of climate change on selected agrobiological and technological traits of the 'Fetească Neagră' cultivar: A comparative study of the Murfatlar vineyard (Romania) and the Codru wine region (Republic of Moldova)

**A. Ranca^{1*}, E. Alexandrov ², A. Tănase¹, I. Dina¹, V. Artem¹, M. Kwon³
and T.S. Cosma ¹**

¹Research Station for Viticulture and Oenology, Murfatlar, România

²Institute of Genetics, Physiology and Plant Protection of the USM,
Republic of Moldova

³The Gyeongsangbuk-do Agricultural Research and Extension Services,
Republic of Korea

*Corresponding author email: auroraranca@gmail.com

ABSTRACT

Over the past decade, shifts in key climatic parameters in both wine-growing regions have brought notable changes to the agrobiological behavior of the indigenous 'Fetească Neagră' cultivar. These include a shorter vegetative period, an earlier onset of full ripening, and an advance in the grape harvest date. Traits such as fertility, productivity, and resilience to stress factors (drought, pathogen pressure) are directly shaped by these climatic shifts. This paper presents a comparative analysis of the evolution of principal climatic factors recorded between 2015 and 2024 and examines their impact on the phenology and grape quality of the 'Fetească Neagră' cultivar cultivated in both regions. The findings indicate an increase in average temperatures during the vegetative phase by 0.8–0.9°C in both areas, coupled with uneven rainfall patterns marked by alternating dry periods and months with normal precipitation. In both vineyards, the vegetative cycle was reduced by 10–15 days, with full ripening now occurring in early September; the average vegetative period lasted around 120 days. Berry development accelerated by about 9 days at Murfatlar, where the ripening phase now begins at approximately 100 days of vegetation. At Codru, the ripening phase was shortened to 17 days instead of the previous 35. In terms of grape yield, no significant differences were observed between the two regions during the study period. However, improvements in quality were recorded: at Murfatlar, must sugar levels increased by 6.6g/L, accompanied by a decrease in acidity; at Codru, must sugar levels rose by 8.39g/L, with acidity stabilizing at 4.5g/L tartaric acid. Correlation analyses highlight the considerable influence of temperature and precipitation on grape quality parameters for the 'Fetească Neagră' cultivar. The challenges posed by climate change for this variety in both regions are clear. To sustain wine quality, adaptive cultivation measures are needed, including improved soil water conservation, canopy management, grape shading, and other responsive viticultural practices.

Keywords: climate change, phenology, grape quality, sustainability, resilience

INTRODUCTION

Climate change represents a major challenge to the viticultural sector, directly affecting the agrobiological and technological characteristics of grapevine cultivars. Extreme climatic phenomena, such as prolonged droughts, increased frequency of high temperatures, and heavy rainfall, significantly influence the biological processes of the plant, leading to changes in phenology, the duration of the vegetative period, and grape chemical composition (Jones *et al.*, 2005; Fraga *et al.*, 2016). In recent decades, the rise in temperatures has caused an earlier ripening of grapes, affecting the sugar-acid balance in the must—an essential factor in shaping the sensory profile of wine (Moriondo *et al.*, 2013; Caffi *et al.*, 2016). In this context, identifying and assessing the impact of climate change on the agrobiological and technological traits of grapevine cultivars—particularly indigenous ones—has become a critical priority for ensuring sustainable viticulture. During the vegetative season, grapevines require daily average temperatures above 10 °C to initiate vegetative growth, followed by sufficient thermal accumulation to ensure full ripening of the grapes (Huglin, 1986). However, extreme temperatures during key phenological stages can cause physiological stress, premature ripening, yield losses, enzymatic inactivation, and poor development of aromatic compounds (Coombe, 1987). In the wine-growing regions of southeastern Europe, including Romania and the Republic of Moldova, the effects of climate change are already evident. These areas, with a long-standing viticultural tradition, are now facing a variety of risks associated with extreme weather events such as persistent droughts and flooding (Lafon *et al.*, 2013). Additionally, changes in precipitation patterns—marked by alternating extended dry periods and torrential rains—have created conditions conducive to major challenges, such as efficient water management and increased pressure from diseases and pests (Fraga *et al.*, 2016). Recent studies have shown that climate change affects not only grapevine phenology but also the chemical composition and sensory profile of wines, by altering concentrations of phenolic compounds, organic acids, and sugars in the grapes (Webber *et al.*, 2018; Brillante *et al.*, 2019). Moreover, significant variations in rainfall patterns and rising average temperatures can lead to yield fluctuations and negatively impact fruit development, with direct consequences on the volume and quality of viticultural production (Schultz, 2010).

The effects of climate change are further amplified by additional factors such as water resource management and the protection of grapevines from biotic and abiotic stress. These issues highlight the urgent need for integrated and sustainable approaches in viticultural practices (Caffi *et al.*, 2016). Traditionally, viticulturists have selected wine grape cultivars based on phenotypic traits that adapt most effectively to local microclimates (Wolkovich *et al.*, 2018) and soil types. Only cultivars that deliver consistent yields under specific climatic conditions and maintain an optimal balance of sugars, acidity, and other compounds with technological and sensory value are retained in cultivation (van Leeuwen and Darriet, 2016). 'Fetească Neagră' is one of the oldest and most valuable indigenous grapevine cultivars in Romania and the Republic of Moldova, holding a unique historical and cultural significance. However, in the context of recent climate change, significant alterations have been observed in the agrobiological and technological behavior of the variety, underlining the necessity of in-depth evaluation of these influences to support a sustainable and high-performing viticulture. The present paper aims to analyze the impact of climate change on the 'Fetească Neagră' cultivar grown in two distinct wine regions of southeastern Europe: Murfatlar (Romania) and Codru (Republic of Moldova). The objective is to highlight differences and similarities in the cultivar's response to climatic variations, with a focus on agrobiological traits and enological potential.

MATERIALS AND METHODS

The study was conducted on the red wine grape cultivar *Vitis vinifera* L. cv. 'Fetească Neagră', cultivated in two renowned vineyards: Murfatlar (Romania) and Codru (Republic of Moldova). The vineyard in the Murfatlar wine center was established in 2008, with vines grafted onto the Berlandieri x Riparia rootstock, Oppenheim selection 4, clone 4 (SO4-4). The vine rows are oriented north–south, with a spacing of 2.2 meters between rows and 1.2 meters between vines within the row. The training system used is Guyot, with a trunk height of 0.8 meters and bilateral cordons. The vineyard in the Codru region was established in 2016, using the same rootstock type (SO4-4). The rows are also oriented north–south, with 3.0 meters between rows and 1.5 meters between vines within the row. The training system consists of a trunk height of 70 cm and two lateral cordons, each bearing four fruiting canes pruned to 3–4 buds. Climatic parameters such as maximum temperature, minimum temperature, and precipitation were recorded using automatic weather stations: iMetos 3.3 (Murfatlar) and PTM-48A (Codru). Based on these data, several climatic indices relevant to viticulture were calculated, including the Huglin Index, Winkler Index, and Martonne Index (Serdinescu, 2020). Must parameters, including sugar content (g/L), total acidity (g/L expressed as tartaric acid), and pH, were determined in accordance with standard methods outlined in the OIV Compendium of International Methods of Analysis of Wines and Musts. The correlations between climatic variables and grape quality parameters were evaluated using the Pearson correlation coefficient. The statistical significance level was set at $p < 0.05$, and only significant correlations were considered. To assess the impact of climate change on the Murfatlar (Romania) and Codru (Republic of Moldova) vineyards during the 2015–2024 period, compared to the 2005–2014 interval, a Principal Component Analysis (PCA) was performed. This analysis was conducted using the XLSTAT Add-in for Microsoft Excel, version 15.5.03.3707 (Addinsoft, New York, NY, USA).

RESULTS AND DISCUSSIONS

A comparative analysis of climatic parameters for the Murfatlar (Romania) and Codru (Republic of Moldova) wine regions during the 2015–2024 period, relative to the previous decade (2005–2014), reveals a series of significant climate changes, summarized in Table 1. In both vineyards, a marked increase in annual average temperature (T_{avg}) was observed: $+0.8^{\circ}\text{C}$ in Murfatlar and $+0.93^{\circ}\text{C}$ in Codru, indicating a clear warming trend in the regional climate. Maximum temperature (T_{max}) increased by 0.7°C in Murfatlar and 1.5°C in Codru, reflecting an intensification of extreme heat stress events, which may negatively affect grapevine physiology and, consequently, the quality of production. Minimum temperature (T_{min}) also recorded significant increases: $+7.0^{\circ}\text{C}$ in Murfatlar, suggesting improved overwintering conditions and reduced risks of severe frost, and $+3.8^{\circ}\text{C}$ in Codru, indicating a similar trend. Regarding the average temperature during the vegetative season (GST_{avg}), Murfatlar recorded a slight decrease (-0.8°C), while Codru experienced a moderate increase ($+0.65^{\circ}\text{C}$). These differences demonstrate that while global warming is a general trend, its effects are modulated by local microclimatic conditions. As for precipitation, annual precipitation (AP) showed a sharp decline in Murfatlar (-75.4 mm), while the reduction in Codru was less pronounced. During the growing season, precipitation (GSP) decreased by 23.83% in Murfatlar, whereas Codru registered a slight increase ($+4.3\%$). Seasonal precipitation distribution shows that around 50% of annual rainfall accumulates during the vegetative season in Murfatlar and approximately 65% in Codru. The Winkler Index (WI), which reflects cumulative growing degree days (GDD) from April 1 to October 31, exceeded 2200 units in both regions, placing Murfatlar and Codru in Winkler Region V ($WI > 2220$), characteristic of very warm climates. This climatological classification suggests a potential

imbalance between sugar accumulation and acid retention, with implications for the sensory profile of the resulting wines. The increase in the Heliothermal Index (IH), by +158.6 units in Murfatlar and +116.5 units in Codru, indicates greater solar radiation and thermal intensity, favorable conditions for the biosynthesis of sugar compounds. Likewise, the average values of the Huglin Index (HI) confirm that both vineyards fall within the "very warm" climate class ($2400 < HI \leq 3000$), underscoring the need to adapt viticultural technologies to ensure sustainable production and maintain quality under new climatic conditions. At Murfatlar, the De Martonne Index (IM) dropped from 23.7 in 2005–2014 to 19.2 in 2015–2024, indicating a 4.5-unit decrease. According to the De Martonne classification, these values place the region in the subhumid category; however, the proximity to the lower limit of this class signals a potential transition toward semiarid conditions (Frich et al., 2002). This decline is correlated with the significant reduction in annual precipitation (AP: -75.4 mm) and that of the active vegetation period (GSP: -77.9 mm), alongside a slight increase in average and maximum temperatures. In the Codru vineyard, the decrease in the Martonne Index (IM) was more moderate, from 22.4 to 20.4 (-2 units), keeping the region within the subhumid class. Although a slight increase in both annual precipitation (+12.6 mm) and growing season rainfall (+15.4 mm) was recorded, the rise in maximum temperatures (T.max: +1.5°C and GST.max: +4.1°C) contributes to enhanced evapotranspiration and the onset of moderate water imbalance, potentially affecting the ecophysiological behavior of the grapevine.

Table 1. Climatic parameters recorded in both wine regions, Murfatlar and Codru

Climatic parameters	Murfatlar, Romania			Codru, Republic of Moldova		
	2005-2014	2015-2024	Diff. 2015-2024 2005-2014	2005-2014	2015-2024	Diff. 2015-2024 2005-2014
T.avg	13,9	14,7	0,8	10,92	11,85	0,93
T.max	40,2	40,9	0,7	24,5	26	1,5
T.min	-22	-15	7	-12	-8,2	3,8
GST.avg	22,3	21,5	-0,8	17,61	18,26	0,65
GST.max	32,4	34	1,6	28,5	32,6	4,1
GST.min	-5	-4,5	0,5	-3,5	-1,3	2,2
AP	567,6	492,2	-75,4	541,3	553,9	12,6
GSP	326,8	248,9	-77,9	357,9	373,3	15,4
IW	2272,9	2437,8	164,9	2150,6	2485,1	334,5
HI	2722,6	2881,2	158,6	2655,6	2772,1	116,5
IM	23,7	19,2	-4,5	22,4	20,4	-2

Figure 1 highlights significant phenological changes in the ‘Fetească Neagră’ cultivar during the 2015–2024 period compared to the 2005–2014 interval in both analyzed wine regions. A systematic advancement of the main phenophases is evident, particularly the onset of the growing season—a phenomenon correlated with the rise in average and extreme temperatures. In the Murfatlar wine center, budburst (BBCH 08–09) occurred up to 12 days earlier, with bud development observed as early as the beginning of April. In the Codru region, the shift was less pronounced but maintained the same trend of earlier phenological development. The most substantial advances were recorded in the veraison (BBCH 85) and full maturity (BBCH 89) stages, with a 15–20-day advancement at Murfatlar and 10–14 days at Codru. As a result of these changes, the total length of the growing season was reduced from 210 to 199 days in Murfatlar and from 215 to 199 days in Codru. In both vineyards, an advancement of the phenological calendar and a compression of key stages within the growing season can be observed, particularly during the second half of the cycle (veraison →

harvest). This behavior reflects the influence of higher average and maximum temperatures recorded over the past decade and suggests an accelerated adaptation of the cultivar to the new climatic conditions. This shortening of the growing season has direct implications for determining the optimal harvest time, the chemical composition of the grapes, and, consequently, the quality of the wine. It underscores the need for adjustments in vineyard management practices and oenological strategies tailored to the specific conditions of each region.

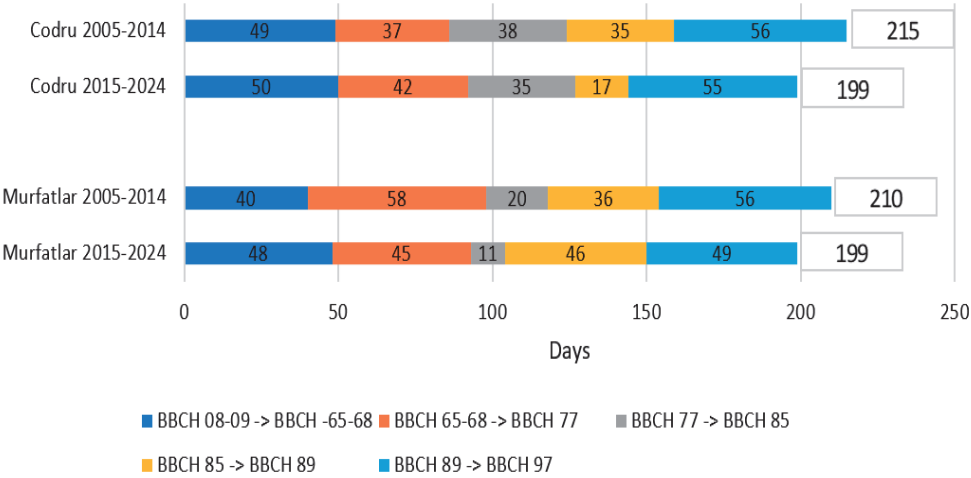


Figure 1. Phenophase development in the Murfatlar and Codru vineyards

The climate-induced changes observed in the Murfatlar and Codru vineyards are directly reflected in the evolution of grape quality parameters, as shown in Table 3. The analyzed parameters—sugar content, total acidity, must pH, and yield per vine—offer valuable insights into the cultivar’s adaptation to new climatic conditions and its oenological potential. Sugar content showed a significant increase in both vineyards: +6.6 g/L in Murfatlar and +8.39 g/L in Codru, correlated with higher temperatures and a shortened vegetative period. These changes are also supported by Jones *et al.* (2022), who describe this phenomenon as one that may lead to wines with higher potential alcohol content. However, such evolution may result in taste imbalances if acidity levels are negatively affected (van Leeuwen and Seguin, 2006). Total acidity showed a slight decrease in both areas: -0.15 g/L in Murfatlar and -0.24 g/L in Codru. This trend is typical for vineyards subjected to warmer and drier summers, conditions that accelerate the degradation of organic acids, particularly malic acid (Teixeira et al., 2018). Changes in must acidity are also reflected in the evolution of pH, which increased visibly by +0.21 in Murfatlar and +0.35 in Codru. This rise indicates a reduction in effective acidity and a possible decline in microbial stability, as a result of accelerated ripening and faster consumption of organic acids (Cholet *et al.*, 2021).

Although pH values remain within acceptable ranges, this trend calls for close monitoring and adjustments in winemaking technology. Yield per vine increased slightly, by +0.1 kg in Murfatlar and +0.15 kg in Codru, which may suggest effective adaptation of the cultivar to the new climatic conditions, as well as optimized cultivation practices and an extended duration of photosynthetically active periods (Bertin *et al.*, 2020).

However, higher productivity does not automatically equate to superior quality, since increased fruit load per vine can dilute aromatic and phenolic compounds, thereby affecting the complexity of the resulting wine (Jackson and Lombard, 2019).

Table 2. Grape quality parameters from both vineyards – Murfatlar and Codru

Quality parameter	Murfatlar, Romania			Codru, Republic of Moldova		
	2005-2014	2015-2024	Difference 2015-2024 2005-2014	2005-2014	2015-2024	Difference 2015-2024 2005-2014
Sugars g/L	216,3	222,9	6,6	210,5	218,89	8,39
Total Acidity g/L tarttric acid	5,02	4,87	-0.15	4,59	4,35	-0.24
pH	3,14	3,35	0,21	3,00	3,35	0,35
Yield/vine (Kg)	1,6	1,7	0,1	1,5	1,65	0,15

The correlation analysis between climatic variables and grape quality parameters for the ‘Fetească Neagră’ cultivar (Table 4) reveals the significant influence of temperature and precipitation on grape quality. Both average and maximum temperatures show strong positive correlations with sugar accumulation and yield per vine, suggesting that a warmer thermal regime promotes physiological processes involved in the synthesis of compounds important for enological purposes. For instance, annual average temperature (T.avg) is highly correlated with sugar content ($r = 0.858$) and yield per vine ($r = 0.818$), indicating a direct relationship between a warmer climate and the productive performance of the grapevine. In contrast, growing season precipitation (GSP) is negatively correlated with these parameters: $r = -0.893$ for sugar content and $r = -0.996$ for must acidity. These results suggest that excessive moisture can dilute the must composition and reduce the qualitative potential of the harvest. This highlights the necessity of achieving a climatic balance between thermal input and rainfall regime to produce grapes with optimal technological traits. Elevated temperatures promote the accumulation of quality parameters, while excessive precipitation may lead to their reduction and, implicitly, to a decrease in the quality of the resulting wine (Jones and Davis, 2000).

Table 3. Pearson correlations between climatic variables and grape quality parameters.

Var	T.avg	T max	GST.avg	AP	GSP	IH	IM	Kg/butuc	Sugars	Total acidity
T.avg	1	0,926	0,990	-0,963	-0,991	-0,977	-0,979	0,818	0,858	0,995
T.max	0,926	1	0,949	-0,970	-0,885	-0,824	-0,852	0,541	0,601	0,922
GST.avg	0,990	0,949	1	-0,951	-0,985	-0,950	-0,973	0,763	0,803	0,996
AP	-0,963	-0,970	-0,951	1	0,918	0,895	0,887	-0,666	-0,724	-0,940
GSP	-0,991	-0,885	-0,985	0,918	1	0,988	0,997	-0,863	-0,893	-0,996
IH	-0,977	-0,824	-0,950	0,895	0,988	1	0,988	-0,923	-0,948	-0,972
IM	-0,979	-0,852	-0,973	0,887	0,997	0,988	1	-0,886	-0,910	-0,988
Kg/Stump	0,818	0,541	0,763	-0,666	-0,863	-0,923	-0,886	1	0,996	0,815
Sugars	0,858	0,601	0,803	-0,724	-0,893	-0,948	-0,910	0,996	1	0,850
Total acidity	0,995	0,922	0,996	-0,940	-0,996	-0,972	-0,988	0,815	0,850	1

**Values in bold are different from 0 with a significance level $\alpha=0.05$*

The principal component analysis (PCA) highlights the impact of climate change on the Murfatlar and Codru vineyards during the 2015–2024 period, in comparison with the previous decade (2005–2014). The first two axes explain 97.23% of the total variability, suggesting an effective discrimination between the analyzed timeframes and regions. On axis F1 (77.40%), thermal variables (T.avg, T.max), pH, sugar content, and yield are strongly associated with Murfatlar 2015–2024, indicating a warmer climate favorable to the rapid accumulation of quality parameters. By contrast, Codru 2015–2024 is influenced by high values of the Heliothermal Index (IH) and growing season precipitation (GSP), suggesting increased water stress and a potential risk of quality degradation in the must.

The regions recorded during the 2005–2014 period are positioned at the opposite end of the spectrum, correlated with more moderate temperatures and thermal indices (e.g., GST.min), reflecting more temperate climatic conditions. This separation clearly illustrates the effects of climate change and its differentiated impact on the technological behavior of the ‘Fetească Neagră’cultivar, thereby justifying the need for adaptive strategies at both the viticultural and oenological levels.

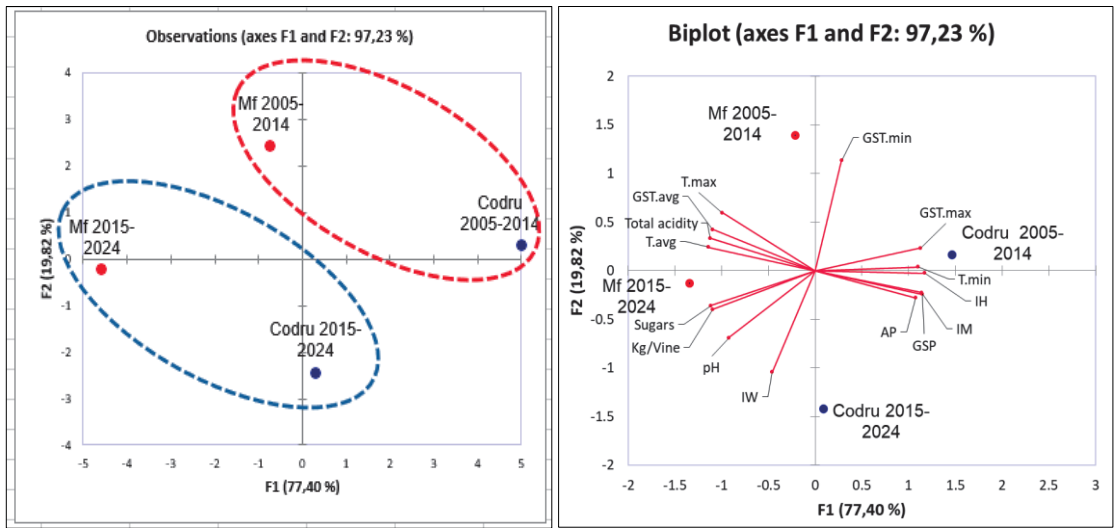


Figure 2. Differentiation of the wine-growing zones (Mf = Murfatlar and C = Codru, along with the analyzed periods 2005–2015 and 2012–2022) based on climatic variables and quality parameters of ‘Fetească Neagră’ grapes.

CONCLUSIONS

The comparative analysis between the Murfatlar and Codru vineyards reveals significant climate changes, marked by increased average and maximum temperatures, along with shifts in precipitation patterns, all of which can influence the quality parameters of ‘Fetească Neagră’grapes. In particular, regions with higher thermal regimes and lower precipitation levels, such as Murfatlar, are experiencing an accelerated ripening process, as well as an increased risk of drought and heat stress. These conditions can directly impact must quality and, consequently, the organoleptic profile of the resulting wine. In contrast, the Codru vineyard, characterized by a more balanced climate and a more even distribution of precipitation, benefits from a more uniform ripening process and a lower risk of damage from extreme climatic factors. These observations suggest that climate change can bring both challenges and opportunities for viticulture. In warmer regions like Murfatlar, it is essential to implement adaptation strategies, such as optimized irrigation management and protection against thermal stress to maintain wine quality. Furthermore, investment in research is necessary to support the development of viticultural and oenological techniques tailored to extreme conditions. Meanwhile, the Codru vineyard, with its more temperate and stable climatic profile, continues to provide favorable conditions for consistent grape ripening and reduced exposure to extreme weather impacts. In conclusion, maintaining the technological and qualitative performance of the ‘Fetească Neagră’cultivar requires adaptation to climate change and the adoption of sustainable vineyard management strategies, especially in light of the accelerated pace of climatic shifts observed in recent decade.

REFERENCES

1. Bertin, S., Bindi, M., and Moriondo, M. (2020). Grapevine phenology and quality in a changing climate: A modeling approach. *Agricultural Systems*, 182, 102857.
2. Brillante, R. D., Barbero, S., and Giordano, A. (2019). Impact of climate change on viticulture in the Mediterranean: A review. *International Journal of Climatology*, 39(4), 2021-2037.
3. Caffi, T., Albajes, R., and Savino, M. (2016). Effects of climate change on grapevine diseases and pest dynamics. *Agricultural and Forest Entomology*, 18(1), 8-16.
4. Cholet, C., Charrier, G., and Mouton, S. (2021). Temperature effects on the grapevine phenology and berry composition: A global perspective. *OENO One*, 55(1), 125-136.
5. Coombe, B.G. (2016). Influence of temperature on composition and quality of grapes. *Acta Hort.* 1987, 206, 23-35.
6. Fraga, H., Santos, J. A., Lema, D., and Silva, T. (2016). Climate change and wine production: A global overview of the impact of climate change on viticulture and wine production. *Climate Change*, 137(1-2), 303-313.
7. Frich, P., Alexander, L. V., Della-Marta, P., Gleason, B., Haylock, M., Klein Tank, A. M. G., and Peterson, T. (2002). Observed coherent changes in climatic extremes during the second half of the twentieth century. *Climate Research*, 19(3), 193-212.
8. Huglin, P. (1986). *Biologie et Écologie de la Vigne*; Lavoisier: Paris, France, 1986; p. 372.
9. Jackson, R. S., and Lombard, P. B. (2019). Environmental and management practices affecting grape composition and wine quality—A review. *American Journal of Enology and Viticulture*, 70(3), 271-284.
10. Jones, G. V., and Davis, R. E. (2000). "Climatic influences on grapevine phenology, grape composition, and wine production and quality." In: *Wine Science: Principles and Applications*, Second Edition, edited by R. A. Lawlor, J. J. van Oort. Academic Press, pp. 256-276.
11. Jones, G. V., Moriondo, M., Bois, B., Hall, A., and Duff, A. (2022). Climate change and wine: Challenges and adaptations. *Nature Reviews Earth & Environment*, 3(5), 329-346.
12. Jones, G. V., White, M. A., Cooper, O. R., and Storchmann, K. (2005). Climate change and wine: Observations, impacts and future implications. *Australian Journal of Grape and Wine Research*, 11(1), 1-10.
13. Lafon, T., Mandi, L., and Gallet, S. (2013). Impacts of climate change on viticulture: Regional analyses in Europe and their implications for the wine industry. *Agricultural Systems*, 118, 75-85.
14. Moriondo, M., Giannakopoulos, C., and Bindi, M. (2013). Projected climate change impacts on grapevine phenology in Europe. *Climate Research*, 58(1), 61-75.
15. Schultz, H. R. (2010). Climate change and viticulture: A European perspective on climate change impacts, adaptation and mitigation. *Journal of Wine Research*, 21(2), 99-111.
16. Serdinescu, A. (2020). *Indicatori sintetici, ecologici și ecofiziologici, utilizați în viticultură*. București: Editura Ceres.
17. Teixeira, A., Eiras-Dias, J., Castellarin, S. D., & Gerós, H. (2018). Berry phenolics of grapevine under challenging environments. *International Journal of Molecular Sciences*, 19(2), 348.
18. van Leeuwen, C., and Darriet, P. (2016). The impact of climate change on viticulture and wine quality. *Journal of Wine Economics*, 11(1), 150-167.
19. van Leeuwen, C.; Seguin, G (2006). The concept of terroir in viticulture. *J. Wine Res.* 2006, 17, 1-10.
20. Webber, H., McIntyre, S., and Bindi, M. (2018). The impact of climate change on viticulture in Europe: A review. *Agricultural Systems*, 167, 1-12.
21. Wolkovich, E.M., García de Cortázar-Atauri, I., Morales-Castilla, I., Nicholas, K.A., Lacombe, T (2018). From Pinot to Xinomavro in the world's future wine-growing regions. *Nat. Clim. Chang.* 2018, 8, 29-37.

Climate change impact on grape ripening in Murfatlar vineyard

I.C. Ciobanu*, V. Artem and I. Dina

Research Station for Viticulture and Oenology Murfatlar,
Romania

*Corresponding author email: stanciu_iulia_cristina@yahoo.com

ABSTRACT

Climate change is exerting a significant influence on viticulture, leading to altered phenological stages, shifts in grape ripening, and major challenges for maintaining wine typicity. This study evaluates the long-term impact of climate variability on grape maturity in the Murfatlar vineyard, southeastern Romania, one of the country's most representative wine regions. A comprehensive dataset was analyzed, integrating harvest initiation dates over two decades (2004–2024), grape composition at harvest for six cultivars across ten vintages (2015–2024), and berry ripening dynamics monitored during the last seven years (2017–2024). Results indicate a consistent advancement of harvest dates, with initiation occurring nearly three weeks earlier in 2024 compared to the early 2000s, at an average rate of 8.7 days per decade. Grape acidity displayed a steady decline across cultivars, reflecting the direct effect of higher temperatures on organic acid degradation. By contrast, sugar accumulation showed pronounced interannual variability without a clear upward trend, suggesting that heat and drought stress often limit carbohydrate assimilation despite accelerated ripening. The 2024 vintage further illustrated this decoupling: early and rapid sugar accumulation was followed by stagnation and dilution due to late-season rainfall, while berry weight decreased, with cultivar-specific responses. Overall, the findings highlight that the primary challenge for Murfatlar viticulture is not excessive sugar accumulation, but the accelerated loss of acidity, which compromises freshness and balance in wines. Adaptive strategies, including canopy and water management, optimized harvest timing, and innovative oenological practices, are therefore essential to safeguard grape quality and preserve the typicity of local cultivars under ongoing climate change.

Keywords: climate change, phenological shifts, grape ripening, Murfatlar, wine typicity

INTRODUCTION

Climate change is increasingly recognized as one of the main drivers shaping the future of viticulture and winemaking worldwide. Rising temperatures, altered rainfall patterns, and more frequent extreme weather events significantly affect vine growth, grape ripening, and ultimately wine quality (Jones *et al.*, 2005; Schultz, 2016). One of the most evident consequences is the shift in grapevine phenology, with earlier budburst, flowering, veraison and harvest dates recorded across many wine regions (Webb *et al.*, 2012; van Leeuwen and Destrac-Irvine, 2017). From an oenological perspective, these changes translate into accelerated sugar accumulation, reduced acidity, and altered aromatic potential of grapes (Mira de Orduña, 2010; Sadras and Moran, 2012). In some regions, varieties traditionally used for balanced wines now reach high sugar levels too early, before full phenolic or aromatic maturity. This imbalance poses a challenge for

winemakers seeking to preserve typicity and quality, while also adapting vineyard and cellar practices to new climatic realities (Hannah *et al.*, 2013).

In Romania, several studies have already documented the influence of climate change on grapevine phenology and fruit composition. Research carried out in different viticultural centers indicates earlier harvests, accelerated sugar accumulation, and a general decrease in grape acidity compared with historical averages (Irimia *et al.*, 2017). At Murfatlar, one of the most important wine-growing areas in southeastern Romania, recent investigations revealed that rising temperatures and reduced rainfall have led to shifts in the timing of key developmental stages, with earlier veraison and harvest, accompanied by higher sugar levels and lower acidity in grapes (Negraru *et al.*, 2022; Artem *et al.*, 2023). In this context, the present study analyzes a 10-year dataset (2015–2024) concerning sugar accumulation and titratable acidity (expressed in tartaric acid) in several grapevine varieties cultivated at Murfatlar. The objectives are threefold: (i) to evaluate interannual variability in grape sugar concentration and acidity at harvest; (ii) to analyze seasonal trends of sugar accumulation and berry weight for the period 2017–2024 in comparison with data from the 2024 vintage; and (iii) to assess the evolution of harvest starting dates over the past two decades.

MATERIALS AND METHODS

The study was conducted in the Murfatlar Viticultural Center, located in the southeastern part of Romania, one of the country's most representative wine regions. The area is characterized by a temperate-continental climate with strong maritime influences, hot and dry summers, and relatively mild winters. According to previous studies, Murfatlar is among the regions most affected by rising temperatures and declining precipitation in recent decades (Negraru *et al.*, 2022; Artem *et al.*, 2023). The dataset used for this research integrates three complementary components:

Harvest starting dates (2004–2024). The official dates marking the beginning of the harvest campaign were collected annually at the Murfatlar Research and Development Station for Viticulture and Oenology. Although these dates are often influenced by a combination of local subjective decisions, climatic variability, and economic factors, the long-term trend remains clear and illustrative for the impact of climate conditions on grape ripening.

Grape quality at harvest (2015–2024). Six grapevine varieties cultivated in the Murfatlar vineyard were analyzed for sugar concentration (g/L) and titratable acidity (g/L tartaric acid) at harvest. These values were compared across vintages and against the 10-year average to highlight temporal patterns.

Berry ripening dynamics (2017–2024). Seasonal monitoring of sugar accumulation (g/L) and 100-berry weight (g) was performed for the same six varieties. Sampling was carried out weekly from veraison until harvest, following the standard protocol applied at the Murfatlar station. The year 2024 was compared with the 2017–2023 period to assess deviations from recent trends.

Data were processed using descriptive statistics and graphical analysis to identify long-term tendencies and inter-annual variability. Comparisons were made both within individual varieties and across the entire group, with emphasis on differences between 2024 and the multiannual averages.

RESULTS AND DISCUSSIONS

Harvest date trends

The evaluation of harvest initiation dates in the Murfatlar wine center over the past two decades (2004–2024) reveals a clear tendency towards earlier grape ripening (Figure 1). Between 2004–2010, harvest generally started between 10 and 20 September, whereas after

2015 several vintages show harvest initiation in late August. The earliest date was recorded in 2024 (22 August), representing an advancement of nearly three weeks compared to the early-2000s average. The linear regression fitted to the dataset confirms this shift, with a slope of -0.87 days/year⁻¹ (≈ 8.7 days per decade⁻¹) and a $R^2 = 0.553$, indicating a moderate but statistically meaningful trend. This progressive advancement is consistent with phenological responses to climate warming reported for many European wine regions (Jones et al., 2005; Hannah et al., 2013).

The local climatic context of Dobrogea further intensifies this effect. This region is characterized by hot, dry summers and pronounced inter-annual precipitation variability (Irimia et al., 2017; Negraru et al., 2022), that typically accelerate sugar accumulation, shorten ripening, and advance optimal harvest windows. Consequently, the advancement of harvest dates observed at Murfatlar reflects both broader global warming trends and the amplification influence of the regional terroir. This phenological compression may complicate the alignment between technological and phenolic maturity and could challenge the maintenance of varietal typicity.

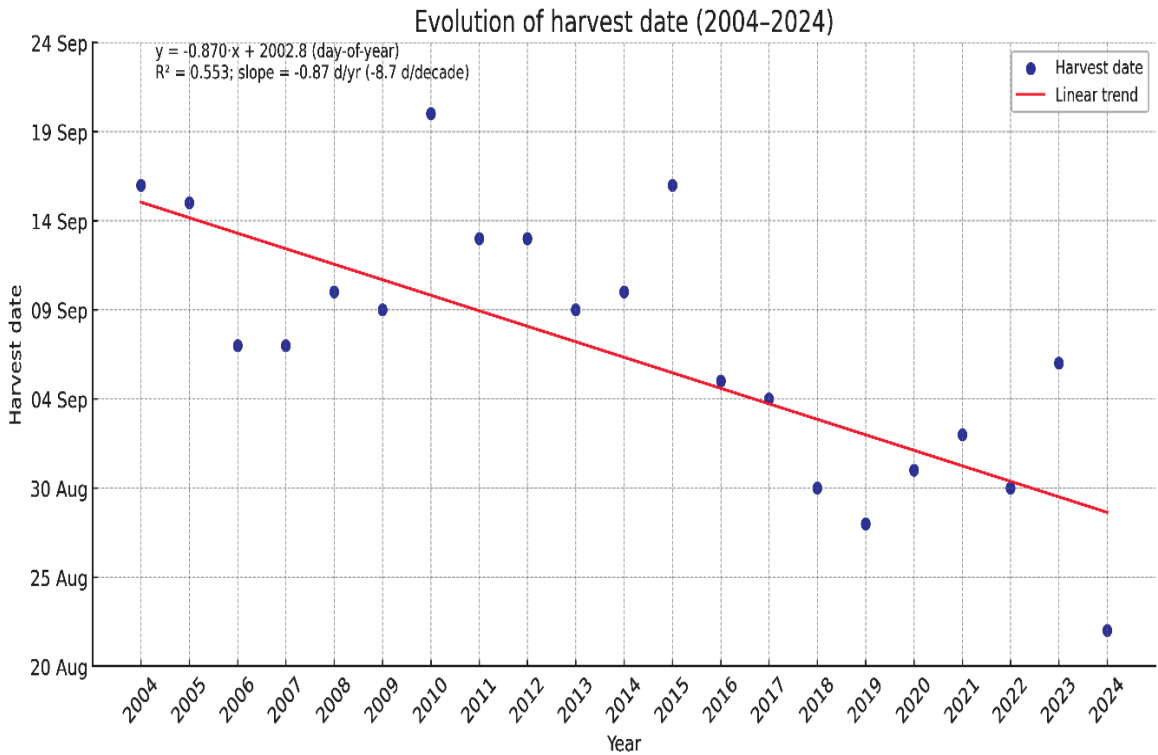


Figure 1. Evolution of harvest date in the Murfatlar wine center (2004–2024).

*Points show the observed harvest dates; the straight line indicates the linear trend ($y = -0.870 \cdot \text{year} + 2002.8$, $R^2 = 0.553$). Negative slope denotes a shift towards earlier harvests (≈ 8.7 days per decade). The regression was fitted on day-of-year and displayed on a calendar-date axis.

Grape quality at harvest

The analysis of the 2015–2024 dataset revealed two distinct patterns regarding grape ripening in the Murfatlar vineyard (Figure 2). First, a consistent decline in titratable acidity was observed across cultivars, indicating a robust and directional response to climatic drivers.

Second, sugar accumulation showed high inter-annual variability without a clear upward trend, despite the widespread expectation that global warming would systematically increase sugar levels at harvest.

Evolution of sugars and acidity at harvest (2015-2024)

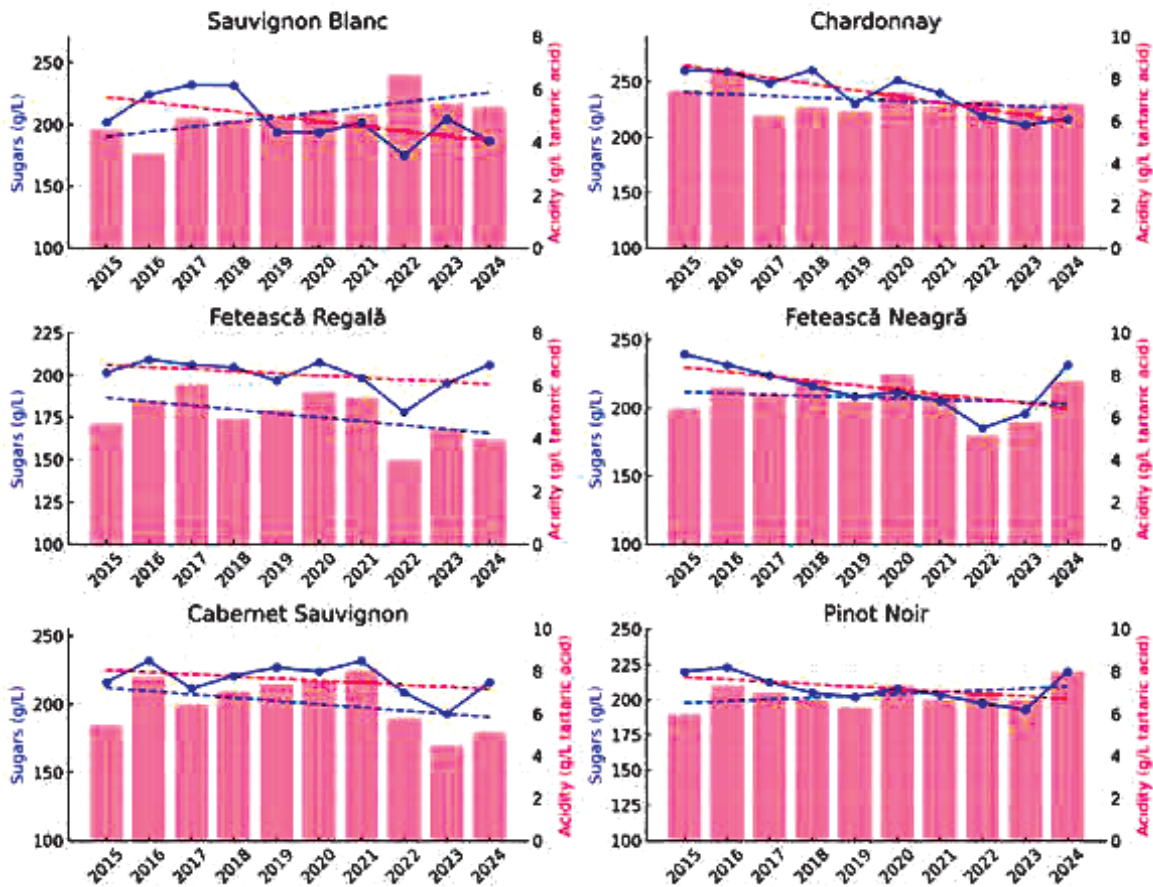


Figure 2. Sugars and acidity dynamics at harvest (2015-2024)

The decline in acidity is consistent with well-documented physiological mechanisms. Warmer ripening periods accelerate the respiration and degradation of organic acids, particularly malic acid, while water deficit can exacerbate this process by enhancing berry transpiration and reducing metabolic stability. As a result, grapes tend to reach harvest maturity at lower acidity, even when sugar accumulation does not substantially increase.

Such decoupling between technological ripeness (sugar concentration) and acid or phenolic balance has been increasingly reported in viticultural areas, especially in Mediterranean climates, where earlier harvests coincide with lower acidity but not necessarily higher sugar levels. This discrepancy arises because higher temperatures accelerate acid degradation, while heat- and drought-induced stomatal closure can limit sugar accumulation—resulting in high variability in sugar content despite significantly declining acid levels. This pattern has been documented in both experimental and survey-based studies of European viticulture under climate warming trends (Poni *et al.*, 2023).

This trend was also highlighted in Romanian vineyards, where higher temperatures and irregular precipitation regimes have led to an earlier onset of maturity and lower acid retention at harvest (Irimia *et al.*, 2017; Artem *et al.*, 2023; Bucur, 2019; Filimon, 2024).

In contrast, sugar levels did not display a systematic increase. Instead, they oscillated between years, reflecting strong vintage effects. Several factors may explain this apparent discrepancy. Under heat and drought stress, stomatal closure limits photosynthesis, reducing the assimilates available for sugar loading into berries. Furthermore, extreme climatic events—such as heatwaves or episodes of heavy rainfall—can either restrict sugar accumulation or dilute must composition. Additionally, biotic stressors (disease pressure)

and vineyard management decisions (harvest timing adjusted to preserve acidity) may also have contributed to maintaining sugars within a relatively stable range.

The regional context of Dobrogea further substantiates these findings. This area is characterized by hot and dry summers, with frequent droughts and irregular rainfall distribution. Climatic studies indicate a gradual warming trend coupled with increased frequency of extreme weather events, confirming the environmental pressures likely driving the observed shifts in grape composition (Negru *et al.*, 2022; Irimia *et al.*, 2017). Thus, the local terroir acts as both a magnifier of climatic variability and a determinant of grapevine metabolic response.

Taken together, these results suggest that the major challenge for Murfatlar viticulture is not excessive sugar accumulation, but the acidity decline. Practical implications include the need to adapt both viticultural and oenological strategies.

The decade-long data from Murfatlar underline a climate-driven decoupling of grape maturity parameters: acidity declines consistently, while sugars remain highly variable. This highlights the importance of integrated adaptation measures to preserve the typicity and competitiveness of local cultivars under ongoing climate change (Negru *et al.*, 2022; *Climate Change Summit Report*, 2023).

In-season dynamics of 100-berry weight and sugar accumulation (2024 vs. multiannual average)

The monitoring of 100-berry weight in 2024 compared with the multiannual mean (Figure 3) reveals pronounced deviations associated with the specific climatic conditions of the year. The 2024 growing season was marked by severe drought during the berry growth phase, followed by episodes of heavy rainfall at the end of August and in early September. This sequence of climatic constraints strongly influenced both cell expansion and final berry size.

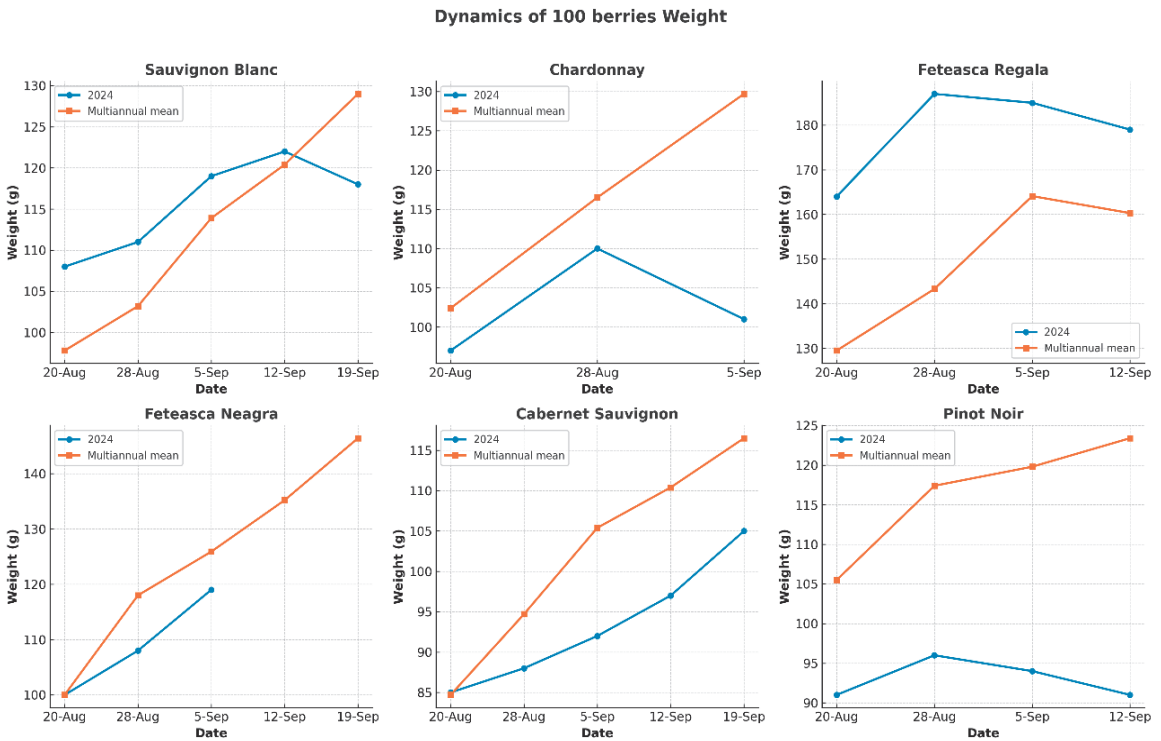


Figure 3. Comparison of 100-berry weight between the 2024 harvest and the multiannual average

In terms of berry weight, most cultivars ('Sauvignon Blanc', 'Chardonnay', 'Cabernet Sauvignon', 'Fetească Neagră') showed lower values than the multiannual mean, with an earlier and more evident decrease towards full maturity. 'Fetească Regală' was the only variety that exceeded the long-term reference, maintaining larger berries for a longer period and showing resilience to climatic stress. 'Pinot Noir' registered the widest gap compared to the multiannual average, with persistently lower values and a steeper decline after veraison, which illustrates its particular sensitivity to climatic extremes.

Monitoring the dynamics of sugar accumulation in 2024, compared with the multiannual mean (Figure 4), indicates pronounced deviations driven by the atypical climatic pattern of the season. All cultivars entered the ripening phase with sugar levels higher than the long-term reference, reflecting the accelerated onset of maturation under the high temperatures recorded during July and early August. However, the progression of sugar accumulation shifted markedly in late August, when reduced sunshine hours and substantial rainfall events slowed down the ripening process and, in some varieties, led to a stagnation of sugar concentration. Among the cultivars evaluated, 'Pinot Noir' consistently maintained higher sugar levels than the multiannual mean across all sampling dates, confirming its sensitivity to thermal conditions and its tendency toward rapid technological maturation during warm seasons. 'Sauvignon Blanc' initially displayed a strong advantage over the long-term reference but was unable to sustain accumulation under the cooler, wetter conditions of early September, resulting in final values below the multiannual average. 'Chardonnay', 'Fetească Regală', and 'Cabernet Sauvignon' also showed signs of stagnation or slight declines in the final stages of ripening, illustrating the combined effects of excess precipitation, reduced radiation and physiological limitations imposed by the climatic anomalies of 2024.

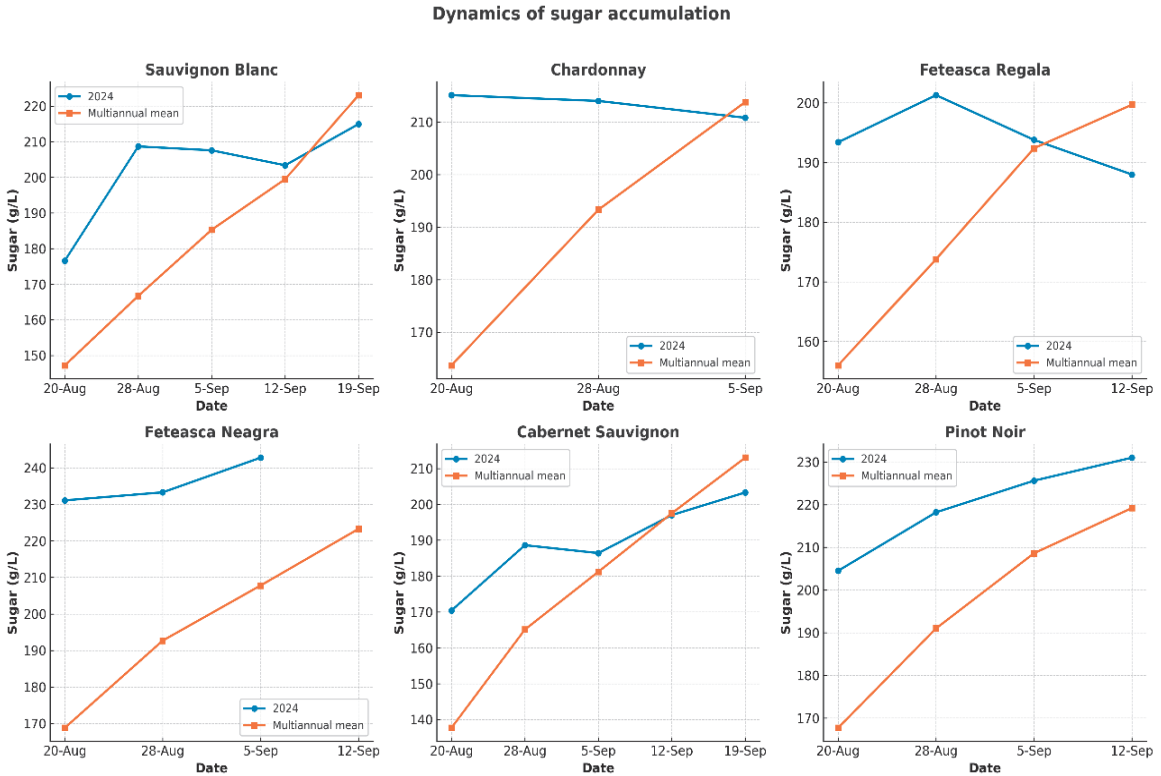


Figure 4. Dynamics of sugar accumulation – comparison between 2024 harvest vs. multiannual average

Taken together, the 2024 vintage shows a markedly atypical ripening dynamic across all cultivars.

An initial phase of accelerated sugar accumulation is observed between 20 and 28 August, followed by a marked reduction in accumulation rate during early-mid September (5–12 September), a period characterized by lower solar radiation and high rainfall.

This shift resulted in cultivar-specific responses. '*Sauvignon Blanc*', '*Fetească Regală*', and '*Cabernet Sauvignon*' exhibited an early-season sugar advantage that was subsequently attenuated, leading to final sugar concentrations on 12–19 September that converged with or dropped below the multiannual mean. Conversely, '*Pinot Noir*' maintained higher sugar levels throughout the September measurements, despite reduced berry mass, indicating a partial decoupling between sugar accumulation and berry growth under late-season climatic constraints.

CONCLUSIONS

The two-decade monitoring of grape ripening at Murfatlar highlights clear climate-driven shifts. Harvest initiation has advanced by nearly three weeks since the early 2000s, reflecting accelerated ripening under warmer and drier conditions. Acidity levels showed a steady decline, whereas sugar accumulation displayed strong inter-annual variability, lacking a consistent increasing trend.

The detailed assessment of the 2024 vintage illustrates how extreme weather shapes grape composition: drought during berry growth limited berry size, while abundant rainfall in late August led to stagnation or dilution of sugars. These combined effects underline the growing risk of decoupling between technological and phenolic ripeness, as well as the challenge of maintaining freshness and balance in local cultivars.

From a practical perspective, the results emphasize the importance of adaptive strategies in both the vineyard and winery. Measures such as optimized canopy and water management, precise harvest timing, and innovative oenological practices are critical to preserve the typicity and competitiveness of Murfatlar wines under ongoing climate change.

REFERENCES

1. Artem, V., Ciobanu, I.C., Ranca, A., Dina, I., Nechita, A., and Zaldea, G. (2023). The influence of climatic factors on the quality of grapes in the vineyards Murfatlar and Iași. *Romanian Journal of Horticulture*, 2(2): 24–29.
2. Bucur, G. M. (2019). The rate of climate change in southern Romania: trends in phenology and grape quality. *BIO Web of Conferences*, 9: 01008. <https://doi.org/10.1051/bioconf/20191501008>
3. Climate Change Summit Report. (2023). Climate change and its influence on Romanian wine: the case of Dobrogea. Retrieved from *Climate Change Summit*.
4. Filimon, R. M. (2024). Long-term evolution of climatic factors and their influence on grape quality in northeastern Romania. *Horticulturae* 2024, 10(7): 705. <https://www.mdpi.com/2311-7524/10/7/705>
5. Hannah, L., Roehrdanz, P.R., Ikegami, M. et al. (2013). Climate change, wine, and conservation. *Proceedings of the National Academy of Sciences*, 110(17), 6907–6912.
6. Irimia, L.M., Patriche, C.V., and Irimia, A. (2017). Climate change impact on climate suitability for wine production in Romania. *Theoretical and Applied Climatology*, 127: 1–14.
7. Jones, G.V., White, M.A., Cooper, O.R., and Storchmann, K. (2005). Climate change and global wine quality. *Climatic Change*, 73: 319–343. <https://link.springer.com/article/10.1007/s10584-005-4704-2>
8. Mira de Orduña, R. (2010). Climate change associated effects on grape and wine quality and production. *Food Research International*, 43: 1844–1855.
9. Negraru (Tănase), A., Botu, M., Ranca, A., Ciocan, M.A., Dina, I., and Beleniuc, G.V. (2022). The impact of climate change on the grapevine in the topoclimate of the Murfatlar wine center. *Journal of Agroalimentary Processes and Technologies*, 28(3): 260–267.
10. Poni, S., Gatti, M., Palliotti, A., Dai, Z., Duchêne, E., Truong, T.-T., Ferrara, G., Matarrese, A. M. S., Gallotta, A., Bellincontro, A., Mencarelli, F., and Tombesi, S. (2018). Grapevine quality: A multiple choice issue. *Scientia Horticulturae*, 234, 445–462. <https://doi.org/10.1016/j.scienta.2017.12.035>
11. Sadras, V.O., and Moran, M.A. (2012). Elevated temperature decouples anthocyanins and sugars in berries of Shiraz and Cabernet Franc. *Australian Journal of Grape and Wine Research*, 18: 115–122.

12. Schultz, H.R. (2016). Global climate change, sustainability, and some challenges for grape and wine production. *Journal of Wine Economics*, 11(1): 181–200.; doi:10.1017/jwe.2015.31
13. van Leeuwen, C., and Destrac-Irvine, A. (2017). Modified grape composition under climate change conditions requires adaptations in the vineyard. *OENO One*, 51(2): 147–154.
14. Webb, L.B., Whetton, P.H., and Barlow, E.W.R. (2012). Modelled impact of future climate change on the phenology of winegrapes in Australia. *Australian Journal of Grape and Wine Research*, 18: 145–155.

Influence of irrigation on the growth and quality of grapevine propagation material

D.G. Dinu, A.C. Florea, D.I. Sumedrea* and A.M. Dumitru

National Research and Development Institute for Biotechnology
in Horticulture, Stefanesti-Arges, Romania

*Corresponding author email: dsumedrea@yahoo.com

ABSTRACT

Efficient water management is essential in grapevine nurseries, where irrigation strongly influences the morphological quality and the suitability of propagation material for successful vineyard establishment. This study evaluated the impact of two irrigation regimes on grafted grapevine seedlings produced under protected nursery conditions in 2025. Four table-grape cultivars ('Argessis', 'Memory', 'Norocel', and 'Ștefănești') grafted onto SO4-4 rootstock were grown under irrigation levels corresponding to 100% and 75% of the Active Soil Moisture Interval (ASMI). Morphological characteristics, including leaf area, main shoot length and diameter, number of roots ≥ 2 mm, main root length, and maximum root diameter, were assessed to evaluate shoot vigor and root morphological characteristics. Water use efficiency (WUE) was also determined to characterize the technological performance of each irrigation regime. The reduced irrigation level (75% ASMI) improved the morphological characteristics of both shoots and roots in most cultivars, increased the proportion of first-quality vines, and resulted in substantially higher WUE. The results obtained have confirmed that a 75% ASMI regime can improve water-use efficiency while sustaining high morphological quality, highlighting its potential as an efficient and sustainable irrigation strategy for nurseries.

Keywords: greenhouse, grapevine propagation, irrigation, grapevine nursery, morphological quality, water use efficiency (WUE)

INTRODUCTION

Grapevines are an important crop worldwide and they can be grown with limited water supply, occupying dry and semidry lands well (Rustioni *et al.*, 2019; Cifre *et al.*, 2005; Chalmers *et al.*, 2004). Growing conditions significantly affect the vine phenotype, including the plant morphology (Dinu *et al.*, 2021). For sustainable water management, it is essential to consider the relationship between root-water uptake and leaf transpiration (Green *et al.*, 2016), as vines absorb water primarily through their roots.

In general, root elongation and increase soil exploration play a central role in adaptation to abiotic stresses (Sun *et al.*, 2020; Mariani *et al.*, 2017), including higher tolerance to drought (Köse *et al.*, 2014; Corso and Bonghi, 2014). It has been demonstrated that one morphological adaptation strategy to drought led to the development of deeper roots capable of reaching wetter soils layers (Bauerle *et al.*, 2008). Furthermore, roots proliferation, through increasing number and branching enhances water conductance (Alsina *et al.*, 2010).

This study aimed to assess the effect of two irrigation regimes on the morphological quality of grafted grapevine vines grown in a protected nursery system.

MATERIALS AND METHODS

The experiment was conducted at National Research and Development Institute for Biotechnology in Horticulture – Stefanesti (INCDBH Stefanesti), in the Laboratory of Ecology and Propagation of Horticultural Species, with the objective of improving techniques for producing grafted grapevine material under protected conditions.

The study included four local table-grape cultivars ('Argessis', 'Norocel', 'Memory', and 'Ștefănești'), which were grafted onto SO4-4 rootstock at the end of February. Scions were hydrated for 24 hours before grafting. A paraffin mixture containing growth stimulators was applied during grafting.

Green paraffin was used before planting for protection, red paraffin was applied immediately after grafting and before forcing, and an additional coating was added before field establishment to limit dehydration and protect young shoots. Grafted cuttings were planted in raised beds covered with polyethylene film, arranged in a double-row system (20 cm width). A total of 3,900 plants were grown in a 300 m² protected area.

After planting, the grafted vines were monitored throughout the entire experimental period under two irrigation treatments: V1 – 100% of the Active Soil Moisture Interval (ASMI) and V2 – 75% of ASMI.

In October, when the grafted vines were harvested for evaluation and commercial classification, the following morphological characteristics were recorded: main shoot length, main shoot diameter, leaf area, number of roots, maximum root length, main root diameter, and number of roots ≥ 2 mm. At the same time, the vines were classified into Quality I and Quality II categories according to nursery morphological criteria, and the percentage of plants in each quality class was determined for both irrigation treatments. Statistical analysis was performed using SPSS (IBM Corporation, Armonk, NY, USA). Pearson correlation was applied to evaluate the relationships between morphological characteristics, vine quality (Quality I and II), and water use efficiency (WUE).

RESULTS AND DISCUSSIONS

The quality classification of grafted vines represents a key indicator in nursery production, as it determines both the commercial value of the planting material and its potential performance after establishment. The results showed clear differences between irrigation treatments, with the reduced irrigation level (75% ASMI) consistently improving the proportion of Quality I vines across all cultivars (Table 1). For 'Argessis', the percentage of Quality I vines increased from 42% under full irrigation (100% ASMI) to 53% under reduced irrigation (75% ASMI), while Quality II vines decreased from 43% to 27%. A similar trend was observed in 'Memory', where Quality I vines increased from 41% (V1) to 54% (V2), accompanied by a reduction in Quality II vines from 59% to 46%.

The most pronounced response was recorded for 'Norocel', which showed a substantial increase in Quality I vines from 47% (V1) to 67% (V2), while Quality II vines decreased from 53% to 33%. For 'Ștefănești', the proportion of Quality I vines increased from 49% to 55%, although the reduction in Quality II vines was less pronounced (from 32% to 36%). Overall, the 75% ASMI regime improved vine quality in all cultivars, suggesting that moderate water restriction promotes a more balanced vegetative and root development, ultimately leading to a higher proportion of commercially valuable planting material.

Leaf area is a key morphological indicator that reflects the plant's vegetative vigor and its capacity to use water resources efficiently, being strongly influenced by growth conditions (Green *et al.*, 2006; Dinu *et al.*, 2021).

Evaluating this parameter under different irrigation regimes provides essential information on cultivar-specific responses to water availability. Evaluating leaf area under different irrigation regimes offers valuable insight into the way grapevine cultivars adjust their growth in response to water availability.

Table 1. Classification of grafted vines (Quality I and II) under different irrigation regimes (ASMI%) for the four cultivars.

Cultivar	Irrigation regime-ASMI (%)	Quality I grafted wines (%)	Quality II grafted vines (%)
'Argessis'	v1-100	42	43
	v2-75	53	27
'Memory'	v1-100	41	59
	v2-75	54	46
'Norocel'	v1-100	47	53
	v2-75	67	33
'Ștefănești'	v1-100	49	32
	v2-75	55	36

The four cultivars showed distinct responses to the two irrigation treatments (Figure 1). The 'Argessis' cultivar recorded the highest leaf area values under both irrigation levels variants (with 152.48 cm² at 100% (V1) and 141.00 cm² at 75% ASMI indicating low sensitivity to reduced irrigation.

The 'Memory' cultivar displayed minimal variation between treatments (64,32 and 63,56 cm² leaf area) suggesting limited development and reduced responsiveness to water reduction. In contrast, 'Norocel' and 'Ștefănești' cultivars showed a positive reaction to moderate water restriction. Leaf area increased from 99.14 cm² (V1) to 147.39 cm² (V2) in 'Norocel', and from 85.00 cm² to 104.12 cm² in 'Ștefănești'. This response suggests a more efficient use of water and better vegetative balance under 75% ASMI regime. In conclusion, the cultivars showed distinct responses to reduced irrigation. 'Argessis' and 'Memory' displayed similar leaf area values under both treatments, indicating low sensitivity to changes in water supply. In contrast, 'Norocel' and 'Ștefănești' responded positively to the 75% ASMI regime, exhibiting increased leaf area under moderate water restriction.

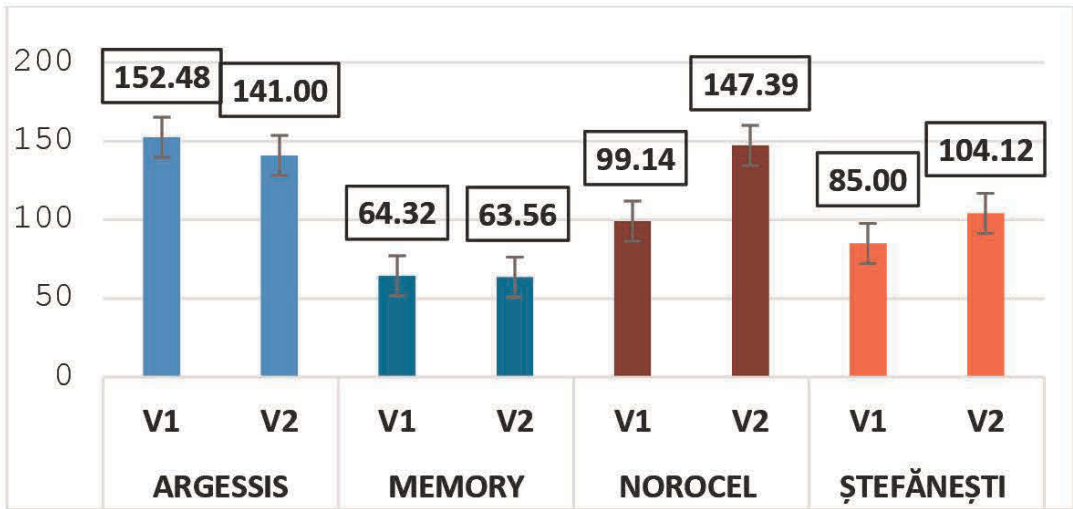


Figure 1. Influence of irrigation regimes on leaf area

Main shoot length is an essential morphological indicator, as it reflects vegetative vigour and the plant's ability to allocate resources efficiently under varying water conditions. Irrigation level can significantly influence shoot development, with cultivar-specific responses often reported in grapevine studies (Dinu *et al.*, 2021).

The 'Argessis' cultivar recorded the highest shoot length values in both experimental variants, reaching 50,08 cm under the influence of irrigation applied at in the at 100% ASMI (V1), respectively 54,46 cm when irrigation was applied at 75% ASMI (V2) indicating stable growth regardless of water reduction.

The 'Memory' cultivar recorded, lowest shoot length values among the cultivars studied with small differences between irrigation regimes: 28.44 cm under the influence of 100% ASMI irrigation (V1) and 24.14 cm at the application of irrigation calculated at 75% ASMI (V2). These results emphasize its reduced vegetative development and limited sensitivity to irrigation level-28,44 cm and 24,14 cm.

More pronounced differences were observed in the "Norocel" cultivar. Under the influence of irrigation applied at 100% ASMI (V1), the shoot length was only 15.44 cm, while the application of irrigation calculated at 75% ASMI (V2) led to a substantial increase, reaching 43.34 cm. A similar response was also recorded in the 'Ștefănești' cultivar. The application of irrigation at 100% ASMI (V1) led to a shoot length of only 14.63 cm, while the irrigation applied at 75% ASMI (V2) led to a considerable increase, reaching 43.69 cm. These results confirm that this cultivar responds more favorably to moderate irrigation than to full water availability.

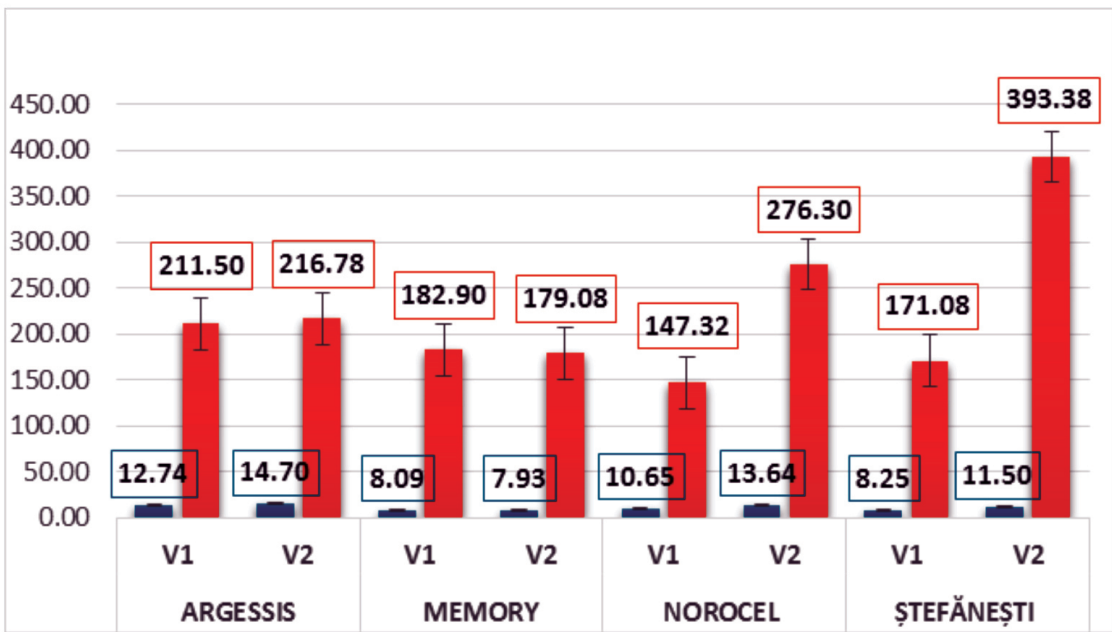


Figure 2. Influence of irrigation regimes on main shoot length and diameter in the four grapevine cultivars

The results obtained highlighted how the analyzed cultivars responded to the experimental variants (irrigation level applied). A significant difference in shoot diameter is observed between the cultivars but also between the two experimental variants (V1 and V2). The 'Argessis' cultivar recorded the highest shoot diameter value, with 12,74 mm under the influence of irrigation applied at 100% ASMI (V1), respectively 14.70 mm when irrigation was applied at 75% ASMI (V2). The resulting increase of +1.96 mm of irrigation at 75%_did

not affect growth, but stimulated shoot thickening. In the 'Memory' cultivar, shoot diameter was relatively constant between irrigation regimes (8.09 mm in V1 and 7.93 mm in V2), suggesting a indicating balanced but limited growth, regardless of water supply. The 'Norocel' cultivar responded positively to reduced irrigation level—with shoot diameter increasing from 10.65 mm (V1) to 13.64 mm (V2).

A similar trend was observed in the 'Ștefănești' cultivar where shoot diameter increasing from 8.25 mm (V1) to 11.50 mm (V2). In contrast, the 'Memory' cultivar recorded similar values between treatment, indicating stable growth but a reduced capacity to adjust to modified humidity conditions. These differences underlines the genetic variability among cultivars and their distinct ability to capitalize available resources.

Regarding the number of roots with a diameter ≥ 2 mm, the results showed clear differences between cultivars and irrigation treatments.—The 'Argessis' cultivar recorded the highest values, in both irrigation regimes, with 7.20 roots under the influence of irrigation applied at 100% ASMI (V1) and 12.80 when irrigation was applied at 75% ASMI (V2), indicating strong root development under moderate moisture conditions.

The 'Memory' cultivar in the number of roots increased markedly from 3.00 mm (V1) to 6.40 mm (V2), suggesting that reduced irrigation stimulated the formation of roots with larger diameter.

A similar trend was observed in the 'Norocel' cultivar—where the number of roots ≥ 2 mm increased from 4.80 roots under 100% ASMI (V1) to 9.00 roots under 75% ASMI (V2), confirming good adaptation to moderate soil moisture. In contrast, the 'Ștefănești' cultivar recorded similar values of roots with a diameter of ≥ 2 mm between the experimental variants (4.80 mm – V1, respectively 6.20 mm – V2).

Overall, most cultivars showed an improvement in root development, under reduced irrigation condition (V2).

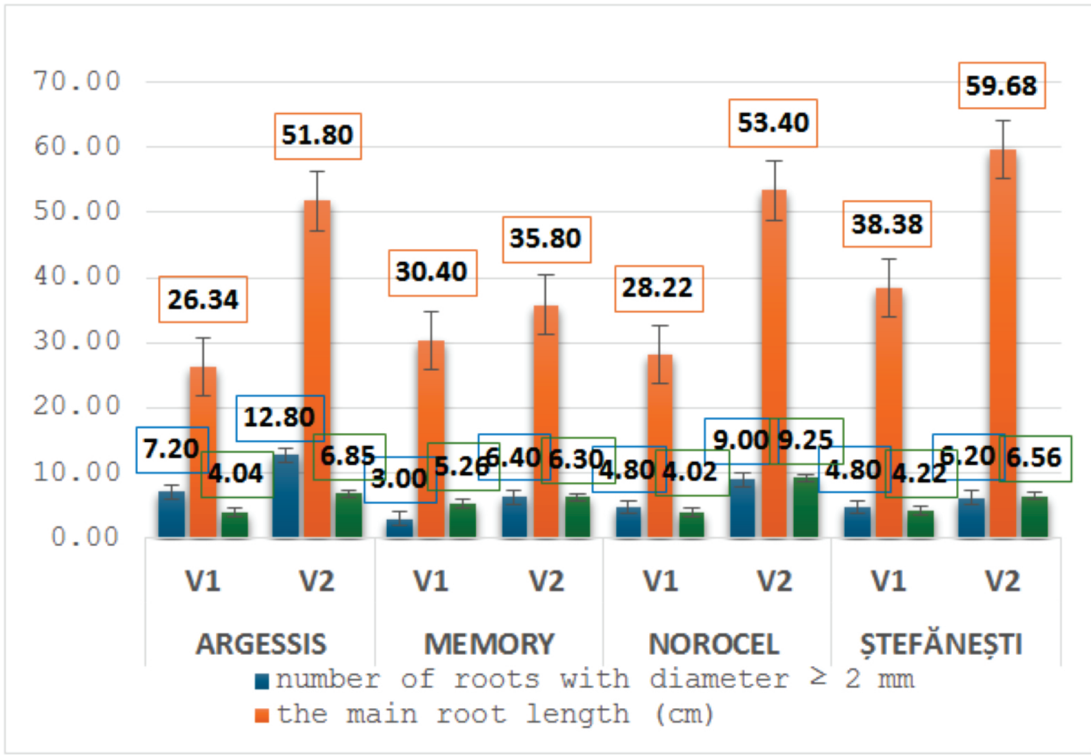


Figure 3. Influence of irrigation variants on the number of roots with diameter ≥ 2 mm, the main shoot length and maximum root diameter

Analyzing the influence of experimental variants on the main root length in the 4 cultivars, significant differences are found between genotypes, with a tendency to intensify root growth under the conditions of applying irrigation calculated at V2, compared to V1 (Figure 3).

The 'Argessis' cultivar recorded a significant increase in the length of the main root from 26.34 cm in the V1 variant to 51.80 cm in the V2 variant, which indicates a positive reaction to the moderate reduction in water intake.

The results indicate that reducing the level of irrigation favored the elongation of the main root, which suggests an effective adaptive strategy by which plants optimize the absorption of water under conditions of limited resources. In the case of experimental variants with reduced dose of water (V2) to complete the life requirement, they explore a larger volume of soil (chemotropism) and as a result, create a more extensive root system than in the case of plants receiving optimal amount of water.

Regarding the maximum root diameter, the results show consistent increases under the application of irrigation calculated at 75% ASMI (V2), with all cultivars displaying thicker roots under reduced water supply.

The 'Argessis' cultivar recorded a marked increase in maximum root diameter when irrigation was reduced to 75% ASMI (V2), rising from 4.04 mm under the influence of irrigation applied at 100% ASMI (V1) to 6.85 mm in V2, representing a +2.81 mm increase.

The 'Memory' cultivar showed a similar trend, with maximum root diameter increasing from 5.26 mm under the influence of irrigation applied at 100% ASMI (V1) to 6.30 mm when irrigation was applied at 75% ASMI (V2), suggesting a good adaptation capacity.

In the 'Norocel' cultivar, increase was even more pronounced, maximum root diameter 9.25 mm in V2, suggesting enhanced root tissue development and a strong adaptive response to reduced irrigation input.

The 'Ștefănești' cultivar recorded the same positive trend, with the maximum root diameter increasing from 4.22 mm (V1) to 6.56 mm (V2).

The results obtained showed that reducing the level of irrigation to 75% resulted in a thickening of the roots in all the cultivar analyzed, suggesting a better efficiency in the use of water resources.

Tabel 1. Correlation (r) between the morphological indicators

Morphologic indicator	Ist Quality	IInd Quality	WUE
Length of the main shoot (cm)	0,57	-0,62	0,71
Diameter of the shoot (mm)	0,02	-0,03	0,04
Leaf area (cm ²)	0,26	-0,21	0,24
Length of the main root (cm)	0,34	-0,35	0,31
Maximum root diameter (mm)	-0,13	0,09	0,03
Number of roots with diameter ≥ 2 mm	0,65	-0,67*	0,77*

The results obtained shows that balanced development of both aerial part and the root system play an essential role in the efficient use of water, especially under the 75% ASMI irrigation regime, where these relationships were more evident.

Extending the Pearson correlation analysis to include Quality II vines revealed several inverse and statistically significant relationships between morphological indicators and this quality category.

Thus, the length of the main shoot and the number of roots with a diameter of ≥2 mm shows significant negative correlations with the percentage of Quality II vines ($r = -0.62$ and $r = -0.67$, $p < 0.05$).

These results suggest that a vigorous shoot development vines and a well-defined root system favor the formation of Quality I vines, thereby reducing the proportion of lower quality plants.

Other indicators, such as main root length also showed negative but nonsignificant correlations ($r = -0.28$ and $r = -0.35$), confirming that vines with weaker morphologically development tend to fall into the Quality II category.

In contrast, the maximum root diameter did not have a significant influence on quality, the correlation values being close to zero ($r = 0.09$).

Overall, these correlations emphasize that the moderate irrigation regime (V2) promotes—to a more balanced morphological development, increases the proportion of Quality I vines, and reduces the share of Quality II vines, thereby improving the overall quality of the planting material Quality II and increasing the proportion of Quality I vines.

CONCLUSIONS

The results of the study show that the irrigation regime of 75% ASMI (V2) supported a more balanced development of the grafted vines compared to full irrigation (V1). Moderate water restriction stimulated root thickening, increased the number of roots with a diameter ≥ 2 mm, and improved main shoot growth in most cultivars. 'Norocel' and 'Ștefănești' showed the strongest positive reactions to reduced irrigation, while 'Argessis' displayed stable development under both irrigation levels. In contrast, 'Memory' exhibited limited variability, indicating a lower sensitivity to changes in water supply. Quality classification confirmed these trends: the 75% ASMI regime increased the proportion of Quality I vines and reduced the number of Quality II vines across all cultivars. Correlation analysis further highlighted the importance of a well-developed root system and balanced shoot growth for achieving higher-quality grafted vines and better water-use efficiency.

Overall, the 75% ASMI irrigation regime ensured efficient resource use and contributed to obtaining high-quality grafted vines, demonstrating its suitability as an optimal technological solution for the conditions analyzed.

REFERENCES

1. Alsina, M.M., Smart, D.R., Bauerle, T., De Herralde, F., Biel, C., Stockert, C., Negron, C., Save, R. (2010). Seasonal changes of whole root system conductance by a drought-tolerant grape root system. *Journal of Experimental Botany*, 62, 99–109.
2. Bauerle, T.L., Smart, D.R., Bauerle, W.L., Stockert, C., Eissenstat, D.M. (2008). Root foraging in response to heterogeneous soil moisture in two grapevines that differ in potential growth rate. *New Phytologist* 179, 857–866.
3. Chalmers, Y., Kelly, G., Krstic, M. (2004). Partial Rootzone Drying of *Vitis Vinifera* cv. 'Shiraz' Winegrapes in a Semi-Arid Climate. *Acta Horticulturae*, 664, 133–138.
4. Cifre, J., Bota, J., Escalona, J., Medrano, H., Flexas, J. (2005). Physiological tools for irrigation scheduling in grapevine (*Vitis vinifera* L.): An open gate to improve water-use efficiency? *Agriculture Ecosystems & Environment*, 106, 159–170.
5. Corso, M., Bonghi, C. (2014). Grapevine rootstock effects on abiotic stress tolerance. *Plant Science. Today*, 1, 108–113.
6. Dinu, D.G., Bianchi, D., Mamasakhlisashvili, L., Quarta, C., Brancadoro, L., Maghradze, D., Cardinale, M., Rustioni, L. (2021). Effects of genotype and environmental conditions on grapevine (*Vitis vinifera* L.) shoot morphology. *Vitis*, 60, 85–91.
7. Green, S.R., Kirkham, M., Clothier, B.E. (2006). Root uptake and transpiration: From measurements and models to sustainable irrigation. *Agricultural Water Management*, 86, 165–176.
8. Köse, B., Karabulut, B., Ceylan, K. (2014). Effect of Rootstock on Grafted Grapevine Quality *European Journal of Horticultural Science*, 79, 197.
9. Mariani, L., Ferrante, A. (2017). Agronomic Management for Enhancing Plant Tolerance to Abiotic Stresses—Drought, Salinity, Hypoxia, and Lodging. *Horticulturae*, 3, 52.

10. Rustioni, L., Cola, G., Maghradze, D., Abashidze, E., Argiriou, A., Aroutiounian, R., Brazão, J., Chipashvili, R., Cocco, M., Cornea, V., et al. (2019). Description of the *Vitis vinifera* L. phenotypic variability in enocarpological traits by a Euro-Asiatic collaborative network among ampelographic collections. *Vitis*, 58, 37–46.
11. Sun, X., Chen, F., Yuan, L., Mi, G. (2020). The physiological mechanism underlying root elongation in response to nitrogen deficiency in crop plants. *Planta*, 251, 84.

Oil content in grapevine seeds of intra- and interspecific provenance genotypes

E. Alexandrov

The Institute of Genetics, Physiology and Plant Protection of the MSU,
Republic of Moldova

*Corresponding author e-mail: alexandrov.eugeniu@gmail.com

ABSTRACT

The aim of this study was to determine and compare the oil content in grapevine seeds from intra- and interspecific genotypes with yellow-green and blue-violet berries. Grapevine processing generates secondary products that represent about 20% of the total processed mass among which grape seeds contain 9-20% oil, rich in antioxidants and essential fatty acids with biological importance for the human body. Owing to this composition, grape seeds serve as a valuable raw material for obtaining grape seed oil. The seeds were extracted from fresh berries at the onset of must fermentation, and oil content was determined using the nuclear magnetic resonance (NMR) relaxometry method. In intraspecific genotypes with yellow-green berries, oil content ranged from 13.8-13.7% ('Fetescă regală', 'Sauvignon'), while interspecific genotypes of the same colour group contained 13.45-14.46% oil ('Alexandrina', 'Sarmis', etc.). For intraspecific genotypes of the same colour group contained 13.5% oil ('Fetească regală') and 16.6% oil ('Merlot'), were recorded, whereas interspecific blue-violet genotypes showed oil contents between 11% ('Ametist') and 15.35% (Sor). The results indicate that oil accumulation in grapevine seeds depends both on the genotype and on the climatic conditions in which the vines are cultivated. Climatic analysis of the cultivation area showed an increase of 3.23°C above the calculated long-term annual temperature norm, suggesting that arid conditions favour higher oil content compared with regions with moderate temperatures.

Keywords: genotype, seeds, oil, grapevine, relaxometry

INTRODUCTION

Every year, winemaking enterprises accumulate impressive quantities of grape pomace, which, as a result of the separation of fractions, extract the seeds for further processing for various purposes. The secondary products, as a result of the processing of the vine grapes, constitute about 20% of the total mass processed, being made up of bunches, the skin of the berries, seeds and various sediments from the must and wine. This is the raw material for the production of grape seed oil, feed products, food products, abrasive materials, oenotannin, etc. As a result of the fermentation of grape juice, to obtain derived products (wine, divin), the deposited sediments allow the production of tartaric acid, as well as alcohol, biogas and organic fertilizers (Glăman *et al*, 2018; Codreanu, 2006; Dorobanțu and Beceanu, 2007).

From an economic and technological point of view, a primary criterion for evaluating oil raw materials is the composition and quality of the oils. The oil obtained from grape seeds consists of saturated fats (palmitic and stearic) as well as unsaturated fatty acids. Grape

seeds contain three times less oil compared to traditional oil crops, such as rapeseed, sunflower, etc. (Antoce. 2007; Prichernomorya 2009).

This work aimed to quantitatively compare the oil content of seeds of intraspecific and interspecific grapevine genotypes.

MATERIALS AND METHODS

The study was carried out at the Institute of Genetics, Physiology and Plant Protection of the MSU, where all seed processing and analytical determinations were performed, in the period 2024. The biological material consisted of seeds obtained from—intraspecific grapevine genotypes ('Feteasca neagră', 'Feteasca regală', 'Merlot', "Sauvignon"), and interspecific rhizogenic grapevine genotypes ('Ametist', 'Alexandrina', 'Tethys', etc. (Alexandrov, 2019; 2023). These genotypes are part of the institutional germplasm collection described in previous studies.

Grape seeds were extracted from freshly harvested berries before the onset of must fermentation, to preserve their natural biochemical composition, particularly the oil fraction.

Through the analyses carried out, according to existing standards, the following were determined: the amount of oil (%) and the weight of the seeds.

The determination of oil content in grape seeds was carried out using the NMR (nuclear magnetic resonance) relaxometer method.

The determination of oil by nuclear magnetic resonance is carried out by comparing the amplitude of the echo signal from the seeds with that from a set of calibration standards. Grape seeds were extracted manually from freshly harvested berries before the onset of must fermentation, to preserve their natural biochemical composition, particularly the oil fraction. After extraction, the seeds were cleaned and prepared for analysis in accordance with the standard. According to existing methodological standards, two parameters were determined Seed oil content (%), and Seed weight (mg).

Oil content was quantified using the nuclear magnetic resonance (NMR) relaxometry method. The determination is based on measuring and comparing the amplitude of the echo signal from the seed samples with that obtained from calibration standards selected according to the natural biochemical characteristics of grapevine seeds.

Temperature and atmospheric precipitation were collected from weather stations; the data obtained were analyzed and statistically processed, based on which the necessary graphs were developed [Atlas, 2021; www.meteo.md].

The climatic parameters (temperature and atmospheric precipitation) corresponding to the cultivation area of the analyzed grapevine genotypes were obtained from official national meteorological stations.

The recorded data, calculated as averages for the study years (2022–2024), were processed and compared with the long-term climatic norms established for the region (covering the period 1950–1985). Data were expressed as mean $\pm$ standard deviation

Statistical analysis was performed using software SPSS, R, STATISTICA.

RESULTS AND DISCUSSIONS

Grape seed oil is known to be rich in antioxidants, particularly vitamin E and contains essential fatty acids such as linoleic acid, which contribute to supporting cardiovascular, circulatory and immune functions. Literature data indicate that grape seeds contain between 9.9 – 20.6% oil on a dry mass [Dorobanțu and Beceanu D., 2007; Prichernomorya, 2009].

The quantity of oil accumulated in depends on the genotype seeds is influenced both by the genotype and the climatic conditions in which the grapevine is cultivated. In the case of grapevine cultivation in the southern wine-growing area with an arid climate, the oil content

in grapevine seeds will be higher than in the case of grapevine cultivation in areas located further north. Grape berries contain between one and four seeds; therefore, 1,000 berries yield approximately 2,000 seeds. From one kilogram of grapes, about 30 g of seeds can be obtained, with a dry matter content of roughly 72% (Codreanu, 2006; Dorobanțu and Beceanu, 2007; Vinogradarstvo, 2009). Determining the oil content in grapevine seeds of intraspecific genotypes with yellow-green berries, it was found that they contain about 13.8-13.7% oil ('Fetească regală', 'Sauvignon'), while seeds of interspecific genotypes with yellow-green berries contain about 13.45-14.46% oil ('Alexandrina', 'Sarmis', etc.) (Table 1.).

Analysis of oil content in seeds from intraspecific genotypes with blue-violet yellow-green berries showed values of 13.8–13.7% (Fetească regală, "Sauvignon"), whereas interspecific genotypes with yellow-green berries contained 13.45–14.46% oil ('Alexandrina', 'Sarmis', etc.) (Table 1).

For intraspecific genotypes with blue-violet berries, oil content reached 13.5% ('Fetească neagră') and 16.6% ('Merlot'). In comparison, interspecific genotypes with blue-violet berries contained 11% ('Ametist') and 15.35% ('Sor') (Table 1).

Considering the average seed weight intraspecific genotypes displayed an average value of 30.4 mg, with an average oil content of 14.4% while interspecific genotypes presented seeds with an average weight of 33 mg and an average oil content of 13.9% (Table 1).

When grouped according to berry colour, seeds from genotypes with yellowish-green berries contained, on average, 14.4% oil, whereas those from genotypes with blue-violet berries contained 13.54% oil.

Previous studies reported that grape seeds collected from fermented grape pomace contain only 9-12%, which is lower than values obtained from seeds extracted before must fermentation (Codreanu, 2006; Dorobanțu and Beceanu, 2007).

Regarding the climatic conditions in the cultivation area of the studied genotypes, the long-term average annual temperature norm for the region is 9.73°C (the period 1950–1985).

The average annual temperature calculated for the study period 2022–2024 was 12.96°C, indicating an increase of 3.23°C above the established climatic norm (Figure 1).

Table 1. Oil content and weight of grapevine seeds

<i>Genotype</i>	<i>Oil, (%)</i>	<i>Seed weight, (mg)</i>
<i>Intraspecific genotypes (yellow-green berry)</i>		
'Feteasca regală'	13,8±0,15	27±0,15
'Sauvignon'	13,7±0,2	38,9±0,25
<i>Intraspecific genotypes (blue-purple berry)</i>		
'Feteasca neagră'	13,5±0,12	28,4±0,13
'Merlot'	16,6±0,18	27,3±0,11
<i>Interspecific genotypes (yellow-green berry)</i>		
'Alexandrina'	14±0,15	32,6±0,14
'Tethys'	13,45±0,12	29,8±0,21
'Sarmis'	13,7±0,21	38,9±0,22
'BC ₃ -536'	14,46±0,14	35,8±0,2
<i>Interspecific genotypes (blue-purple berry)</i>		
'Ametist'	11±0,11	32,85±0,24
'Sor'	15,35±0,13	28,4±0,13

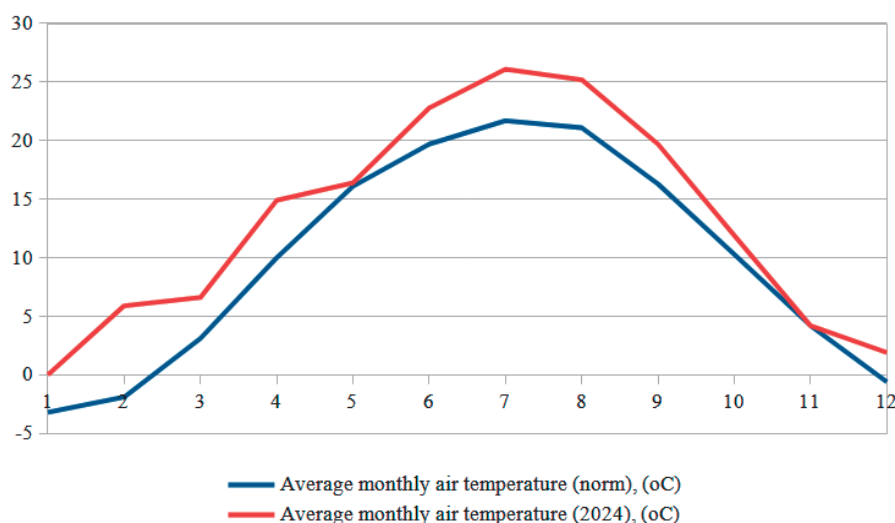


Figure 1. Average monthly temperature during 2024 compared to the monthly norm (1950-1985), Chisinau

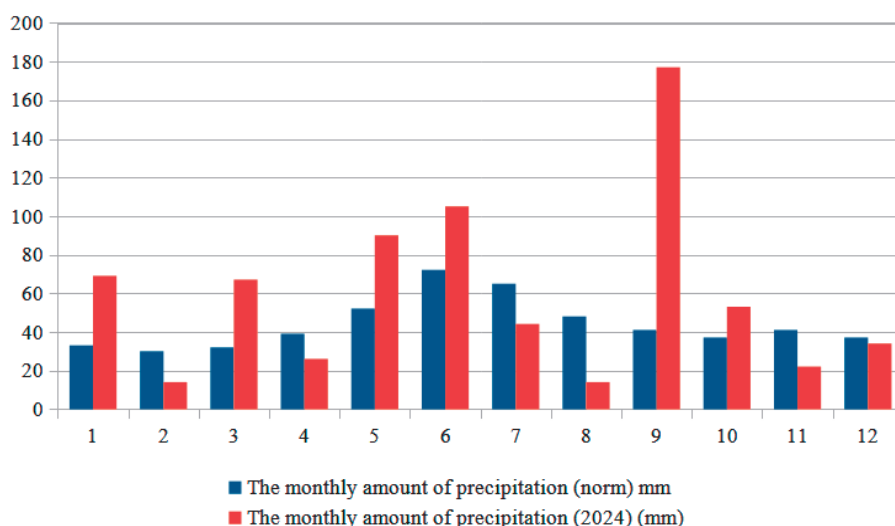


Figure 2. Amount of monthly atmospheric precipitation during 2024 compared to the norm (1950-1985), Chisinau

The annual precipitation norm for the studied area is 527 mm. In the year 2024, the total amount of atmospheric precipitation reached 715 mm. These data indicate an excess of 188 mm relative to the multiannual norm.

The analysis of daily precipitation recorded throughout 2024 reveals a marked irregularity in the temporal distribution of atmospheric precipitation (Figure 2). The average annual normal temperature for the territory of the Republic of Moldova, calculated for the reference period 1950–1985, is 9.46 °C. Analysis of the average annual temperature data for the period 2002–2024 indicates a mean value of 10.88 °C. Consequently, an increase of 1.42 °C above the climatic norm is recorded for this interval across the territory of the republic (Figure 4).

Based on the analysis of monthly atmospheric precipitation for the period 2002–2024 in the territory of the Republic of Moldova, it was found that precipitation amounts were approximately half of the calculated norm for 1950–1985. Consequently, a pronounced soil moisture deficit persists throughout the region (Figure 3).

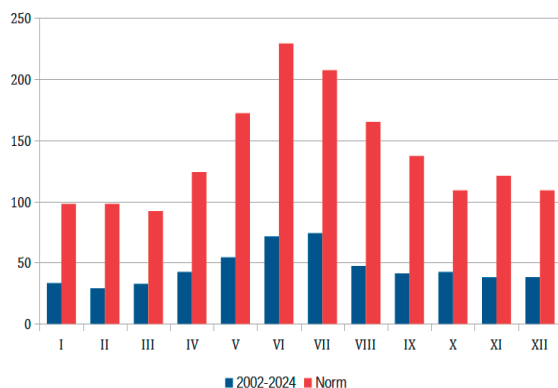


Figure 3. Monthly atmospheric precipitation for 2002–2024 compared with the calculated climatic norm for 1950–1985, Republic of Moldova.

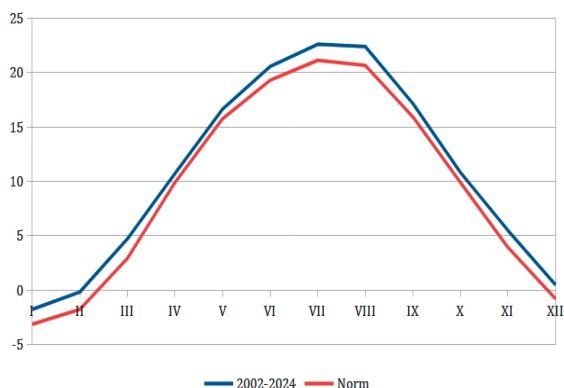


Figure 4. Average annual temperature in the Republic of Moldova (2002–2024) compared with the calculated climatic norm (1950–1985).

The annual distribution of atmospheric precipitation exhibits pronounced non-uniformity. Analysis of monthly precipitation data indicates extended periods of 2–3 months across large areas with little or no rainfall. Additionally, precipitation events are highly localized, occurring over relatively small areas (Alexandrov E. 2019; 2023; 2024).

CONCLUSIONS

Grapevine seeds, taking into account the fact that they contain valuable derivatives of oily substances, so necessary to maintain the activity of the human body, demonstrate that they can serve as raw material for obtaining and using these seed oils.

A greater amount of oil can be extracted from grape seeds extracted from the grapevine before the fermentation process begins than from seeds extracted after the grapevine fermentation process has taken place.

Grapevine seeds of intraspecific genotypes with yellow-green berries contain 13.8–13.7% oil ('Fetescă regală', 'Sauvignon'), while seeds of interspecific genotypes with yellow-green berries contain 13.45–14.46% oil ('Alexandrina', 'Sarmis', etc.).

Grapevine seeds of intraspecific genotypes with blue-violet berries contain 13.5% oil ('Feteasca regală') and 16.6% oil ('Merlot'), while seeds of interspecific genotypes with blue-violet berries contain 11% oil ('Ametist') and 15.35% oil (Sor).

Based on the color of the berries of the studied grapevine genotypes, it was found that grapevine seeds of genotypes with yellowish-green berries contain an average of 14.4% oil, and seeds of grapevine genotypes with blue-violet berries contain an average of 13.54% oil. For the period 2002–2024, an increase in the average temperature of 1.42 °C compared to the normal temperature is observed.

Atmospheric precipitation is manifested by non-uniformity, predominantly local and short-lived.

ACKNOWLEDGEMENTS

The research was carried out under the sub-programme 011102. *Increasing and conserving genetic diversity, agricultural crop breeding in the context of climate change.*

REFERENCES

1. Alexandrov, E. (2023) A way of mitigation and adaptation to climate change. Scientific Papers Series Management, Economic Engineering in Agriculture and Rural Development. Vol. 23(1). p. 39–42.

2. Alexandrov, E. (2019) Organic viticulture. Real opportunities for implementation. Scientific Papers. Series "Management, Economic Engineering in Agriculture and Rural Development", Vol. 19(1). p. 25-30.
3. Alexandrov E. (2024) Climate change a necessity in the process of improvement and cultivation of the grapevine. Romanian Journal of Horticulture. Vol. 5. p. 149-156.
4. Antocea A. (2007) Oenologie. Chimie și analiză senzorială. Craiova. 810 p.
5. Atlas. Schimbările climatice și starea actuală a peisajelor. Chișinău, 2021. 100 p.
6. Codreanu, V., 2006, Anatomia comparată a viței de vie (*Vitis* L.) Chișinău, 251 p
7. Ghid climatic al Republicii Moldova. Date pe termen lung. Chișinău, 2024. 190 pag.
8. Glăman Gh., Dejeu, L., Brîndușe, E., Șerdinescu, A., Ion, M. Et al. (2018) Ampelografia României, vol. IX. Soiuri noi de viță-de-vie și portaltoi creați în România. Editura Ceres, București 2018.
9. Dorobanțu P., Beceanu D. (2007) Studii privind uleiul extras din semințe de struguri, aparținând unor soiuri diferite de *Vitis vinifera*. Lucrări științifice USAMV Iași, Seria Agronomie, vol. 51. p. 30-35.
10. Виноградарство Северного Причерноморья. Арциз, 2009. 232 стр. (Vinogradarstvo Severnogo Prichernomorya.)
11. Панов П., Харчук О. Модернизированный ЯМР-релаксометр для изучения состояния воды в растениях и определения содержания масла в семенах. (Modernizirovanny YAMR-relaxometr dliya izucheniya sostoyaniya vody v rasteniah i opredeleniya soderzhaniya masla v semenah). În: Materialele Conferinței științifice internaționale (Ed. a V-a) "Genetica, fiziologia și ameliorarea plantelor". Chișinău, 23-24.10.2022. p. 144-146.
12. www.meteo.md



Floriculture, Ornamental Plants, Landscape architecture

Studies on the propagation and behavior of some biotypes of *Mirabilis jalapa* L.

**E. Hărșan¹, Hărșan E.², I. Moldovan², L. Draghia^{3*} and
M. Cantor^{1*}**

¹Department of Horticulture and Landscape Design, Faculty of Horticulture and Business in Rural Development, University of Agricultural Sciences and Veterinary Medicine of Cluj-Napoca, Romania

²Horticultural Research Station, University of Agricultural Sciences and Veterinary Medicine Cluj-Napoca, Romania

³Faculty of Horticulture, Iasi University of Life Sciences, Romania

*Corresponding author email: maria.cantor@usamvcluj.ro; lucia.draghia@iuls.ro

ABSTRACT

Nowadays, worldwide, but also in Romania, there is an enormous demand for *Mirabilis jalapa* L., a popular ornamental plant for landscaping design, due to the beauty of its flowers, but also for its use in traditional medicine. This study highlights some contributions regarding generative propagation in *Mirabilis jalapa* L., which is appreciated, especially as a decorative plant, alone or in different combinations, but also for its uses, mainly in medicine. Experience shows that *Mirabilis jalapa* L. can be easily used as a teaching plant in the practical study of plant morphology and physiology, as well as in the demonstration of certain genetic particularities. In this experiment, seeds from 4 biotypes with flowers of different colors (red, pink, white, and yellow) were tested for germination percentage and number of viable plants, under greenhouse conditions. The morphological characteristics of theseeds and the plants obtained from these were also evaluated. The maximum germination percentage was 100% for the red flower variant, followed by the yellow flower variant, with a percentage of 95%. The germination interval is between 6 and 20 days, with a maximum at 15 days after sowing. The most vigorous was the white-flowered variant, which had a maximum height (110.3 cm) and also the largest diameter (60.9 cm). The seeds are relatively large, generally ranging between 6.6 and 8.8 mm in height and 4.0 and 5.7 mm in diameter, respectively.

Keywords: four o'clock flower, annual plant, propagation, morphological characteris

INTRODUCTION

Mirabilis is a genus of the Nyctaginaceae family, mainly native to America, and commonly known as the four o'clock flower. It is a perennial bushy herb that propagates by flowers or leaves. The genus *Mirabilis* comprises approximately 50 species distributed primarily in tropical and temperate regions of the world, and of these, the most cultivated as an annual plant is *M. jalapa* L.

In Romania, when grown outdoors in the field, it behaves like an annual plant due to the climatic conditions during the winter period (Toma and Petra, 2020). However, due to climate change, it could perhaps be cultivated as a perennial plant, both in the coastal area and in the Danube Delta area, where the cold period is shorter, the minimum temperatures are closer to zero degrees, and the ground frost is superficial. *M. jalapa* L. is native to the dry

tropical regions of Belize, El Salvador, Guatemala, Honduras, Mexico Central, Mexico Gulf, Mexico Northeast, Mexico Northwest, Mexico Southeast, Mexico Southwest, Nicaragua, but it has been naturalized in many parts of the world (Asia, Africa, the United States, the Middle East, Europe, as well as in Australia) (<https://powo.science.kew.org/taxon/urn:lsid:ipni.org:names:162591-2>)(Figure 1). It was introduced to Europe in the 16th century, after the Spanish conquest of Mexico, when the seeds of *M. jalapa* reached Spain, then England and then spread throughout the European continent, being cultivated as an ornamental plant (Engels *et al.*, 1974; <https://ro.guide-herb.com/kogda-sejat-mirabilis>). In Romania, *M. jalapa* L. is mainly present in the area of large localities (Alba Iulia, Bucharest, Cluj-Napoca, Craiova, Constanța, Galați, Iași, Pitești, Suceava, Târgu Mureș, Timișoara etc.)(Figure 2).

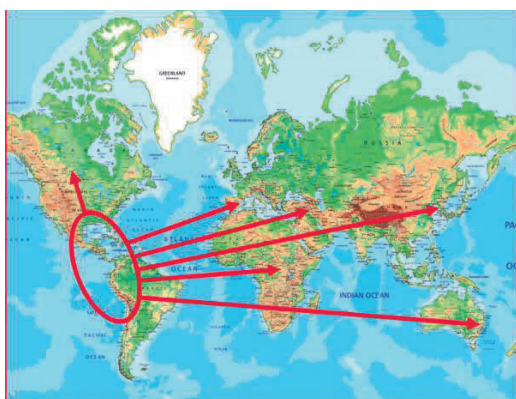


Figure 1. The origin and global spread of *M. jalapa* L.

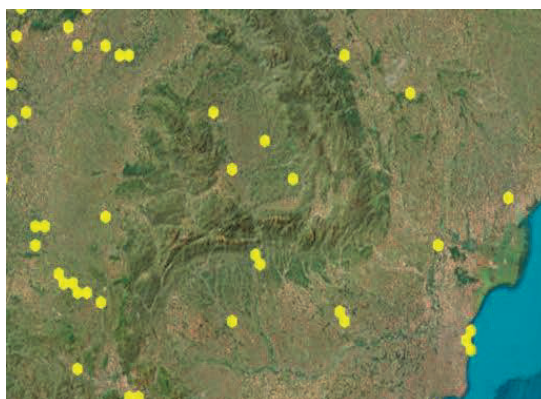


Figure 2. The spread of *M. jalapa* L. in Romania

Source: <https://identify.plantnet.org/ro/the-plant-list/species/Mirabilis%20jalapa%20L./data>

Mirabilis himalaica is indigenous to the Himalayas, being the only species native outside the northern hemisphere, most species of the genus being native to the tropical and subtropical areas of South America (Singh *et al.*, 2012). Some species are ornamental and are cultivated in the world's warmer parts. Several of the American species are widespread.

The flower plant (scientifically known as *Mirabilis jalapa*) is now found in most parts of the world. Its trumpet-shaped blooms often emerge in the late afternoon, around four o'clock, and remain open until the next morning, hence the name "Four O'Clock Flower". The common name of 'four o'clocks' was given to *M. jalapa* because the flowers of this plant do not open until late in the day. In Romanian the common name is 'frumoasa nopții', 'barba împăratului', 'noptițe', and in French 'belle de nuit' (Toma, 2009; Șelaru, 2007).

M. jalapa L. was used for health care and medicinal purposes for several thousand years. The root was used by indigenous peoples for medicinal purposes, as a hallucinogen, and as a purported aphrodisiac, while the flowers produce an edible red dye for coloring food (<https://hort.extension.wisc.edu/articles/four-oclocks-mirabilis-jalapa>).

This plant was widely used as traditional, herbal based natural therapies from ancient times in different countries. It is now a widely available weed cultivated for medicinal and ornamental purposes (Liyaet *al.*, 2021).

Numerous phytochemical products, including alkaloids, brassicasterol, carbohydrates, flavonoids, glycosides, phytosterols (beta-sitosterol and stigmasterol), oleanolic acid, trigonelline, and ursolic acid, have been identified in this plant's constituent parts.

The anti-inflammatory, antimicrobial (antiviral, antibacterial, and antifungal), antioxidant, cytotoxic, antitumor, antinociceptive, analgesic, antihistamine, immunomodulatory, anti-tubercular induced hepatotoxic effect, antiparasitic, dermatological, hypoglycemic, and antihyperlipidemic qualities of this plant have all been investigated. In the process of

creating gold nanoparticles, it is also employed as a reductant and has many other pharmacological effects (Raza *et al.*, 2024; Al-Snafi *et al.*, 2021; Disha *et al.*, 2024; Peiris *et al.*, 2022; Ramesh and Mahalakshmi, 2014; Saha *et al.*, 2020). It has traditionally been used in the treatment of gastrointestinal disorders, muscle pain, abdominal colic, and diarrhea in ancient Mexico, Japan, China, and Brazil. In the aerial parts of the *M. jalapa*, triterpenes and flavonoids are found. Flowers mostly contain anthocyanins and flavonoids. Carbohydrates, resin, and alkaloids are found in roots. Tricosan-12-one, n-hexacosanal, β -sitosterol, and tetracosanoic acid were isolated from the leaves of the *M. jalapa*. Seeds contain β -sitosterol, β -amyrin, and β -sitosterol-D-glucoside. The presence of various bioactive compounds validates the whole plant for different medicinal practitioners (Kaladhar, 2010). It has been extensively used in almost all folk remedies worldwide for treating a variety of conditions. It has also been used as reducing agent for the production of gold nanoparticles (Liya *et al.*, 2021). *Mirabilis* plants contain antimicrobial peptides, with the potential for their use in the preparation of biopesticides (Maulina *et al.*, 2018). Edible crimson dye from flowers; is used as a colorant for cosmetics, cakes, and jellies. Pounded seeds are used in Malaya, China, and Japan for making cosmetic powder. Powdered root, is also used for cosmetic preparations, mixed with rice powder and sandalwood. In China, the flowers are also used for cosmetic purposes (Campbell *et al.*, 2019; <https://www.stuartxchange.com/AlasCuatro>). As an ornamental plant, *M. jalapa* L. has gained significant attention for its remarkable ability to hyper-accumulate heavy metals such as Cd, Sn, Cu, Pb, and Zn (Li *et al.*, 2020a; Li *et al.*, 2020b; Sun *et al.*, 2011; Li *et al.*, 2025). Beyond its capacity for metal uptake, *M. jalapa* demonstrates tolerance and even degradation capabilities for organic pollutants, including petroleum hydrocarbons and nitrobenzene (Zhou *et al.*, 2012). The use of ornamental plants for phytoremediation of polluted soil can not only remove pollutants from the environment, but also green, beautify, and purify the environment (Li *et al.*, 2020; Li and Xu, 2022).

M. jalapa is also known as a model organism in botanical and genetic studies (Van Dijk and Elis, 2022; Zhang *et al.*, 2022).

M. jalapa L. is right at home in a cottage garden, in borders and beds, or can be added to large containers, impressing with the multitude of color combinations it offers. If possible, place them along lighted walkways where the night-blooming flowers and fragrance can be appreciated. It combines nicely with Asiatic lilies or other plants, creating an interesting contrast between large and small flowers (<https://hort.extension.wisc.edu/articles/four-oclocks-mirabilis-jalapa/>).

In Romania, it is not very widespread, but it is cultivated as an ornamental plant. Dwarf varieties are suitable for landscaping plant bands, flower beds, rondos, and borders, while tall varieties can be used for solitary use, in groups next to shrubs and subshrubs, and in mixed borders (Toma and Petra, 2020). It is not resistant to cold, therefore during the cold season it is recommended to grow it under glass (Mc Hoy, 1997). The ease of propagation by generative or vegetative methods, the low requirements for environmental factors, and the resistance to diseases and pests make *M. jalapa* L. a species recommended to be used as a nocturnal alternative in parks, plant bands, curbs, borders, or even on terraces in pots or planters. They emit a sweet-smelling fragrance throughout the night, and then close in the morning.

M. jalapa is easiest to grow from seed. The seeds are large (caper-sized) and can be sown singly in pots or cells, in warmth, in spring. The seedlings are large and easy to look after if protected from frost until planting out in late May in Romania.

Planted in good soil in full sun, they quickly make large, bushy plants, 60-90cm high and wide. The foliage is narrowly heart-shaped, slightly cupped, and rich green, and the flowers appear at the ends of the shoots in small clusters. The colour of the flowers varies from white to yellow, pink, red, and orange, but what makes the plant remarkable is that two blooms on the same stem can be different colours. In fact, many seed strains offer plants

with flowers that are striped with two contrasting colours or solid-coloured flowers of two hues on the same plant (<https://thebikinggardener.com/2014/08/14/something-truly-wonderful-mirabilis/>). An interesting feature of *M. jalapa* is that the same plant can have flowers of different colors, or individual flowers with color combinations in the form of stripes, spots, sections. In addition, the phenomenon of color change as the plant reaches maturity (for example, from yellow to dark pink, or from white to light purple) frequently occurs (Engels *et al.*, 1974; Rozina, 2016).

In recent years, there has been a great deal of interest in propagating *M. jalapa* plants through tissue culture (Gaber, 2015; Rahman *et al.*, 2021).

This study places particular emphasis on the propagation of the *M. jalapa* species, which could be introduced into landscaping and floriculture on a larger scale in our country.

MATERIALS AND METHODS

The experiments were performed at the Horticultural Research Station-University of Agricultural Sciences and Veterinary Medicine Cluj-Napoca and consisted of testing the possibilities of propagating some biotypes of *M. jalapa* through the generative method and its influence on plant growth and development. Fruits of *M. jalapa* are coriaceous obovoid, called achenes, which become wrinkled and dark brown to black at maturity, and tuberous stems are prominent (Argade and Solanki, 2021).

Seed is covered with a thin fruit wall, tight-fitting, spheroid, 4–6 mm (<http://w3.biosci.utexas.edu/prc/K12/pages/Mirabilis>).

The biological material consisted of seeds purchased commercially. The experiment was monofactorial organized with seeds from four biotypes with flowers of different colors (V₁- red flower; V₂- pink flower; V₃- white flower; V₄- yellow flower), arranged in four repetitions, using 40 seeds of each cultivar.

For sowing or planting tubers, commercially purchased substrate mixed with perlite in a ratio of 10:1 was used, and the soil pH was permanently maintained at 6.8. The temperature inside remained around 21-22°C during the day, dropping to 4-5°C at night, and the relative humidity was 55%.

The seeds were sown in plastic pots, in the greenhouse the end of February 2023, and during the summer the fruits were harvested for re-sowing the following year. In the fall, after the plants died, the tuberous roots formed during the almost eight months of vegetation were harvested. The seeds were kept in paper envelopes at room temperature and the tubers in a pot, covered with soil, at positive temperatures and were sprayed with water once a month to avoid dehydration.

Before sowing, 20 seeds were prepared from each basic biotype (red, white, yellow, and pink), to be analyzed. Germination of seeds was determined. Data on different germination parameters were recorded after germination at 6-day intervals until no further germination occurred. Final germination percentage (%), seedling length, was recorded after 20 days of planting. The germinated seeds were counted daily for 20 days. Seedlings were considered to have emerged when cotyledons were visible at the soil surface. Specific observations of four o'clock plant genotypes were made on the obtained plants regarding morphological biometric data that targeted plant height and diameter, number of flowers per plant, and flower diameter.

Statistical analysis

All the experiments were carried out in single row block method with four replications. Experimental data were analyzed using Microsoft Office-Excel and by a statistical package ANOVA, mean values were considered significantly different if the calculated P value was at least 5% ($P < 0.05$) (Ardelean *et al.*, 2002).

RESULTS AND DISUSSIONS

Results of generative propagation

Seed propagation of *M. jalapa* has a greater share in Romania, but has not been studied to date, and propagation by tuber roots is almost unknown and practiced only by amateur gardeners. For the morphological analysis of the seeds obtained, 20 seeds were randomly selected from each experimental variant and measurements were made regarding their diameter and height (Table 1).

Table 1. Morphological characteristics of the seeds of *M. jalapa* L.

No. crt.	Flower color of the variant							
	Red		Pink		White		Yellow	
	H (mm)	D (mm)	H (mm)	D (mm)	H (mm)	D (mm)	H (mm)	D (mm)
1	8.0	4.7	8.1	4.9	7.8	4.9	7.7	5.4
2	7.1	4.4	7.4	4.2	8.2	5.2	7.7	5.7
3	7.0	4.2	7.9	5.1	8.1	4.6	7.8	4.8
4	7.4	4.7	8.0	5.0	7.8	4.5	7.8	5.3
5	7.3	4.7	7.8	4.8	8.4	4.6	7.5	5.3
6	7.5	5.0	7.9	4.6	8.1	5.2	7.8	5.1
7	7.2	5.0	7.6	4.7	7.7	4.5	7.7	5.0
8	7.0	4.7	7.8	4.9	8.4	4.8	7.6	5.2
9	7.9	4.7	7.7	4.8	8.1	4.7	7.9	5.5
10	8.4	5.0	7.7	5.0	7.9	4.7	7.5	4.7
11	7.0	4.8	8.0	4.6	7.9	5.0	7.4	5.5
12	7.3	5.0	7.4	4.8	8.8	5.2	7.5	4.6
13	7.4	5.0	7.8	4.9	7.8	4.9	7.6	4.7
14	6.6	4.5	7.9	5.2	8.2	4.9	7.9	4.9
15	7.4	4.7	7.7	4.6	8.3	4.8	7.9	5.2
16	7.4	4.4	8.1	4.8	7.8	5.0	7.6	4.8
17	7.0	4.5	8.1	4.7	7.7	4.0	7.6	5.0
18	7.1	4.8	8.2	4.7	8.4	4.8	7.7	5.3
19	7.3	4.9	7.9	4.8	8.0	4.7	7.8	4.8
20	7.2	4.2	8.0	4.9	8.2	4.7	7.7	4.8
Total	146.5	93.9	157.0	96.0	161.6	95.7	153.7	101.6
Mean	7.325	4.695	7.85	4.80	8.08	4.785	7.685	5.08

H = seed height; *D* = seed diameter

From the analysis of the data in Table 1, it can be seen that the seed sizes are relatively large, generally ranging between 6.6 and 8.8 mm in height and 4.0 and 5.7 mm in diameter, respectively. From the analysis of the data recorded in the table, it results that the seeds of the red-flowered variety are, on average, the smallest in both assessed criteria (*H*=7.325 mm, *D*=4.695 mm). The seeds of the white-flowered biotype have the highest height (*H*=8.08 mm) and the seeds of the yellow-flowered variety have the highest average diameter (*D*=5.08 mm). The average sizes of the seeds practically correspond to the sizes of the plants of each variety, there being a certain correlation. The smallest, those with red flowers, develop plants with a height of 45-50 cm, while the white ones generate plants with a height of almost 90 cm. On average, the seed height/seed diameter ratio is 1.5975, very close to the golden number, the number of universal harmony, the divine proportion, which is 1.618. For the two characteristics studied, a summary statistic was also made based on the 160 measured values, from which the minimum, maximum, media, and standard deviation values resulted (Table 2).

Table 2. Summary statistics of height and diameter values of *M. jalapa* L. seeds

Statistical data	Height values	Diameter values
Mean	7.735	4.84
Standard Error	0.043686889	0.03382756
Median	7.8	4.8
Mode	7.8	4.7
Standard Deviation	0.390747418	0.302562892
Sample Variance	0.152683544	0.091544304
Kurtosis	0.410407235	0.845523527
Skewness	0.227172356	0.097982993
Range	2.2	1.7
Minimum	6.6	4
Maximum	8.8	5.7
Sum	618.8	387.2
Count	80	80

The seeds, separated by flower color, were kept in water for 24 hours for soaking and rehydration to improve germination, after which they were sown in plastic pots (40 alveoli for each variety in four replications) at the end of February.

Seed germination was observed, and germination (%) of *Myrabilis* varied significantly depending on the biotypes tested.

Seed germination was observed daily, and counting was performed after 6, 10, 15 and were terminated after 20 days of incubation period; final germination percentage was calculated and used for the interpretation of results (Table 3). The highest germination percentage of V1-biotype red flower was observed at 25°C.

Regarding the duration of germination, the determinations made showed that the first seedlings appeared after only 6 days after sowing, in all the varieties studied.

Table 3. The dynamics of *M. jalapa* plants' emergence

Variants (biotypes)	Seeds sown	Plants emergence	Plants emergence after*								Total lost plants no/%
			6days		10days		15days		20days		
			no	%	no	%	no	%	no	%	
V1- red flower	40	40 a	19	47.5	29	72.5	37	92.5	40	100.0 a	-
V2- pink flower	40	31 b	11	27.5	18	45.0	26	65.0	31	77.5b	9/22.5
V3- white flower	40	34 b	15	37.5	24	60.0	28	70.0	34	85.0 b	6/15.0
V4- yellow flower	40	38 a	16	40.0	27	67.5	32	80.0	38	95.0 a	2/5.0
Total	160	143	61	38.1	98	61.2	123	76.9	143	89.4	17/42.5
Mean	40	35.8	15.25	38.1	24.50	61.2	30.75	76.9	35.75	89.4	4.25/10.6

* Values marked by a different letter(s) are significantly different at $p < 0.05$, according to the LSD test

Analyzing Table 3, out of a total of 160 seeds sown in the 4 variants, it can be seen that 143 were viable, at a percentage of 89.4%, while 17 seeds did not germinate and were non-viable, at a percentage of 10.6%. Germination was staggered over a period of 20 days, with sowing taking place at the end of February.

The dynamics of emergence during the observation period showed important differences in terms of the number of emerged plants. After 6 days from the moment of sowing, variant V₁ (plants with red flowers) presents the most emerged plants, on average 19 plants, an aspect that is maintained in the other monitoring days. This is followed by V₄ (with yellow flowers), with the highest number of emerged plants, respectively 16. Variant V₂ (with pink flowers) recorded the lowest values, the number of emerged plants being only 11 plants.

After 20 days from sowing, all 40 seeds with red flowers germinated, while 17 seeds in the other three variants did not germinate, the lowest number of 2 non-viable seeds being in the variant with yellow flowers.

It is worth noting that after 10 days, except for V₂ with red flowers, more than half of the seeds were viable and most of the plants sprouted, most with red flowers (29 plants). The total germination rate peaked on the 15th day, respectively 92.5 % for V₁ (variant with red flower). The research showed that the germination rate of *M. jalapa* seeds decreased significantly with the prolongation of storage time, and the seeds germinated satisfactorily within 9 months. The germination rate peaked on day 6 (Li and Xu, 2022).

Germination (%) of *Mirabilis* varied significantly depending on the tested variants.

The highest germination percentage in the red color biotype was observed at variant V₁.

Analyzing the results obtained as a whole, it is found that after 10 days from sowing, an average germination of 61.2% is ensured, after 15 days of 76.9% and at the end of the period of 89.4%. In the variant with red flowers, all plants germinated, ensuring a percentage of 100%, followed by those with yellow flowers in a percentage of 95%.

With significant differences from these is the variant with pink flowers, in which the germination percentage reached only 77.5% and variant 3 with white flowers, with a percentage of 85%.

At the end of the germination period (after 20 days), not only the total germination was evaluated, but also the percentage of normal seedlings or seedlings with anomalies (deformed roots, cotyledons, or stems), respectively ungerminated seeds (Figure 3).

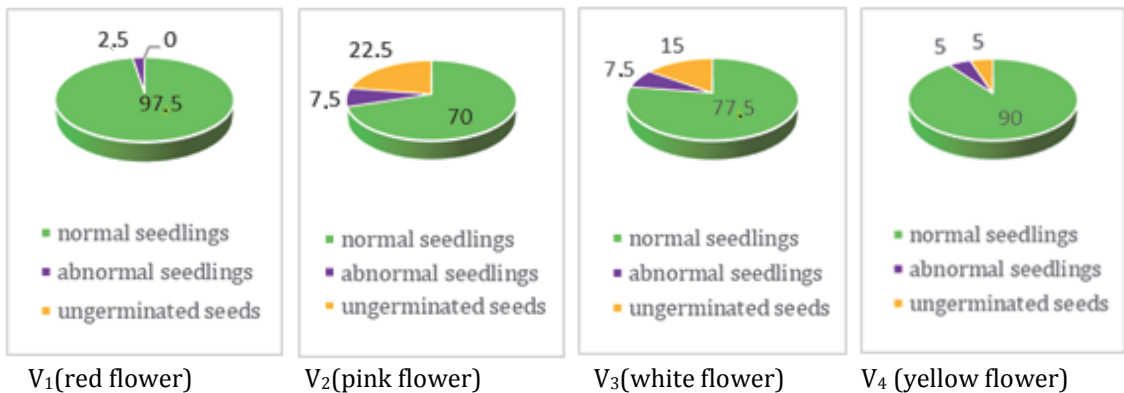


Figure 3. Seeds germination and seedling viability on *M. jalapa* (%)

The results show that out of the total number of germinated seeds, a certain percentage of the seedlings formed showed a series of anomalies that led to poor development and their premature death (Figure 3). These values ranged between 2.5 and 7.5%, respectively 1-3 seedlings from each variant.

The losses recorded after germination were 7.5% (3 seedlings each) for the variants with pink flowers (V₂) and white flowers (V₃), 5% (2 seedlings) for the variant with yellow flowers (V₄), and 2.5% (one seedling) for the variant with red flowers (V₁).

Results on plant growth and development

Observations and determinations were made on the obtained plants regarding their main characteristics: plant height and diameter, number of flowers per plant, flower diameter, and colour of flowers (Table 4; Figures 4 and 5).

Table 4. The main morphological characters of *M. jalapa* plants

Variants (biotypes)	Height/ plant (cm)	Diameter/plant (cm)	No. flower/ plant	Diameter flower (cm)
V1- red flower	55.4c	40.2c	123ab	3.5c
V2- pink flower	70.8b	50.4b	96c	3.5c
V3- white flower	110.3a	60.9a	110b	4.5a
V4- yellow flower	80.0b	60.8a	146a	4.0b
Mean	79.1	53.1	118.8	3.88

* Values marked by a different letter(s) are significantly different at $p < 0.05$, according to the LSD test

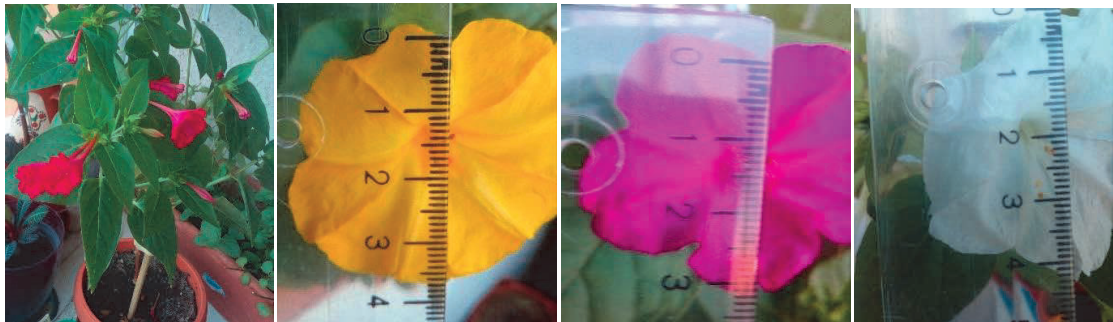


Figure 4. Diameter of flower (cm)

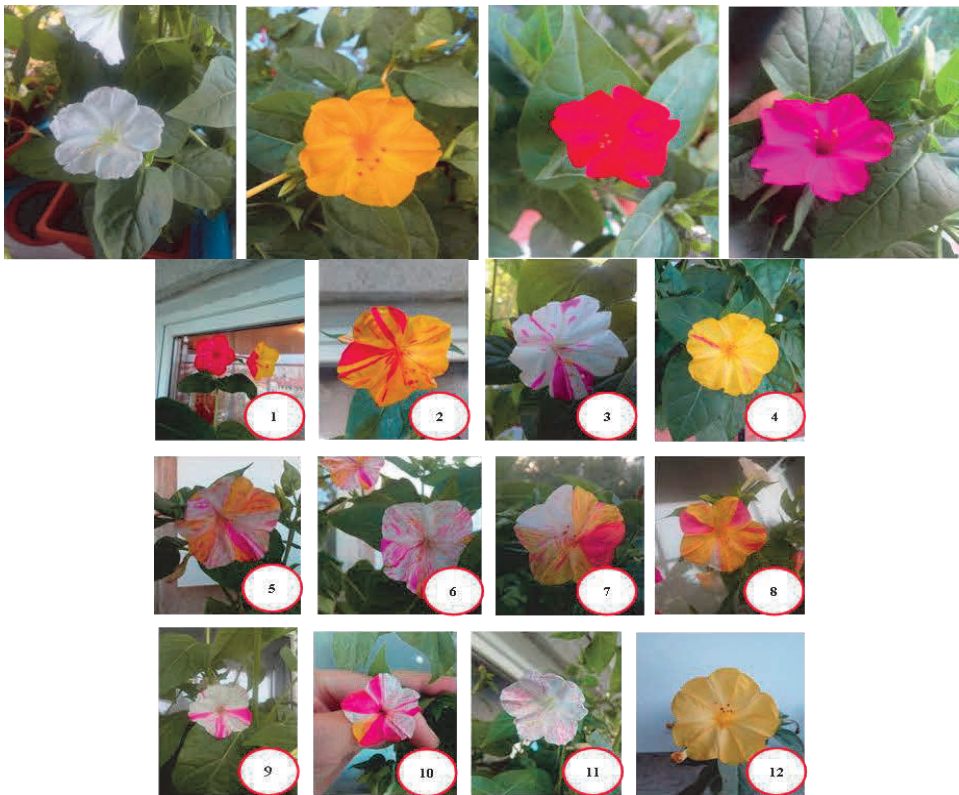


Figure 5. The color diversity of flowers of *M. jalapa* L.

Based on the data presented in Table 4, it was found that *M. jalapa* plants reached a height ranging from 55.4 cm for the red-flowered variant, which had the lowest height, to a maximum of 110.3 cm for the white-flowered variant, which was the most vigorous and also had the largest diameter, 60.9 cm. Regarding plant height, the statistical analysis of the data shows that there were significant differences between variants V_1 and V_3 and nonsignificant

differences between V_2 and V_4 . In the case of plant diameter, there were nonsignificant differences between variants V_3 and V_4 . The number of flowers per plant, during the maximum flowering period (August), had an average of 118.8 flowers. It is worth noting that the yellow-flowered variant had the highest number of flowers per plant (146 flowers), while the pink-flowered variant had only 96 flowers. The flowers at opening had a diameter ranging between 3.5 (red flower) and 4.5 (white flower). Regarding the number of flowers per plant, there are very significant differences between all the analyzed variants, and those regarding flower diameter are not significant between V_1 and V_2 .

The diversity of color combinations obtained is spectacular and amazing, from distinct colors on the same flower to color combinations and designs in the most varied forms (Figure 5).

CONCLUSIONS

This study highlights the potential of seed propagation of *M. jalapa* under greenhouse conditions, in plant pots, and some morphological aspects of the obtained plants. The results obtained highlight a good germination percentage of over 77%, reaching 100% for the red flower biotype, but there may also be small losses due to some anomalies. Monitoring the plants regarding their main characteristics (height, diameter, number of flowers) highlights some properties that justify their use for ornamental purposes.

Grown in pots, it can give terraces or balconies a touch of freshness and originality. Surprising colors and color combinations can be a good reason to cultivate it for those who are passionate about curiosities in the world of flowers.

Given the results of current studies on seed propagation, studies are needed, especially related to vegetative propagation through cuttings or tuber roots, focusing on future climatic conditions in the country.

M. jalapa has a strong environmental adaptability and reproductive capacity and deserves to be promoted and spread throughout the country.

REFERENCES

1. Al-Snafi, A.E., Talab, T.A., Jabbar, W.M. and Alqahtani, A.M. (2021). Chemical constituents and pharmacological activities of *Mirabilis jalapa*-A review. International Journal of Biological and Pharmaceutical Sciences Archive. 01(02): 034–045. <https://doi.org/10.30574/ijbpsa.2021.1.2.0303>.
2. Ardelean, M., Sestras, R. and Cordea, M. (2002). Tehnică experimental horticolă (Experimental horticultural technique). Ed. AcademicPres Cluj-Napoca. (In Romanian).
3. Argade, A.S. and Solanki, H. (2021). A brief review on: *Mirabilis jalapa* plant. International Journal of Pharmaceutical Research and Applications. 6(3): 519-525. Doi: 10.35629/7781-0603519525.
4. Campbell, K. (2019). *Mirabilis jalapa* extract can offer big relief for sensitive skin. ASCP SKIN DEEP:36-37.
5. Disha N.S., AshokKumar B.S., Mamatha H.S. and Khalid M. (2024). Phytochemical composition and neuroprotective potential of *Mirabilis jalapa*: a review. Journal of medical pharmaceutical and allied sciences. 13(6):6855 – 6870.
6. Engels, J.M.M., Kester, V.N.M., Vosselman, W.L., Spitters, C.J.T. and Zeven, A.C. (1975). Investigations of the inheritance of flower variegation in *Mirabilis jalapa* L. Euphytica. 24:1-5.
7. Gaber, M.K. (2015). In Vitro Propagation and Somatic Embryogenesis of Marvel of Peru (*Mirabilis jalapa* L.) from Nodal and Leaf Explants. Alexandria Science Exchange Journal. 36 (4): 314-324.
8. Kaladhar, S.V.G.K.D. and Siva, K.N. (2010). Antimicrobial studies, biochemical and image analysis in *Mirabilis jalapa*. International Journal of Pharmacy & Technology. 2(3):683-693.
9. Li, Q., and Xu, F. (2022). Effects of biological characteristics of *Mirabilis jalapa* L. under different soil substrates, Proc. SPIE vol. 12458, International Conference on Biomedical and Intelligent Systems (IC-BIS 2022), 124580J. <https://doi.org/10.1117/12.2659666>.
10. Li, Q., Wang, H., Wang, Z., Li Y., Ran, J. and Zhang, C. (2020a). Re-investigation of cadmium accumulation in *Mirabilis jalapa* L.: evidences from field and laboratory. Environ Sci Pollut. Res. 27:12065–12079. <https://doi.org/10.1007/s11356-020-07785-6>.

11. Li, X., Zhu, W., Meng, G., Guo, R. and Wang, Y. (2020b). Phytoremediation of Alkaline Soils Co-Contaminated with Cadmium and Tetracycline Antibiotics Using the Ornamental Hyperaccumulators *Mirabilis jalapa* and *Tagetes patula*. *Environ Sci Pollut Res Int* 27(12):14175-14183. doi: 10.1007/s11356-020-07975-2.
12. Li, Y., Liu, J., Feng, X., Xue, Z., Liu, R., Gao, M. and Guo, J. (2025). Reveal Resistance Mechanisms of *Mirabilis jalapa* L. when Exposed to Galaxolide and Polystyrene Microplastics Stress, From Individual, Cellular and Molecular Level. *Plant Physiology and Biochemistry*, Vol. 223:109803, Doi.Org/10.1016/J.Plaphy.2025.109803.
13. Liya, F.I., Yasmin, Mt.F., Chowdhury, N.S., KhasruCharu, T. and Fatema, I.B. (2021). *Mirabilis jalapa*: A review of ethno and pharmacological activities. *Advancement in Medicinal Plant Research*. 9(1): 1-10.
14. Maulina, D., Sumitro, S.B., Amin, M. and Lestari, S.R. (2018). Identification of Peptides Compounds from *Mirabilis jalapa* L. (Caryophyllales: Nyctaginaceae) Potentially as a Biopesticide. The 2017 International Conference on Mathematics, Science, and Education, IOP Conf. Series: Journal of Physics: Conf. Series 1093 (2018) 012009. doi: 10.1088/1742-6596/1093/1/012009.
15. Mc, Hoy P. (1998). *Best Plants for Your Garden*. Thunder Bay Press San Diego SUA. pp.35.
16. Peiris, D.S.H.S., Fernando, D.T.K., Senadeera, S.P.N.N., Chandana, A.K. and Ranaweera, C.B. (2022). *Mirabilis jalapa* Linn.: A Folklore Ayurvedic Medicinal Plant in Sri Lanka. *Asian Plant Research Journal*. 10(2): 21-41.
17. Rahman M.S., Nandi N.C. and Goswami B. (2021). *In vitro* regeneration of *Mirabilis jalapa* L. *Bangladesh J. Sci. Ind. Res.* 56(1):25-28. DOI: <https://doi.org/10.3329/bjsir.v56i1.52692>.
18. Ramesh, N.B. and Mahalakshmi, A.M. (2014). An Ethan pharmacological Review of Four O'Clock Flower Plant (*Mirabilis jalapa* Linn.). *Journal of Biological & Scientific Opinion*. 2(6):344-348. <http://dx.doi.org/10.7897/2321-6328.02679>.
19. Raza, A., Faijan, M., Tahseen Mohd, A. and Patel, D. (2024). A Review of *Mirabilis jalapa*: Traditional Ayurvedic Herbal Remedies. *Int. J.Sci.Res.Sci.&Technol.* 11(6):255-265. Doi.org/10.32628/IJSRST24116168.
20. Rozina, R. (2016). Pharmacological and biological activities of *Mirabilis jalapa* L. *International Journal of Pharmacological Research*. 6(5).Doi:10.7439/ijpr.
21. Saha, S., Deb J. and Deb, N.K. (2020). Review on *Mirabilis jalapa* L., (Nyctaginaceae): A medicinal plant. *International Journal of Herbal Medicine*. 8(2): 14-18.
22. Singh, M., Sanjiv, A., Mittal, K. and Kalia, A.N. (2012). *Mirabilis jalapa*- A Review. *International Journal of Pharmaceutical, Medical and Applied Sciences*. 1(3):22-43. ISSN: 2277-9558.
23. Sun, X., Zhou, Q., Ren, W., Li X., Ren, L. (2011). Spatial and Temporal Distribution of Acetochlor in Sediments and Riparian Soils of the Songhua River Basin in Northeastern China. *J. Environ. Sci. (China)*. 23(10):1684-90. doi: 10.1016/s1001-0742(10)60595-5.
24. Şelaru, E. (2007). *Cultura florilor de grădină* (Garden flower culture). Ed. Ceres Bucharest. pp. 572-573. (In Romanian).
25. Toma, F. (2009). *Floricultură şi artă florală. Specii utilizate pentru decorul parcurilor şi grădinilor* (Floriculture and floral art. Species used for the decoration of parks and gardens). Ed. INVEL-Multimedia Bucharest. Vol. 4: 70-71. (In Romanian).
26. Toma, F. and Petra, S. (2020). *Floricultură şi compoziţii florale* (Floriculture and floral arrangements). Ed. Total Publishing Bucharest. pp 206-207. (In Romanian).
27. van Dijk P.J. and Ellis T.H.N. (2022). Mendel's reaction to Darwin's provision of hypothesis of pangenesis and the experiment that could not wait. *Heredity*. 129:12-16. <https://doi.org/10.1038/s41437-022-00546-w>.
28. Zhang, H., Zhao, X., Zhao, F., Han, J. and Sun, K. (2022). Mendel's controlled pollination experiments in *Mirabilis jalapa* confirmed his discovery of the gamete theory of inheritance in *Pisum*. *Heredity*. 159:19. <https://doi.org/10.1186/s41065-022-00232-1>.
29. Zhou, J.Y., Zhou, S.W., Zeng, S.Y., Zhou, J.Y., Jiang, M.J. and He, Y. (2012). Hypoglycemic and Hypolipidemic effects of ethanolic extract of *Mirabilis jalapa* L. Root on normal and diabetic mice. *Evid Based Complement Alternat Med*. 1-10.
30. <https://powo.science.kew.org/taxon/urn:lsid:ipni.org:names:162591-2>
31. <https://ro.guide-herb.com/kogda-sejat-mirabilis>
32. <https://identify.plantnet.org/ro/the-plant-list/species/Mirabilis%20jalapa%20L./data>
33. <https://www.stuartxchange.com/AlasCuatro>
34. <https://hort.extension.wisc.edu/articles/four-oclocks-mirabilis-jalapa>
35. <https://thebikinggardener.com/2014/08/14/something-truly-wonderful-mirabilis/>
35. <http://w3.biosci.utexas.edu/prc/K12/pages/Mirabilis>

Reducing of water consumption of seashore paspalum using a hydrogel composite

M. A. E. Elashwah^{1*} and W. F. Abobatta²

¹Horticulture Research Institute Agricultural Research Center
Ornamental Plants and Landscape Gardening Research Department Giza, Egypt

²Horticulture Research Institute, Agricultural Research Center, Giza,
Citrus Research Department, Egypt

*Corresponding author email: elashwah50505050@gmail.com

ABSTRACT

In order to lower the water intake of *Paspalum vaginatum* Swartz while preserving its growth and quality, a trial was carried out at the Nursery of the Horticulture Research Institute in Giza, Egypt, throughout the 2023 and 2024 seasons. The main factors were first, irrigation periods (every 1, 3, 5, and 7 days); second, soil mixing with hydrogel (0, 30, and 50 g/m²), and third, the interaction factors between them. Results indicated that long irrigation intervals led to a significant decrease in turf color quality, turf length, fresh and dry weight per area, and fresh and dry root weight. It also decreased cover density, chlorophyll content, and percentages of nitrogen, phosphorus, and potassium. It significantly increased the proline content of the turf. The addition of hydrogel significantly improved vegetative and root characteristics, turf quality and increased cover density, besides plant nitrogen, phosphorus, and potassium content. The interaction between irrigation periods and hydrogel applications leads to a significant increase in the quality of the grass and its vegetative and root specifications, in addition to the nutritional elements. Proline levels in the grass were also reduced when hydrogel was at a rate of 30 or 50 g/m². The best results were achieved with 50 g/m², eliminating most of the negative effects on the grass when irrigated every 3 or 5 days. Generally, to reduce irrigation water consumption, stimulate coastal paspalum growth, and increase its cover density and greenness, it is recommended to mix the soil with 50 g/m² before planting.

Keywords: Growth, hydrogel, lawns, irrigation periods, *Paspalum vaginatum*.

INTRODUCTION

The use of non-potable water sources for turf grass has increased as a result of freshwater scarcity and conservation efforts, necessitating management and innovative strategies to minimize freshwater use and optimize its benefits. Furthermore, it is critical to maximize landscape irrigation, particularly in regions with limited water supplies (Fadil, *et al.*, 2025). The agricultural sector uses 70% of the world's freshwater, but water availability for farming is going down due to climate change and population growth. To improve water use and meet agricultural needs, water management strategies and precision agriculture technologies are necessary (FAO Statistics, 2020).

Smart irrigation systems and precision agriculture help farmers use resources efficiently and lessen water stress on crops. These systems take into account soil moisture, weather, and plant health to manage when, where, and how much water is delivered. Traditional irrigation methods can lead to over- or under-watering, which can cause nutrient loss, waste

water, and reduce both yield and quality (Bwambale *et al.*, 2021). The *Paspalum turfgrass* is useful for a variety of purposes. important functional roles, as well as providing beauty. Turfs are essential to lowering the soil's susceptibility to wind and water erosion, in addition to managing air pollution, dust, and sun glare. Outdoor activities include football and hockey. Turf is frequently used in golf and lawn tennis. The advertisement sodding is the method used to manufacture products around the world, stolonizing, plugging, and sprigging and/or rhizomes throughout the spring and summer (Nooh and El-Naggar, 2021). Paspalum, specifically *Paspalum vaginatum*, is a type of grass in the Poaceae family, previously called Graminae. It is part of warm-season grasses and is commonly known as Bahia grass. Paspalum includes various species such as dallis grasses, and these grasses thrive in tropical and subtropical regions. Paspalum can reproduce through rhizomes and has leaf blades that are 10 to 19 cm long and 3 to 8 mm wide, often with a blue-green color (El-Naggar, *et al.*, 2024). Hydrogel is a type of polymer that can help reduce the need for regular watering in agriculture, particularly in areas with limited water according to Al-Jabari, *et al.*, (2019). This soil treatment improves the physical, chemical, and biological qualities of the soil, leading to better plant growth (Abobatta, 2018). Hydrogel application boosted plant survival duration because it created sufficient soil moisture. As a result, using it in the soil, especially in drought-stricken, dry, and semi-arid regions, is beneficial for water conservation in the rhizosphere Ayangbenro and Babalola, 2020). Gel-forming composites help retain water, improve water movement, and support soil structure in agriculture and landscaping. They enhance the ability of soils to hold chemicals that protect plants and resist erosion (Novoskoltseva, *et al.*, 2021).

MATERIALS AND METHODS

To achieve the objective of this research, field experiments were conducted during the 2023 and 2024 consecutive seasons at the nursery of the Ornamental Plants and Landscape Research Department at the Horticulture Research Institute in Giza. During both seasons, 36 plots (1.0 x 1.0 m each) were established to study the effect of a hydrogel compound in soil on the response of paspalum (*Paspalum vaginatum* Swartz) to different irrigation intervals. The combined effects of hydrogel concentrations (Super-AB-A-200), respectively Factor A with three graduations: a1- zero (control), a2- 30 g/m², and a3- 50 g/m²)-and irrigation intervals, respectively Factor B with graduations: b1- 1 daily irrigation; b2- irrigation applied every 3 days, b3- irrigation applied every 5, and b4- irrigation applied every 7 days on *Paspalum growth* were investigated with three replicates per treatment. Hydrogel specification in (Table 1).

Table 1. The characteristics of Super-AB-A-200 polymer

Characteristics	Super-AB-A-200 polymer
Shape	granular
Density	1.4–1.5 (gr cm ⁻³)
Size of particles	50–150 (µm)
Maximum stability in soil	7 (year)
Practical capacity of water uptake	220 (g g ⁻¹)

The experimental field was prepared on March 1 by digging the soil (25 cm depth) and applying a sand layer (10 cm). Hydrogels (0, 30, and 50 g/m²) were incorporated into an additional 10 cm sand layer, followed by a 5 cm sand cover. Seashore paspalum sods (1 × 1 m) were planted and covered with a thin (1 cm) sand layer. Irrigation was applied at 10 L/m² daily for two weeks, then at 10 L/m² at intervals of 1, 3, 5, and 7 days thereafter. Fertilization was performed twice (three weeks after planting and two months later) using

ammonium nitrate (125 g, 33.5% N), calcium superphosphate (66 g, 15% P₂O₅), and potassium sulfate (22 g, 39.84% K). Grass response was evaluated based on four cutting cycles (May 1, June 1, July 1, and August 1). The recorded parameters included plant height, fresh and dry biomass (shoot and root) per m², and turf density (%), expressed as tillers/area × 100. Turf quality was visually assessed according to RHS standards (0–9 scale), with ≥ 6 considered acceptable. Total chlorophyll content was measured after the final cut (Wettstein, 1957), proline accumulation was analyzed following Bates et al. (1973), and N, P, K contents were determined according to Page *et al.* (1982). All of the statistical analyses were performed based on a completely randomized experimental design (CRD), and data processing was performed by two-way analysis of variance (two-way ANOVA), in accordance with the experimental structure that included two factors. Duncan's multiple range tests were used to evaluate the means of all therapies at a 5% probability level.

RESULTS AND DISCUSSIONS

The results showed that as irrigation water deficit increased, grass quality declined below 6 degrees (Table 2). Grass color and coverage density improved at 50 g/m² of hydrogel. The best grass color and density were noted with daily irrigation at this amount. Turf irrigated every seven days with 50 g/m² hydrogel saw a 37% increase in density in the first season and about 42% in the second season. Hydrogel application also raised turf height, which decreased with longer dry periods, reaching its peak with 50 g/m² across different irrigation intervals.

Table 2. Effect of Hydrogel, irrigation periods and their interaction on turf color, covering density and plant height on *Paspalum vaginatum*

Hydrogel (g) (A)	Irrigation periods (days) (B)									
	b1-1	b2-3	b3-5	b-7	Mean (A)	b1-1	b2-3	b3-5	b-7	Mean (A)
Turf colors (in 9° scale)										
Season 2023						Season 2024				
a1-0.0	7.13e	6.80g	5.80i	5.53j	6.32c	7.47c	7.10d	5.87f	5.5g	6.48c
a2-30	7.57c	7.20e	6.10h	6.00h	6.72b	8.00b	7.37c	6.33r	6.30r	7.00b
a3-50	8.00a	7.77b	7.33d	7.00f	7.53a	8.20a	8.00b	7.47c	7.17d	7.71a
Mean (B)	7.57a	7.26b	6.61c	6.18d		7.89a	7.49b	6.56c	6.32d	
Covering density (%)										
Season 2023						Season 2024				
a1-0.0	94.00a	83.77b	70.47d	59.33e	76.89c	93.97a	82.77b	68.43bc	55.17f	75.08c
a2-30	95.01a	92.17a	79.43bc	75.27cd	85.48b	92.10a	90.73a	75.53cd	70.27de	82.16b
a3-50	98.03a	95.47a	94.57a	81.33bc	92.35a	96.83a	94.67a	94.17a	78.23bc	90.98a
Mean (B)	95.70a	90.47b	91.49c	72.00d		94.30a	89.39b	79.38	67.89d	
Plant height (cm)										
Season 2023						Season 2024				
a1-0.0	20.37f	18.53g	12.63j	10.16k	15.42c	22.27e	20.37g	14.43j	12.35k	17.35c
a2-30	23.40c	22.47d	20.33f	15.57i	20.44b	24.63c	23.90d	22.03ef	17.13i	21.93b
a3-50	25.67a	24.27b	20.83e	17.58hh	22.09a	26.10a	25.13b	21.90f	18.93h	23.02a
Mean (B)	23.14a	21.76b	17.93c	14.43d		24.33a	23.13b	19.46c	16.14d	

The 0.05 probability level was used in Duncan's Multiple Range Test (Duncan, 1955) to indicate that means assigned the same letter are not significantly different.

In the study, it was found that the amount of water used for irrigation affected the fresh and dry weight of grass over two years (Table 3). The best results came from applying 50 g/m² of hydrogel, with fresh weights reaching 1319. 18 g/m² in the first year and 1395. 63 g/m² in the second year. These weights were significantly higher than those from irrigation only once every week. Similarly, dry weight was also much greater with daily irrigation at 197. 90 g/m² compared to 111. 63 g/m² for weekly irrigation. The harmful effects of drought include all aspects of plant growth, and this is agreed upon by Shahba *et al.*, (2014), who found that the quality of three seashore paspalum cultivars degraded linearly when less irrigation water was used, the visual quality of the turf declined, but the leaf proline level increased noticeably. According to the findings, hydrogel enhances the qualities of sandy soil for plant growth by absorbing and holding water in the soil for a longer period of time, which lowers the need for irrigation.

Table 3. Effect of Hydrogel, irrigation periods and their interaction on herb fresh and dry Weights on *Paspalum vaginatum*

Hydrogel (g) (A)	Irrigation periods (days) (B)									
	b1-1	b2-3	b3-5	b-7	Mean (A)	b1-1	b2-3	b3-5	b-7	Mean (A)
Herb fresh weight (g/m ²)										
Season 2023						Season 2024				
a1-0.0	1265.00c	1020.33e	790.00g	605.33h	920.17d	1337.23d	1123.60g	888.33i	550.00k	974.79c
a2-30	1306.67b	1298.33bc	1026.97e	773.83g	1101.45b	1405.00b	1370.43c	1169.23f	851.83j	1199.13b
a3-50	1385.87a	1298.53bc	1138.27d	862.53f	1171.30a	1444.67a	1440.00a	1258.33e	983.10h	1281.523a
Mean (B)	1319.18a	1205.73b	985.08c	747.23c		1395.63a	1311.34b	1105.30c	794.98d	
Herb dry weight (g/m ²)										
	Season 2023					Season 2024				
a1-0.0	177.10d	142.68f	106.65i	78.63j	126.26c	186.90d	157.23f	123.97h	71.37i	134.87c
a2-30	196.00c	194.75c	158.15e	119.17h	167.02b	196.70c	191.86cd	163.69e	119.26h	167.88b
a3-50	220.60a	206.23b	181.19d	137.10g	186.28a	221.47a	201.60b	192.97cd	137.63g	188.42a
Mean (B)	197.90a	181.22b	148.66c	111.63d		201.69a	183.56b	160.21c	109.42d	

The 0.05 probability level was used in Duncan's Multiple Range Test (Duncan, 1955) to indicate that means assigned the same letter are not significantly different.

Results in hand indicated that reducing irrigation intervals negatively affects the shoot and root systems of *Paspalum vaginatum*. The fresh and dry weight of the paspalum grass declined, the plant height decreased, and the grass color quality dropped below a level of six when it was subjected to prolonged drought. These results agree with previous studies by Jespersen *et al.*, (2019), who reported that the biomass of shoot and root systems of *Paspalum vaginatum* declined with increasing drought severity, the phosphorus level in the aerial mass rose, and the nitrogen content in plants remained unchanged.

The results obtained showed that the fresh weight of the roots increased with increasing irrigation regime and hydrogel concentrations (Table 4). On average of factor B (hydrogel combination) was registered. This result is similar to that when hydrogels were combined with sandy soil or used alone, the water content of the subsurface layer increased. Additionally, compared to the control group, hydrogel-treated sand markedly boosted the above- and below-ground biomass of *Agrostis stolonifera*. Compared to the control group, the 0.4% hydrogel modification to the sand boosted the water usage efficiency of the grass by a factor of eight. In hydrogel-amended soil, the hydrogel increased the effectiveness of

water use by encouraging the development of a thick root system and root aggregation, which improved root contact with moisture (Agaba, *et al.*, 2011).

Table 4. Effect of hydrogel, irrigation periods and their interaction on roots fresh and dry weights on *Paspalum vaginatum*

Hydrogel (g) (A)	Irrigation periods (days) (B)									
	b1-1	b2-3	b3-5	b-7	Mean (A)	b1-1	b2-3	b3-5	b-7	Mean (A)
Roots fresh weight (g/m²)										
Season 2023					Season 2024					
a1-0.0	265.23e	251.83f	222.70g	204.63h	236.10c	274.73f	260.33g	230.33h	213.67i	244.77c
a2-30	316.16b	291.43d	288.10d	252.10f	286.95b	323.33b	299.33d	294.33d	261.00g	294.50b
a3-50	348.67a	317.33b	300.33c	271.33e	309.43a	355.33a	323.33b	312.33c	283.33e	318.58c
Mean (B)	310.02a	286.86b	270.38c	242.69d		317.80a	294.33b	279.00c	252.67d	
Roots dry weight (g/m²)										
Season 2023					Season 2024					
a1-0.0	45.01e	37.79g	28.74h	24.57i	34.03c	48.70f	41.05h	33.16i	27.79j	37.57c
a2-30	56.86c	46.17e	40.83f	27.79h	42.91b	58.61c	52.04e	46.11g	33.45i	47.55b
a3-50	76.52a	63.55b	51.09d	40.73f	57.97a	80.76a	67.07b	55.10d	47.94f	62.72a
Mean (B)	59.47a	49.16b	40.22c	31.03d		62.69a	53.39b	44.79c	36.39d	

The 0.05 probability level was used in Duncan's Multiple Range Test (Duncan, 1955) to indicate that means assigned the same letter are not significantly different.

The chlorophyll content of grass can be considered the primary determinant of grass color quality. Mixing the soil with hydrogel increased the chlorophyll content of the grass, which decreased due to the reduction in water amount. In addition, the herb's proline content (Table 5). As the water supply to the grass decreased for one, two, three, and five days at a time, proline accumulation gradually increased, reaching its highest content in the leaves when watered every 7 days. The water stored in the hydrogel beads, on the other hand, decreased the proline concentration of the grass. The findings revealed overall, longer irrigation gaps reduce grass height, resilience, visual quality, uniformity, root length, and weight while increasing proline levels (Darini, *et al.*, 2015).

Regarding the interaction between irrigation intervals and hydrogel, leaf proline content decreased in all interaction treatments. Grasses irrigated every 7 days decreased their proline content from 1111.67 and 1205.67 in both seasons to 443.33 and 455.00 $\mu\text{g}\cdot\text{g}^{-1}$ fresh wt. in both seasons, respectively, after the addition of 50 g hydrogel/m². This was consistent across all interaction treatments. The water-holding capacity of sandy soil was enhanced by adding hydrogel. When the hydrogel dose was increased, the water-holding capacity and plant retention of available water rose gradually, while the drainage capacity fell when compared to the control treatments. The hydrogel additive slowed the rate at which the soil dried out, restricting the amount of water that plants could access. When drought occurred, the hydrogel treatment had a beneficial impact on plant development, increasing the length of time that plants survived by 66% (Baran, *et al.*, 2015). Under water-scarce conditions, hydrogel can reduce irrigation frequency by half, saving approximately 50% of water, and preventing excessive nutrient leaching, thus enhancing growth (Tan, *et al.*, 2021).

Table 5. Effect of hydrogel, irrigation periods and their interaction on total chlorophyll content (a + b) and proline content on *Paspalum vaginatum*

Hydrogel (g) (A)	Irrigation periods (days) (B)									
	b1-1	b2-3	b3-5	b-7	Mean (A)	b1-1	b2-3	b3-5	b-7	Mean (A)
Total chlorophyll content (a + b) (mg/100 g as fresh weight)										
Season 2023						Season 2024				
a1-0.0	2.96e	2.74h	2.32c	2.21k	2.56c	3.04d	2.83e	2.45g	2.31h	2.66c
a2-30	3.24c	3.10d	2.90f	2.55i	2.95b	3.28b	3.19c	3.10cd	2.65f	3.07b
a3-50	3.38a	3.31b	3.12d	2.81g	3.16a	3.40a	3.33ab	3.15c	2.91e	3.20a
Mean (B)	3.19a	3.05b	2.78c	2.52d		3.24a	3.12b	2.90c	2.63d	
Proline content (µg·g⁻¹ fresh wt)										
Season 2023						Season 2024				
a1-0.0	327.00f	434.33d	823.00b	1111.67a	674.08a	316.33i	407.33e	918.00b	1205.67a	711.83a
a2-30	332.67f	356.67e	438.33d	653.33c	445.25b	353.33g	373.33f	411.33e	681.33c	454.83b
a3-50	330.00f	323.33f	331.33f	443.33d	357.00c	352.00g	331.33h	333.67h	455.00d	368.00c
Mean (B)	329.89d	271.44c	531.00b	737.11a		340.56d	370.67c	554.33b	780.67a	

The 0.05 probability level was used in Duncan's Multiple Range Test (Duncan, 1955) to indicate that means assigned the same letter are not significantly different.

It is known that drought negatively affects the plant's absorption of nutrients, while hydrogel retains water and nutrients. This was evident with increasing hydrogel concentration, as nitrogen, phosphorus, and potassium absorption rates progressively rose, with considerable variance. In contrast, the N, P, and K uptake rates dropped with longer irrigation intervals. The addition of hydrogel at 30 or 50 g/m² was effective in raising the N, P, and K absorption rate in the face of limited irrigation water, as the N concentration in both seasons reached 2.45 and 2.36, respectively, compared to 2.07 and 2.00 for grass that was irrigated every seven days (Table 6).

Table 6. Effect of hydrogel, irrigation periods and their interaction on nitrogen, phosphorus, and potassium on *Paspalum vaginatum*

Hydrogel (g) (A)	Irrigation periods (days) (B)									
	b1-1	b2-3	b3-5	b-7	Mean (A)	b1-1	b2-3	b3-5	b-7	Mean (A)
Nitrogen (%)										
Season 2023						Season 2024				
a1-0.0	2.56f	2.49g	2.34i	2.07k	2.36c	2.43g	2.37h	2.24j	2.00k	2.26c
a2-30	2.89c	2.64e	2.55f	2.25f	2.58b	2.76c	2.53e	2.49f	2.31i	2.52b
a3-50	3.04a	2.99b	2.75d	2.45h	2.80a	3.11a	2.90b	2.68d	2.36h	2.76a
Mean (B)	2.83a	2.71b	2.54c	2.26d		2.77a	2.60b	2.47c	2.22d	
Phosphorus (%)										
Season 2023						Season 2024				
a1-0.0	0.225h	0.215j	0.201k	0.182l	0.206c	0.215i	0.211j	0.210j	0.168k	0.201c
a2-30	0.268d	0.256f	0.236g	0.222i	0.246b	0.271d	0.262f	0.243g	0.231h	0.252b
a3-50	0.301a	0.298b	0.288c	0.259e	0.287a	0.322a	0.307b	0.300c	0.266e	0.299a
Mean (B)	0.265a	0.256b	0.242c	0.221d		0.270a	0.260b	0.251c	0.222d	
Potassium (%)										

	Irrigation periods (days) (B)									
	Season 2023					Season 2024				
a1-0.0	2.58e	2.36g	1.91i	1.50j	2.09c	2.78e	2.55g	2.06i	1.65j	2.26c
a2-30	3.07c	3.00c	2.37g	2.15h	2.65b	3.36c	3.28c	2.59g	2.37h	2.90b
a3-50	3.44a	3.24b	2.82d	2.49f	3.00a	3.72a	3.50b	3.05d	2.69f	3.24a
Mean (B)	3.03a	2.87b	2.37c	2.05d		3.29a	3.11b	2.57c	2.23d	

The 0.05 probability level was used in Duncan's Multiple Range Test (Duncan, 1955) to indicate that means assigned the same letter are not significantly different.

Hydrogel not only holds water but also the fertilizers applied to the soil, releasing them later. The turf to which hydrogel was applied had greater amounts of N, P, and K than the soil to which hydrogel was not added, according to the findings of this study. In agreement with these results. Also, Martin del Campo, *et al.*, (2021) on *Agrostis stolonifera* L-93 turf, used in golf courses, found that adding hydrogel to the soil succeeded in retaining water for a long period and significantly reducing nitrogen fertilizer leaching.

The impact of hydrogel on the most prevalent grass species (*Festuca rubra*, *Festuca ovina*, and *Lolium pyrene*) cultivated under regulated drought conditions was examined in a study by (Hadam, 2010), who found that this water-absorbing substance enhanced the tested species' germination, growth, and dry matter content. Also data in (Table 6) showed that the percentage of phosphorus rose to 0.259 and 0.266 with the addition of 50 g/m² hydrogel, compared to 0.182 and 0.168 for the same treatment, and the concentration of K reached 2.49 and 2.69%, respectively, compared to 1.50 and 1.65 in the first and second seasons (Table 6).

All other findings point to a notable rise in the absorption rates of nitrogen, phosphorus, and potassium across all hydrogel treatments. Del Campo, *et al.*, (2019) also found that adding hydrogel to soil for grass on golf courses reduced nitrogen and phosphorus leaching. It also increased these nutrients in the grass compared to soil without hydrogel or with only peat.

CONCLUSIONS

Longer irrigation intervals negatively affected turf quality, length, and weight, reduced cover density and nutrient content, while increased proline levels. Applying hydrogel at 50 g/m² mitigated these adverse effects, retained water, and promoted turf growth and quality by increasing plant development, coverage density, color quality, total chlorophyll content, and N, P, and K levels. Therefore, it is recommended to add hydrogel at a rate of 50 g/m² to reduce irrigation water consumption while maintaining grass quality.

REFERENCES

1. Abobatta, W. F. (2018). Impact of hydrogel polymer in agricultural sector. *Adv. Agric. Environ. Sci.* 1: 59–64. DOI: 10.30881/aaeoa.00011
2. Agaba, H., Oriquiriza, L. J., Obua, J., Kabasa, J. D., Worbes, M. and Hüttermann, A. (2011). Hydrogel amendment to sandy soil reduces irrigation frequency and improves the biomass of *Agrostis stolonifera*. *Agricultural Sciences*, 2(04), 544-550. DOI: 10.4236/as.2011.24071
3. Al-Jabari, M., Ghyadah, R.A. and Alokely R. (2019). Recovery of hydrogel from baby diaper wastes and its application for enhancing soil irrigation management. *J. Environ. Manag.* 239: 255–261. doi.org/10.1016/j.jenvman.2019.03.087
4. Ayangbenro, A.S. and Babalola, O.O. (2020). Reclamation of arid and semi-arid soils: The role of plant growth-promoting archaea and bacteria. *Curr. Plant Biol.* (25): 00173.
5. Baran, A., Zaleski, T., Kulikowski, E. and Wiczeorek, J. (2015). Hydrophysical and biological properties of sandy substrata enriched with hydrogel. *Polish Journal of Environmental Studies*, 24(6): 2355-2362. DOI: 10.15244/pjoes/59258
6. Bates, L.S., Walden, R.P. and Teare, T.D. (1973): Rapid determination of free proline for water stress studies. *Plant and Soil*, 39: 305-307. doi.org/10.1007/BF00018060

7. Bwambale, E., Abagale, F.K. and Anornu, G.K. (2021). Smart irrigation monitoring and control strategies for improving water use efficiency in precision agriculture: A review. *Agric. Water Manag.* 260: 107324. doi.org/10.1016/j.agwat.2021.107324
9. Darini, A. K., Naderi, R., Khalighi, A. and Taheri, M. (2015). Effect of superabsorbent polymer on lawn under drought stress condition. *Agric. Sci. Dev.* 4, 22-26.
10. Del Campo, M. M., Esteller, M. V., Morell, I., Expósito, J. L., Bandenay, G. L. and Díaz-Delgado, C. (2019). A lysimeter study under field conditions of nitrogen and phosphorus leaching in a turf grass crop amended with peat and hydrogel. *Science of the total environment*, 648: 530-541.
11. Domański, P. (1992). System badań i oceny traw gazonowych w Polsce [System of research and assessment of lawn grasses in Poland]. *Biul. IHAR* 183: 251-263.
12. Duncan, D.B. (1955). Multiple range and multiple F test. *Journal of Biometrics*, 11:1-42.
13. El-Basha, S. Y., Abdel-Aziz, A. A., Bedair, O. M. and Akl, M. M. (2019). Mathematical model to predict the distribution of soil moisture in the root zone of turf landscape. *Arab Universities Journal of Agricultural Sciences*, 27(1): 105-114. Doi: 10.21608/ajs.2019.43071
14. El-Naggar, A., Imhmd, G. and Hamid, E. (2024). Effects of some natural soil amendments and water requirements on the growth and chemical constituents of some lawns (*paspalum vaginatum*, L.). *Scientific Journal of Flowers and Ornamental Plants*, 11(2): 43-54. doi: 10.21608/sjfop.2024.376813
15. Fadil, S. Hassan F., Fetouh M., El-Serafy, R. and Moussa, M. (2025). Rhizobacteria inoculation enhanced the growth performance of Paspalum turfgrass under saline onditions. *Journal of Sustainable Agricultural and Environmental Sciences*, 4(2): 8-16. DOI: 10.21608/jsaes.2025.386499.1138
16. Food and Agriculture Organization (FAO)(2021). The State of Food and Agriculture. Overcoming water challenges in agriculture; FAO: Rome, Italy, 2020.
17. Hadam, A. (2010) Effect of hydrogel on the turf grass species growing under drought stress. *Challenges of modern technology*, 1(1): 3-6.
18. Jankowski, K., Jankowska, J., Ciepiela, G. A., Wisniewska-Kadzajan B., Kolczarek R. and Sosnowski J. (2014). Effect of hydro gel and soil cover on root mass produced by the lawn mixtures in the years 2007–2009. *Bulgarian Journal of Agricultural Science*, 20(6): 1295-1301.
19. Jankowski, K., Jankowska, J. and Sosnowski, J. (2011). Coloring of lawns established on the basis of red fescue, depending on application of superabsorbent and various fertilizers. *Acta Scientiarum Polonorum. Agricultura*: 10(3), 67-75.
20. Jespersen, D., Leclerc, M., Zhang, G. and Raymer, P. (2019). Drought performance and physiological responses of bermudagrass and seashore paspalum. *Crop Science*, 59(2): 778-786.
21. Khodadadi Dehkordi, D. (2018). Effect of super absorbent polymer on soil and plants on steep surfaces. *Water and Environment Journal*, 32(2): 158-163. doi.org/10.1111/wej.12309
22. Little, I.M. and Hills, F.J. (1978). *Agricultural experimentation, design and Analysis*. John Willey & Sons Inc., New York., USA., 368
23. Mahdi M.Z. (1953). The influence of management on botanical composition and quality of turf. Ph.D. Thesis, Fac. Agric., California Univ., Los Angeles, USA.
24. Martin del Campo, M. A., Esteller, M. V., Morell, I., Expósito, J. L., Bandenay, G. L. and Morales-Casique, E. (2021). Effect of organic matter and hydrogel application on nitrate leaching in a turfgrass crop: a simulation study using HYDRUS. *Journal of Soils and Sediments*, 21(2): 1190-1205. DOI:10.1007/s11368-020-02847-1
25. MSTAT Development Team (1989). *MSTAT user's guide: a microcomputer program for the design management and analysis of agronomic research experiments*. Michigan State University, East Lansing, USA.
26. Nooh A.E., El-Naggar A.H. (2021). *Technology of production of ornamental plants*. Knowledge facility publisher, Alexandria, Egypt: 391 p.
27. Novoskoltseva, O.A., Panova, I.G., Loiko, N.G., Nikolaev, Y.A., Litmanovich, E.A. and Yaroslavov, A.A. (2021). Polyelectrolytes and Polycomplexes for stabilizing sandy grounds. *Polym. Sci. Ser. B.* 63: 488–495. doi.org/10.1134/S1560090421050092
28. Page A.I., Miller R.H. and Kenney D.R. (1982). *Methods of Soil Analysis, Part II*. Amer. Soc. of Agronomy, Madison, Wisconsin, USA. 1159 p. DOI:10.2134/agronmonogr9.2.2ed
29. Shahb, a M. A., Abbas, M. S. and Alshammary, S. F. (2014). Drought resistance strategies of seashore paspalum cultivars at different mowing heights. *HortScience*, 49(2): 221-229. DOI: 10.21273/HORTSCI.49.2.221
30. Tan W. K., Zhu J., Lim J. Y., Gao Z., Loh C. S., Li J. and Ong, C. N. (2021). Use of okara-derived hydrogel for enhancing growth of plants by minimizing leaching and locking nutrients and water in growing substrate. *Ecological Engineering*, 159: 106122. https://doi.org/10.1016/j.ecoleng.2020.106122
31. Wettstein, D.V. (1957). Chlorophyll lethal under sub mikroskoshrel der plastieden. *Ex. PF Celi. Res.*, (12): 427-433. doi.org/10.1016/j.joule.2018.07.015

Romanian Journal
OF
HORTICULTURE
Postharvest

Chitosan-based melatonin coating effects on apricot postharvest life

E. Bal

Tekirdağ Namık Kemal University, Faculty of Agriculture, Department of Horticulture, Tekirdağ, Turkey

*Corresponding author e-mail: ebal@nku.edu.tr

ABSTRACT

Apricot (*Prunus armeniaca* L.) is a climacteric and perishable fruit prone to rapid postharvest deterioration due to microbial decay, moisture loss, and chilling injury. This study investigated the combined effects of chitosan (CT) and melatonin (MLT) coatings on the postharvest quality of apricot fruits (cv. Roxana) stored at 0-1 °C for 28 days. Treatments included control, melatonin (1000 µmol l⁻¹), 1% chitosan, and a combined CT+MLT coating. Weight loss, soluble solids content (TSS), titratable acidity (TA), fruit firmness, respiration rate, total phenolic content, antioxidant content, decay rate and internal browning index were measured at 7-day intervals. CT+MLT-treated fruits showed superior retention of firmness, slower increase in TSS, reduced TA loss, and delayed respiration peaks. Furthermore, CT+MLT coating significantly preserved total phenolic content and antioxidants, which declined in control fruits over time. Importantly, CT+MLT and MLT alone effectively inhibited internal browning and decay throughout the storage period, with no incidence observed until the end of storage. These findings suggest that chitosan coating enriched with melatonin provides a synergistic effect in preserving apricot fruit quality during cold storage, by delaying senescence, maintaining bioactive compounds, and minimizing postharvest losses.

Keywords: *Prunus armeniaca* L., postharvest quality, Chitosan coating, Melatonin, storage life, bioactive compounds

INTRODUCTION

Apricot (*Prunus armeniaca* L.) is a nutritious fruit that is known for its health benefits and is highly demanded by consumers due to its high content of bioactive phytochemicals such as carotenoids, flavonoids, phenolics and antioxidants (Dragovic-Uzelac *et al.*, 2007). Apricot is a climacteric fruit that ripens quickly, is highly perishable and has a limited postharvest life. Depending on apricot cultivar, it can be stored at 0°C for 2-4 weeks after harvest (Crisosto and Kader, 1999). Postharvest losses of apricots due to rapid ripening, microbial decay, and chilling injury due to long-term storage at low temperatures pose significant economic challenges. In order to extend the storage and marketing period of apricot fruits, it is of great importance to delay and slow down these changes.

Edible coating applications have the potential to improve fruit quality and extend the shelf life of products because they provide selective barriers to oxygen, carbon dioxide and flavor compounds (Pham *et al.*, 2023). In addition, edible coatings can serve as carriers of various active ingredients such as natural or chemical antimicrobial agents, antioxidants, flavors, enzymes, functional ingredients, or nutrients (Dhall 2013).

Chitosan (CT) is a naturally abundant biopolymer that plays a key role in edible coating applications. CT coatings with semi-permeable properties can change the internal atmosphere by reducing water loss, showing antimicrobial activity and delaying fruit ripening. The semi-permeable CT film is durable, flexible and does not tear easily and forms a very good oxygen barrier, providing significant advantages to chitosan compared to other polymers (Shahidi *et al.*, 1999). It has been reported in many studies that postharvest CT coating has a significant effect on improving the sensory properties of fruits and preserving their quality (Romanazzi and Moumni, 2022; Saberi Riseh *et al.*, 2023). Melatonin (MLT) is a signaling molecule that plays a role in many plant growth and development processes (Khan *et al.*, 2024). In addition, treatment with exogenous melatonin at an appropriate dose can significantly regulate changes in postharvest respiration, ethylene release, antioxidant enzyme activity and other related physiological parameters of fruits, delay aging and extend shelf life (Saud *et al.* 2023). Studies have shown that postharvest melatonin application can delay the weight loss in sweet cherries (Wang *et al.*, 2019), control the softness of apricot (Medina-Santamarina *et al.*, 2021; Zhang *et al.*, 2024), increase the disease resistance of peach (Gao *et al.* 2016), inhibit ripening in pears (Zhai *et al.*, 2018), alleviate the chilling damage of nectarine and peach (Bal 2020) and promote the accumulation of total phenolics and ascorbic acid, resulting in higher antioxidant capacity (Bal 2019; Luo *et al.*, 2022). At present, no information is available regarding the effect of chitosan coating and melatonin on the postharvest quality of apricots. Thus, the study aimed to investigate the effects of combining chitosan with melatonin on decay, physiological properties and quality attributes of apricots (*Prunus armeniaca* cv. 'Roxana') stored at 0-1°C during storage periods.

MATERIALS AND METHODS

Apricot cv. 'Roxana' fruits were harvested at the firm maturation stage by hand-plucking, from a well-managed demonstration orchard in Turkey. Fruits were immediately taken to the laboratory and selected as homogeneous in size and color, free from visible defects. After that, fruits were randomly distributed into 4 equal parts for postharvest treatments, and then treated as follows: (1) Control: Fruits were immersed in distilled water. (2) MLT treatment: 1000 $\mu\text{mol l}^{-1}$ melatonin was dissolved in ethanol (10 ml) and then added deionized water up to the required volume. (3) CT coating: Chitosan solution was prepared by dissolving 1% (w/v) chitosan in 0.5 % (w/v) acetic acid solution (0.5 % v/v) at 45 C. Glycerol (1 %) and Tween 20 (0.1 %) were added to the solution as surfactant, respectively. After stirring, the fruits were soaked in 1% (w/v) chitosan coating solution for 5 min. (4) CT+MLT coating: Fruits were immersed in a mixed solution containing 1% chitosan and 1000 $\mu\text{mol l}^{-1}$ melatonin. All fruits were air-dried after the treatments and were packed in porous clamshell punnets and stored under 0-1°C at $90 \pm 5\%$ RH for 28 days.

Physical and biochemical analyses were performed on fruit samples removed from cold storage at the beginning of storage and at 7-day intervals. Weight loss of apricot fruits was recorded using an electronic weighing scale at periodic intervals of 7 days during cold storage. TSS and TA were determined using the juice extraction of plum fruit. TSS was measured using the handheld refractometer and TA was measured in terms of malic acid by the titration method (AOAC 2016). Fruit firmness was measured as the penetration force into the fruit flesh (expressed as Newtons (N)), and a penetrometer with a 7.9 mm probe was used to test the firmness. The respiration rate was analyzed with a CO₂ gas analyzer. For 1 hour, 500 g of fruits were enclosed in tightly sealed hermetic flasks. The sensor was used to measure CO₂ concentration, which was then used to determine the respiration rate. The amount of total phenolic substance was determined by the spectrophotometric method using Folin-Ciocalteu reagent, according to Singleton *et al.* (1999). The antioxidant

activity of the samples was analyzed by using the DPPH assay according to the procedures of Brand-Williams *et al.* (1995). The total number of rotten fruits was measured as a percentage compared to the number of fruits placed at the beginning. Fruits were visually analyzed for signs of internal browning (IB), using the following scale: 0, no browning; 1, very light browning; 2, light browning; 3, moderate browning; 4, severe browning. The experimental design was completely randomized. Groups of three replicates of 500 g of fruit per treatment for the cold storage were established. The data were analysed using *SPSS (Version 11.0)* and are reflected as the means with their standard error.

RESULTS AND DISCUSSIONS

After harvest, fruits continue to lose water through their skin, which can lead to shrinkage and weight loss, affecting the fruit's appearance and texture (Lufu *et al.*, 2020). The results showed a significant increase in the percentage of fruit weight loss during the storage in all treatments (Figure 1). Coating apricot fruits with chitosan alone or in combination was highly effective in providing a physical barrier against moisture loss; therefore, a reduced weight loss was observed in chitosan-coated fruit effective in providing a physical barrier against moisture loss. The addition of melatonin to the chitosan-based coating did not significantly alter the moisture barrier functions. At the end of the storage period, weight loss was 15.7% in control fruits and 10.2% in MLT application, while weight loss was 5.0% in CT+MLT coated fruits and 5.7% in CT coated fruits, respectively. These findings were in line with researches of Algarni *et al.* (2022) and Morsy *et al.* (2019) which showed that weight loss in chitosan-coated fruit was lower than in non-coated apricot fruit. Moreover, MLT and CT may affect the weight loss through their synergistic effect in reducing respiration and water loss from the fruit surface.

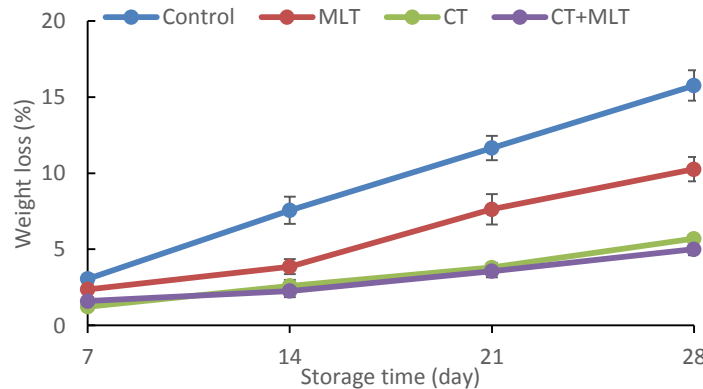


Figure 1. Effects of postharvest Melatonin and Chitosan treatments on weight loss of apricots

According to TSS (%) levels in apricots increased as fruit ripened over the postharvest period (Figure 2a). However, CT and CT+MLT coating significantly delayed the increase in TSS compared to controls, especially between days 21 and 28 postharvest. While the initial TSS value in all apricot samples was 9.8%, the TSS content in control fruits at 28 days of storage was 13%, which was higher than the CT+MLT coating group. This result is consistent with the findings that the retention of TSS content in coated apricots reflected the change in the internal environment of the fruits by coating application, which could eventually reduce the internal O_2 of the fruit and/or suppress ethylene production by increasing CO_2 (Morsy and Rayan, 2019; Riva *et al.*, 2020). TA was 1.65% at initial storage and the faster decrease in acidity led to faster senescence (Figure 2b). The lowest decrease in TA was observed at the same level of significance at the end of storage in fruits coated with CT (1.34%) and CT+MLT (1.36%). Conversely, lower TA levels were found in control

fruits compared to treated fruits (1.21%), suggesting that edible coatings form a semipermeable film around the fruit, thereby delaying the ripening process. An overall decrease in organic acids might be expected due to the consumption of organic acids as metabolic substrates during respiration (Fonseca et al., 2002). However, the rate of TA loss was found to be reduced by the incorporation of MLT into the CH coating, probably by slowing down metabolic activities as reported in banana (Al-qurashi et al., 2023) and pomegranate (Mwelase et al., 2022).

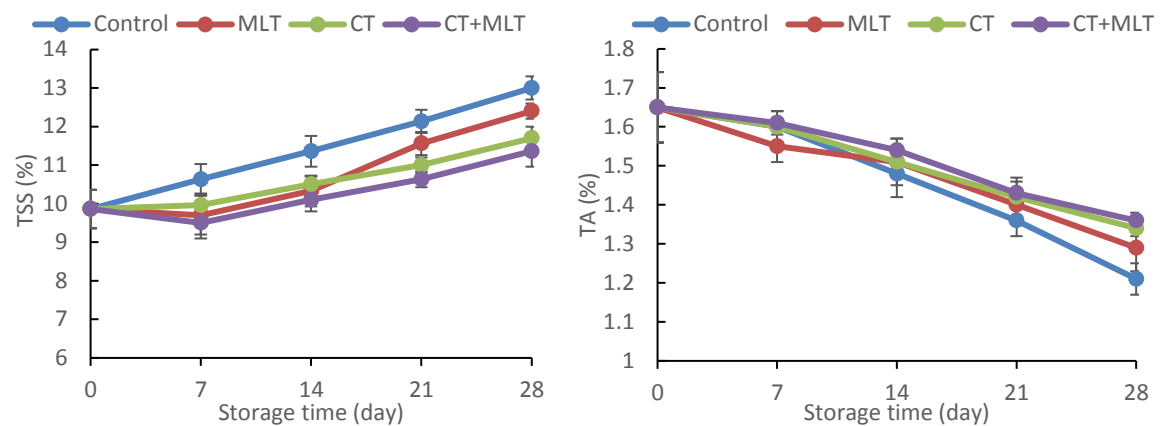


Figure 2. Effects of postharvest Melatonin and Chitosan treatments on TSS (a-left) and TA (b-right) of apricots

Apricot firmness decreased from 42.6 to 12.4 N as the postharvest ripening time increased from 0 to 28 days (Figure 3). The pattern of change in firmness in all fruits during postharvest storage was qualitatively similar, but firmness was significantly higher in MLT and CT-coated fruits compared to control fruits during 28 days of storage. Previous study on apricot fruit has shown that degradation of cell wall components leads to decreased fruit firmness as storage time increases (Morsy and Rayan, 2019). At the end of cold storage, samples with CT and CT+MLT treatments represented smaller firmness loss of apricot (19.2and 20.3 N, respectively) compared with MLT (14 N) and control (12.4 N). Previous research also reported that ML reduced fruit softening and preserved apricot quality during storage by suppressing cell-wall degrading enzyme activity (Medina-Santamarina *et al.*, 2021; Zhang *et al.*, 2024) and also apricot coated with CTS resulted in maintenance of higher fruit firmness and soluble solids content (Han *et al.*, 2024).

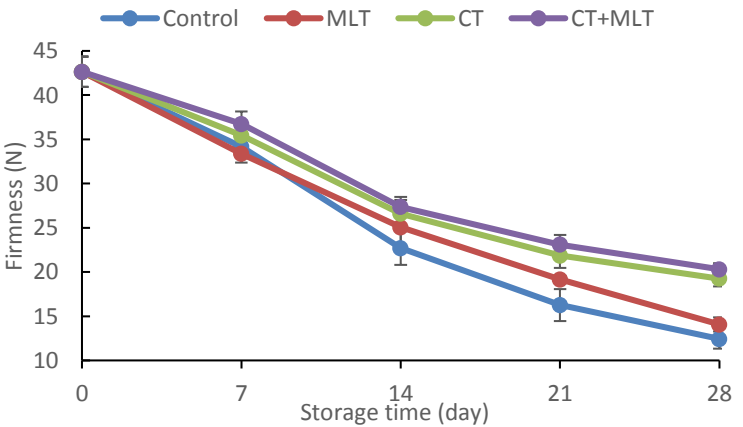


Figure 3. Effects of postharvest Melatonin and Chitosan treatments on firmness of apricots

During storage, an increase and then a decrease in respiration rates were observed in groups (Figure 4). Compared with the control and MLT treated fruits, the respiratory peaks of CT and CT+MLT treated fruits were markedly suppressed throughout the storage period. Chitosan-based coatings slowed down the respiration rate of fruits because it formed a thin layer on the surface of the fruit by acting as a physical barrier that regulates gas and atmospheric exchange (Shahidi *et al.*, 1999). The findings revealed that while CO₂ production in control and MLT applied fruits reached the highest values of 27.6 and 26.3 mg kg⁻¹ h⁻¹ on the 14th day, it reached the highest values of 20.4 and 19.03 mg kg⁻¹ h⁻¹ on the 21st day in CT and CT+MLT treatments. These results indicate that CT and CT+MLT treatments are more effective than the other two treatments in inhibition of respiration rates during storage, which are in agreement with the previous reports suggesting the beneficial effects of chitosan and chitosan coating combined with melatonin treatments in reducing respiration rates in sweet cherry (Ba,l 2024) and broccoli (Tan *et al.*, 2023).

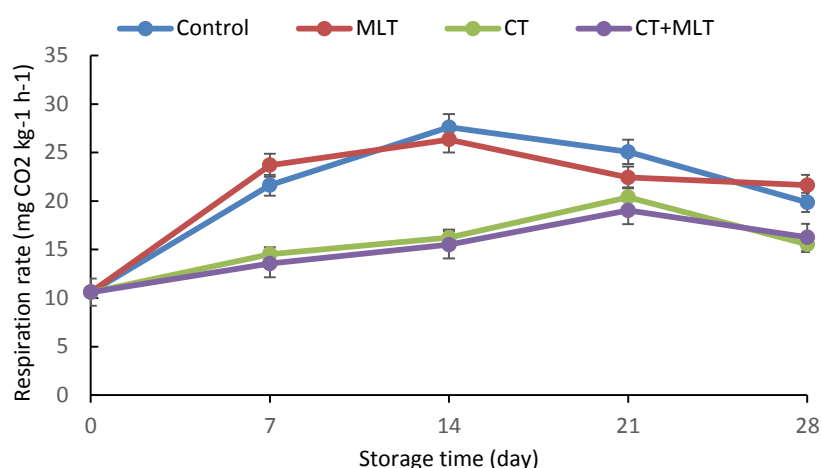


Figure 4. Effects of postharvest Melatonin and Chitosan treatments on respiration rate of apricots

According to the results, phenolic compounds in all treatments showed an increasing trend at the beginning of treatment and then decreased with storage time (Figure 5). However, CT and MLT treatments alone or in combination significantly reduced the phenol loss of apricot fruit compared with controls. After 28 days of storage, the phenolic content of MLT, CT, and CT+MLT treated apricot fruits was 111.3 mg 100 g⁻¹, 115.3 mg 100 g⁻¹, and 122.6 mg 100 g⁻¹, respectively, which were obviously lower than control samples (96.6 mg 100 g⁻¹).

The effectiveness of chitosan coated fruits combined with melatonin can be attributed to its potential to delay oxygen permeation on the fruit surface and reduce the enzymatic oxidation of phenols by melatonin treatment. The inhibitory effect of chitosan coating on polyphenoloxidase (PPO) activity was consistent with previous studies on sweet cherry (Pasquariello *et al.*, 2015) and plum (Iqbal *et al.*, 2025). Some literature also reported that melatonin treatments remarkably enhanced the phenolic content of plum (Bal 2019) and inhibited the rise of PPO activity of sweet cherry (Michailidis *et al.*, 2021).

In the study, antioxidant content of apricots exhibited trends similar to phenolics with a typical peak value (Figure 6). The antioxidant content-of the fruits gradually increased up to 7 and 14 days and then decreased, but were found to be higher in treated fruits than in control. This result is consistent with Wang *et al.* (2019), who reported that CT coating reduces the decline in antioxidant contents and melatonin, as an antioxidant or signaling molecule, has been reported to regulate the activity of the antioxidant system and mitigate cellular damage.

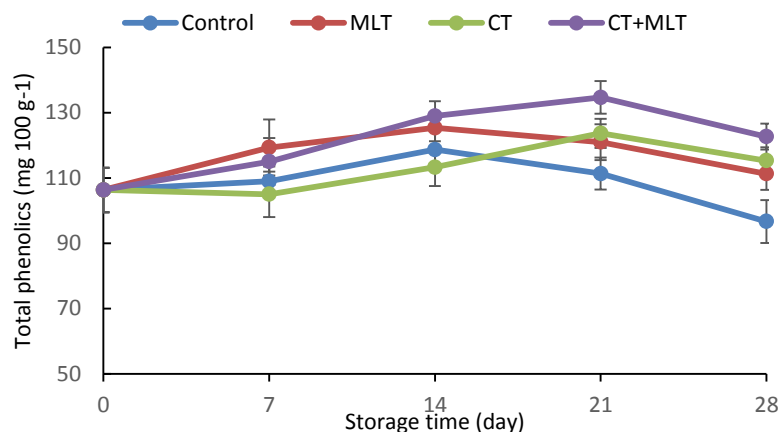


Figure 5. Effects of postharvest Melatonin and Chitosan treatments on total phenolics of apricots

At the end of storage, the CT and MLT combination ($29.06 \mu\text{mol g}^{-1}$) exhibited the highest antioxidant compared to each of them alone, and only CT+MLT treated fruits were found to be higher than the initial value ($27.96 \mu\text{mol g}^{-1}$). Due to the capability of CTS to carry functional substances (Lin *et al.*, 2008), adding melatonin into CTS-based coating showed a synergistic effect on improving non-enzymatic antioxidant defense systems and proved the relationship between MLT and CT described by Arshad and Haghshenas (2025) and Bal (2024).

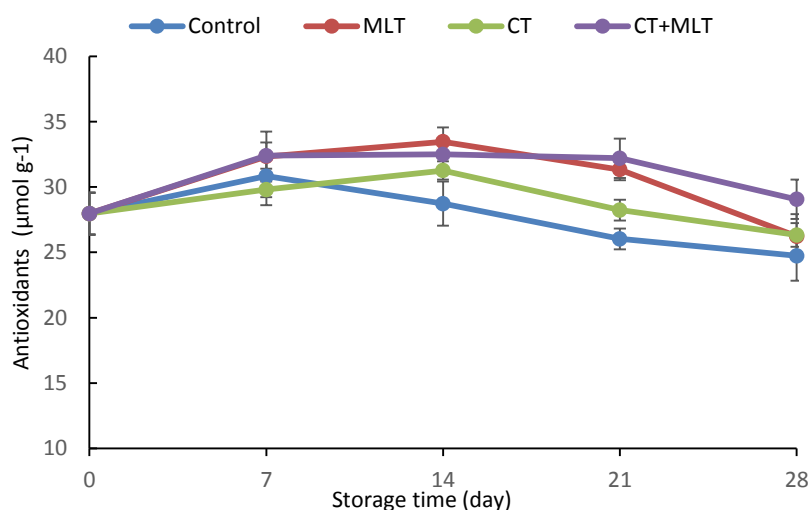


Figure 6. Effects of postharvest Melatonin and Chitosan treatments on antioxidants of apricots

In present research, apricot seldom showed rotting. The results showed that decay was first observed in the control fruits after 21 days of storage, and then, at the end of the storage period, low levels of mold were detected only in the control (5.3%) and CT-treated fruits (2%), (Figure 7a). This trend was also observed in the internal browning analysis results (Figure 7b). IB symptoms as very light browning was first detected after 21 days of storage in control and CT treatment, while in CT + MLT and MLT treatments was no observed during 28 days of storage.

These results were consistent with the observation by Demartino *et al.* (2002) that apricots could develop some physiological problems, including juiceless flesh and internal browning after 2-3 weeks of storage at $1-4^{\circ}\text{C}$.

The results of recent studies showed that the exogenous melatonin and chitosan coating treatments could reduce decays and physiological disorders of postharvest fruit during cold storage (Gao *et al.*, 2016; Bal 2021; Romanazzi and Moumni, 2022; Saberi Riseh *et al.*, 2023). It has also reported that melatonin triggers signaling molecules that play a role in disease resistance (Hao *et al.*, 2025) and chitosan has potential as an edible antifungal coating material (Dai *et al.*, 2025).

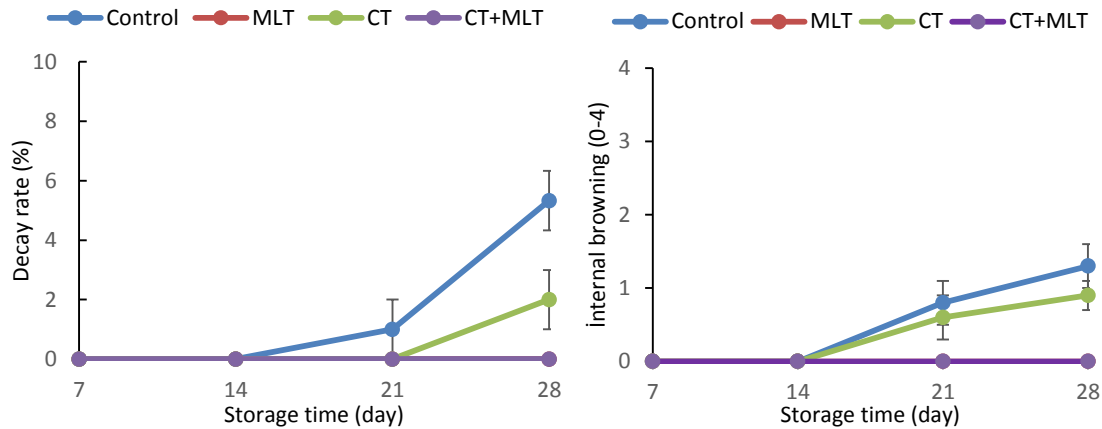


Figure 7. Effects of postharvest Melatonin and Chitosan treatments on decay rate (a) and internal browning (b) of apricots

CONCLUSIONS

This study demonstrated that the application of chitosan-based coatings, particularly when enriched with melatonin, significantly preserved the postharvest quality of apricot fruits (cv. Roxana) during cold storage at 0-1 °C for 28 days. The combined CT+MLT treatment was the most effective in reducing weight loss, maintaining fruit firmness, delaying increases in TSS, and reducing the loss of TA (%). Additionally, this treatment notably suppressed respiration rate and minimized decay and internal browning, indicating its ability to delay senescence and physiological disorders. These findings suggest that chitosan coatings incorporated with melatonin represent a promising, natural, and safe postharvest strategy to extend shelf life, reduce storage losses, and maintain the nutritional and sensory quality of apricots.

REFERENCES

- Algarni, E. H. A., Elnaggar, I. A., Abd El-wahed, A. E.-w. N., Taha, I. M., AL-Jumayi, H. A., Elhamamsy, S. M., Mahmoud, S. F., & Fahmy, A. (2022). Effect of Chitosan Nanoparticles as Edible Coating on the Storability and Quality of Apricot Fruits. *Polymers*, 14(11), 2227. <https://doi.org/10.3390/polym14112227>
- Al-qurashi, A. D., Awad, M. A., Elsayed, M. I., & Ali, M. A. (2023). Postharvest melatonin and chitosan treatments retain quality of 'Williams' bananas during ripening. *Journal of Food Science and Technology*, 61, 84–96.
- AOAC. (2016). *Official Methods of Analysis* (20th ed.). The Association of Official Analytical Chemists.
- Arshad, M., & Haghshenas, M. (2025). Melatonin and Chitosan Coating Effects on Banana Postharvest Life and Physiological Traits. *International Journal of Horticultural Science and Technology*, 12(1), 31–42.
- Bal, E. (2019). Physicochemical changes in 'Santa Rosa' plum fruit treated with melatonin during cold storage. *Journal of Food Measurement and Characterization*, 1–8.
- Bal, E. (2020). Effect of melatonin treatments on biochemical quality and postharvest life of nectarines. *Journal of Food Measurement and Characterization*, 15, 288-295.
- Bal, E. (2024). Impact of Chitosan-Melatonin Composite Coating on Postharvest Quality of Sweet Cherry. *Applied Fruit Science* 66, 763–770. <https://doi.org/10.1007/s10341-023-00994-6>.
- Brand-Williams, W., Cuvelier, M. E., & Berset, C. (1995). Use of a free radical method to evaluate antioxidant activity. *Food Science and Technology*, 28, 25–30.
- Crisosto, C.H. and A.A. Kader. (1999). Apricots postharvest quality maintenance guidelines. department of pomology university of California. <http://www2.uckac.edu/postharv/PDF%20files/Guidelines/.pdf>.

10. Dai, L., Wang, X., Zhang, J., & Li, C. (2025). Application of Chitosan and Its Derivatives in Postharvest Coating Preservation of Fruits. *Foods*, 14(8), 1318. <https://doi.org/10.3390/foods14081318>
11. Demartino, G., Massantini, R., & Botondi, R. (2002). Temperature affects impact injury on apricot fruit. *Postharvest Biology and Technology*, 25, 145–149.
12. Dhall, R. K. (2013). Advances in Edible Coatings for Fresh Fruits and Vegetables: A Review. *Critical Reviews in Food Science and Nutrition*, 53(5), 435–450. <https://doi.org/10.1080/10408398.2010.541568>
13. Fonseca, S. C., Oliveira, F. A. R., & Brecht, J. K. (2002). Modelling respiration rate of fresh fruits and vegetables for modified atmosphere packages: A review. *Journal of Food Engineering*, 52, 99–119.
14. Gao, H., Zhang, Z., Chai, H., Cheng, N., Yang, Y., Wang, D., Yang, T., & Cao, W. (2016). Melatonin treatment delays postharvest senescence and regulates reactive oxygen species metabolism in peach fruit. *Postharvest Biology and Technology*, 118, 103–110.
15. Han, Y., Liu, Y., Li, Z., Sun, J., Song, F., & Chen, J. (2024). Effects of chitosan grafted with gallic acid treatment on the postharvest physiology and biochemistry of Saimaiti apricots. *J. Stored Products Res.*, 108, 102390.
16. Hao, X., Ren, J., Xu, M., Sun, B., Li, R., Yang, S., & Xu, W. (2025). Melatonin in plant pathogen defense: a review of its role in horticultural crops. *Horticulture Research*, 12.
17. Iqbal, S. Z., Waseem, M., Naz, F., Iqbal, M., Ayub, M. A., Mohammed, O. A., & Abdull Razis, A. F. (2025). Application of Chitosan and Pectin-Based Nanoemulsion Coating for the Preservation of Plum (*Prunus domestica*) Fruit. *Food Science & Nutrition*, 13.
18. Khan, M., Hussain, A., Yun, B.-W., & Mun, B.-G. (2024). Melatonin: The Multifaceted Molecule in Plant Growth and Defense. *International Journal of Molecular Sciences*, 25(12), 6799.
19. Lin, L., Wang, B., Wang, M., Cao, J., Zhang, J., Wu, Y., & Jiang, W. (2008). Effects of a chitosan-based coating with ascorbic acid on post-harvest quality and core browning of 'Yali' pears (*Pyrus bertschneideri* Rehd.). *Journal of the Science of Food and Agriculture*, 88, 877–884.
20. Lufu, R., Ambaw, A., & Opara, U. L. (2020). Water loss of fresh fruit: Influencing pre-harvest, harvest and postharvest factors. *Scientia Horticulturae*, 272, 109519.
21. Luo, Z., Zhang, J., Xiang, M., Zeng, J., Chen, J., & Chen, M. (2022). Exogenous melatonin treatment affects ascorbic acid metabolism in postharvest 'Jinyan' kiwifruit. *Frontiers in Nutrition*, 9.
22. Medina-Santamarina, J., Zapata, P. J., Valverde, J. M., Valero, D., Serrano, M., & Guillén, F. (2021). Melatonin Treatment of Apricot Trees Leads to Maintenance of Fruit Quality Attributes during Storage at Chilling and Non-Chilling Temperatures. *Agronomy*, 11, 917.
23. Michailidis, M., Tanou, G., Sarrou, E., Karagiannis, E., Ganopoulos, I., Martens, S., & Molassiotis, A. (2021). Pre- and Post-harvest Melatonin Application Boosted Phenolic Compounds Accumulation and Altered Respiratory Characters in Sweet Cherry Fruit. *Frontiers in Nutrition*, 8.
24. Morsy, N., & Rayan, A. M. (2019). Effect of different edible coatings on biochemical quality and shelf life of apricots (*Prunus armenica* L. cv Canino). *J. Food Measurement and Characterization*, 13, 3173–3182.
25. Mwelase, S., Opara, U. L., & Fawole, O. A. (2022). Effect of chitosan-based melatonin composite coating on the quality of minimally processed pomegranate aril-sacs during cold storage. *J. Food P. Preser.*, 46, e17096.
26. Pasquariello, M., Patre, D.D., Mastrobuoni, F., Zampella, L., Scortichini, M., & Petriccione, M. (2015). Influence of postharvest chitosan treatment on enzymatic browning and antioxidant enzyme activity in sweet cherry fruit. *Postharvest Biology and Technology*, 109, 45–56.
27. Pham, T. T., Nguyen, L. L. P., Dam, M. S., & Baranyai, L. (2023). Application of Edible Coating in Extension of Fruit Shelf Life: Review. *Agri. Engineering*, 5(1), 520–536.
28. Riva, S. C., Opara, U. O., & Fawole, O. A. (2020). Recent developments on postharvest application of edible coatings on stone fruit: A review. *Scientia Horticulturae*, 262, 109074.
29. Romanazzi, G., & Moumni, M. (2022). Chitosan and other edible coatings to extend shelf life, manage postharvest decay, and reduce loss and waste of fresh fruits and vegetables. *Current Opinion in Biotechnology*, 78, 102834.
30. Saberi Riseh, R., Vatankhah, M., Hassanisaadi, M., & Kennedy, J. F. (2023). Chitosan-based nanocomposites as coatings and packaging materials for the postharvest improvement of agricultural product: A review. *Carbohydrate Polymers*, 309, 120666.
31. Saud, S., Jiang, Z., Chen, S., & Fahad, S. (2023). Interaction of melatonin on post-harvest physiology and quality of horticultural crops. *Scientia Horticulturae*, 321, 112286.
32. Shahidi, F., Arachchi, J. K. V., & Jeon, Y. (1999). Food applications of chitin and chitosans. *Trends in Food Science & Technology*, 10, 37–51.
33. Singleton, V. L., Orthofer, R., & Lamuela-Raventós, R. M. (1999). Analysis of total phenols and other oxidation substrates and antioxidants by means of folin-ciocalteu reagent. In *Methods in Enzymology*, 299, 152–178.
34. Tan, Y., Li, L., Li, Y. C., Wang, X. J., & Wang, Y. (2023). Effect of chitosan coating combined with melatonin on the quality and chlorophyll change of fresh-cut broccoli. *Food Science*, 44(21), 184–203.
35. Wang, F., Zhang, X., Yang, Q., & Zhao, Q. (2019). Exogenous melatonin delays postharvest fruit senescence and maintains the quality of sweet cherries. *Food Chemistry*, 301, 125311.
36. Zhai, R., Liu, J., Liu, F., Zhao, Y., Liu, L., Fang, C., Wang, H., Li, X., Wang, Z., Ma, F., & Xu, L. (2018). Melatonin limited ethylene production, softening and reduced physiology disorder in pear (*Pyrus communis* L.) fruit during senescence. *Postharvest Biology and Technology*, 139, 38–46.

The development and industrial implementation of technology for producing biologically active supplements

L. Yushan

National Institute for Applied Research in Agriculture and Veterinary Medicine,
Department of Food Technology and Microbiology, Kishinev, Republic of Moldova

*Corresponding author email: iushan.larisa76@gmail.com

ABSTRACT

This study presents the development and industrial implementation of technology for producing supplements rich in biologically active substances from grape and pumpkin seeds. The process involves drying seeds to optimal moisture levels, crushing to fine particle size, and processing via supercritical CO₂ extraction. Eight types of fat-soluble extracts were obtained and encapsulated in gelatin capsules. Quantitative and qualitative analyses determined the content of key bioactive compounds: linoleic and linolenic acids, α -tocopherol, β -carotene, and phenolic substances.

Keywords: gelatin encapsulation, grape and pumpkin seeds, liposoluble extracts, supercritical CO₂-extraction.

INTRODUCTION

The Republic of Moldova's agricultural sector offers diverse vegetable raw materials valued in domestic and international markets for their biologically active substances with beneficial health effects. Oil extraction from seeds of pumpkin, flax, milk thistle and grapes represents the most commonly used method for obtaining essential bioactive compounds (Batool *et al.*, 2022; Zhou *et al.*, 2022; Aziz *et al.*, 2020).

The bio-climatic and socio-economic conditions of the Republic of Moldova enable industrial utilization of grape and pumpkin seeds, as sufficient quantities accumulate annually at fruit and vegetable processing enterprises.

Pumpkin seeds contain 10.9-30.9% oil, with total unsaturated fatty acids comprising 73.1-80.5% of the lipid fraction. The predominant fatty acids are linoleic, oleic, palmitic, and stearic acids (Batool *et al.*, 2022). Pumpkin seed oil contains numerous biologically active substances including vitamins B₁, B₂, C, P, flavonoids, and various fatty acids (palmitic, stearic, oleic, linoleic). These compounds normalize metabolism, facilitate toxin elimination, and exhibit antiulcer, antisclerotic, and antiseptic properties.

Grape seeds prepared for industrial processing contain approximately 10-18% high-quality oil. The biological activity of grape oil stems from its complex of bioactive substances, notably resveratrol and bioflavonoids—oligomeric proanthocyanidins—which function as antioxidants preventing cellular degeneration. Grape oil also contains vitamin E, which acts synergistically with P-group vitamins to modify membrane phospholipids (Zueva, 2004). Bioactive substances from plant materials can be extracted using two main categories of techniques: conventional and innovative methods.

Conventional techniques, long-established and based on solvent extraction power and heat treatment, include Soxhlet extraction, hydro distillation, and maceration. Recently developed innovative techniques involve assisted extraction processes: microwave, enzymatic, ultrasonic, and supercritical fluid extraction.

Supercritical fluid extraction offers distinct advantages: lower viscosity and higher diffusion coefficient compared to liquid solvent extraction, providing superior mass transfer; reduced processing time; environmental friendliness due to minimal sample and solvent requirements; minimal losses through supercritical fluid reuse and recycling (Liviu, 2017). The bioavailability of bioactive substances can be optimized through encapsulation of lipid extracts obtained with supercritical fluids (CO₂ or N₂) using gelatin. This innovative process implementation offers comprehensive benefits. The purpose of this study is to research and implement technology for producing supplements rich in biologically active substances from native raw materials.

MATERIALS AND METHODS

Research was conducted at the Department of Food Technology and Microbiology of the PI National Institute for Applied Research in Agriculture and Veterinary Medicine, Republic of Moldova (IP NIARAVM).

Research materials included: Chardonnay (white cultivar) grape seeds; Cabernet Sauvignon (red cultivar) grape seeds; Pumpkin seeds.

Seeds were dried, crushed, and subjected to supercritical CO₂ extraction to obtain lipid extracts and pomace for bioactive substance analysis

Fatty Acid Analysis: Saturated, monounsaturated, and polyunsaturated fatty acids in lipid extracts were determined using gas chromatography with molecularly imprinted polymers for volatile compound capture. Analysis was performed at "Agro.Biolab Laboratory" LLC, Italy.

Mineral Analysis: Mineral substances in pomace were determined by flame atomic absorption spectroscopy at "Agro.Biolab Laboratory" LLC, Italy.

Bioactive Compound Analysis: Linoleic and linolenic acids, α -tocopherol were determined by UV spectrophotometry following lipid extract saponification.

Total phenolic substances and carotenoids were determined by visible spectrophotometry using Folin-Ciocalteu reagent in hexane solutions and hexane:acetone: ethanol extraction mixture, respectively.

Analyses were conducted at the IP NIARAVM, Food Quality Control Laboratory.

For each parameter analyzed, the determinations were performed in n independent repetitions (n =3).

The results were statistically processed and presented as mean $\pm$ standard deviation (mean $\pm$ SD), to highlight the analytical and biological variability of the samples.

RESULTS AND DISCUSSIONS

The technological process for obtaining supplements with fat-soluble extracts from white and red grape seeds and pumpkin seeds was developed using supercritical CO₂ extraction and gelatin encapsulation equipment.

The manufacturing technology for vegetable lipid extract supplements involves complex procedures outlined in the following flowchart (Figure 1).

Process operations

Raw material reception. Raw materials included 'Chardonnay' grape seeds, 'Cabernet-Sauvignon' grape seeds and pumpkin seeds supplied by "Oil Eco Grup" LLC. Fresh seeds exhibited moisture content above 14%, necessitating drying prior to processing.

Seed drying. Drying was performed in hot air installations at 70°C until optimal moisture content of 8-10% was achieved. Proper drying is essential for storage and processing; excessive moisture intensifies hydrolysis, increases seed acidity, and triggers biological processes leading to seed degradation. Optimal moisture produces powdery, friable

grinding with high oil absorption capacity. Higher moisture levels impair shredding and result in sticky grinding, complicating subsequent processing.

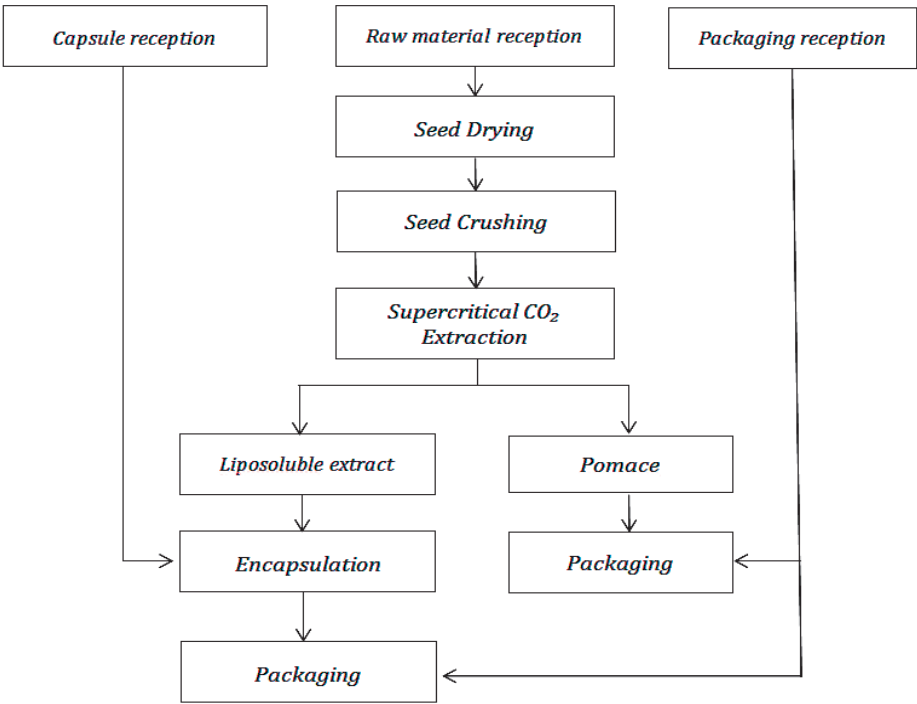


Figure 1. Manufacturing technology flowchart for supplements

Seed crushing. Grape and pumpkin seeds were crushed to fine particle size (Figures 2-4), breaking oleoplasm cell membranes and releasing oil as fine droplets from disrupted intercellular structures. Technical and technological limitations restrict grinding efficiency to 70-80% cell breakage, leaving oil trapped in intact cells. The specific morphological structure of seeds (hard shell, soft kernel) produces non-homogeneous grinding, with variety and species origin playing determining roles.



Figure 2. Chrushed white grape seeds



Figure 3. Chrushed red grape seeds



Figure 4. Chrushed pumpkin seeds

Grinding induces structural changes, creating conditions for oil oxidation and intensifying respiration and fermentation processes through protective shell removal. These factors reduce grinding stability, limiting retention time to very short intervals.

Supercritical CO₂ Extraction

Using pilot-scale supercritical extraction equipment, fat-soluble extracts (Figure 5) and pomace (Figure 6) were obtained according to extraction regimes (Table 1). The CO₂

extraction process involves setting pressure and temperature parameters, fixing extraction duration, and monitoring solvent flow rate. Supercritical CO₂ fluid functions as a lipophilic solvent, extracting volatile molecules (sesquiterpenes, monoterpenes, terpenols), tocopherols (vitamin E), glycerides, tocotrienols, phytosterols, carotenoids, and squalenes.



Figure 5. Extracts of seeds: white grape, red grape, white and red grape, pumpkin



Figure 6. Pomace of seeds: white grape, red grape, pumpkin

For the eight supercritical extractions, extractor cell loading varied from 366-386 g for pumpkin seeds and 506-576 g for grape seeds. Extract masses obtained in separator I ranged from 117.0-120.0 g for pumpkin seeds and 51.0-59.0 g for grape seeds. Higher extract yields from pumpkin seeds reflect their greater lipid content compared to grape seeds.

Encapsulation. Seamless soft gelatin capsules were produced using AC-powered equipment (220V) with a 3,600-capsule/hour capacity. Production was based on gelatinous mass physical properties. Capsules formed at the exit of the encapsulator cell, where a fat-soluble CO₂ extract and a gelatin mass were heated to 60-70°C under air pressure.

Table 1. Extraction regimes from grape and pumpkin seeds

Seed sample	Extraction regimes	Pressure, MPa	Temperature, °C	Time, min	Flow rate, L/h	Raw material, g	Extract Mass Separator I, g	Extract Mass Separator II, g	Pomace, g	Yield, %
White grape 'Chardonnay'	1	22.6	52.0	60	53	552	59	5	488	11.59
	2	36.2	48.7	120	54- 56	506	56	32	418	17.39
	3	26.0	49.3	190	58	576	57	5	514	10.76
Red grape 'Cabernet – Sauvignon'	1	25.6	48.8	120	54- 57	563	57	12	494	12.26
	2	35.6	49.1	120	53	561	56	14	491	12.48
Mixed White/Red	1	35.0	49.3	120	54	560	51	17	492	12.14
Pumpkin seeds	1	25.9	53.3	120	50	386	117	11	258	33.16
	2	30.2	59.6	150	50-52	366	120	4	242	33.88

Oil pulsation in cells created jet separation, with surface tension forces producing spherical shapes. Capsules (0.4g fill weight) solidified gradually in cooled vegetable oil flow (10-12°C). Post-formation capsules were transferred to oil-filled trays with regulated CO₂ extract and gelatin mass consumption. Visual inspection on illuminated tables confirmed proper parameters: a rounded shape without stripes, mechanical damage, or air bubbles (Figure 7).

**Figure 7.** Seamless soft gelatin capsules

Packaging. Inspected capsules are packaged in blisters or glass/plastic containers with lids in rooms equipped with general ventilation and air purification systems.

Bioactive Substance Content Analysis

Gas chromatography analysis at "Agro.Biolab Laboratory" LLC, Italy, determined fatty acids content in fat-soluble extracts (Table 2). Linoleic acid predominated: 71.7-72.2% in grape seed extracts and 43.9-44.0% in pumpkin seed extracts. Linolenic acid content was 0.51-0.52% in grape extracts and 0.33% in pumpkin extracts. Grape extracts demonstrated 37-40% higher linoleic (ω -6) and linolenic (ω -3) acid content than pumpkin extracts, establishing these CO₂-extracted lipid fractions as valuable bioactive substance sources with significant oxidoreductive potential.

Table 2. Fatty acid content in fat-soluble seed extracts

Fatty acid	Fatty acid content in fat-soluble seed extracts, %			
	White grape	Red grape	Mixed White/Red	Pumpkin
Lauric acid (C 12:0)	< *LOQ	< *LOQ	< *LOQ	< *LOQ
Myristic acid (C 14:0)	0.05±0.01	0.04±0.01	0.04±0.01	0.10±0.01
Palmitic acid (C 16:0)	7.30±0.05	7.70±0.06	7.47±0.04	11.75±0.07
Palmitoleic acid (C 16:1)	0.15±0.01	0.19±0.01	0.16±0.02	0.14±0.03
Stearic acid (C 18:0)	4.32±0.04	3.24±0.04	3.79±0.04	5.95±0.03
Oleic acid (C 18:1)	15.80±0.13	16.20±0.20	15.9±0.17	37.10±0.11
Linoleic acid (C 18:2)	71.80±0.26	72.20±0.30	72.0±0.29	44.00±0.23
Linolenic acid (C 18:3)	0.53±0.01	0.51±0.02	0.51±0.01	0.33±0.01
Arachidic acid (C 20:0)	< *LOQ	< *LOQ	0.12±0.01	0.53±0.08
* - LOQ: Limit of detection LOQ = 0.01				

Mineral substance analysis of three pomace samples revealed varying concentrations of Ca, K, Mg, Fe, and Zn (Table 3). Grape seed pomace showed higher calcium content, while pumpkin seed pomace contained higher levels of iron, magnesium, potassium, and zinc.

Table 3. Mineral Content in Post-Extraction Seed Pomace

Mineral	Mineral content in post-extraction seed pomace, mg/kg		
	White grape	Red grape	Pumpkin
Calcium (Ca)	5880±1.31	6104±1.90	1133±1.99
Iron (Fe)	60.2±0.67	75.5±0.59	183±0.72
Magnesium (Mg)	1139±1.31	1155±1.30	5737±2.61
Potassium (K)	20.4±0.14	22.8±0.26	140±0.91
Zinc (Zn)	623±1.30	599±1.31	1808±1.99
*Note- Values represent the mean ± standard error (SD), n=3			

Spectrophotometric analysis at IP NIARAVM, Food Quality Control Laboratory, determined bioactive substance content in grape and pumpkin seed extracts (Table 4).

Table 4. Bioactive substance content in seed extracts

Seeds extract	α-tocopherol, mg/100 g	β-caroten, mg/100g	Phenolic Substances, mg/100 g *	Linoleic acid, %	Linolenic acid, %
White grape	93.7±0.30	-	95.2±0.11	69.8±0.33	0.4±0.02
Red grape	45.8±0.20	-	45.4±0.13	68.6±0.20	0.3±0.01
White and Red grape	94.7±0.21	-	97.3±0.16	70.2±0.35	0.4±0.02
Pumpkin, variant I	97.4±0.40	0.72±0.02	-	41.7±0.20	0.3±0.01
Pumpkin, variant II	89.8±0.40	0.88±0.02	-	40.4±0.27	0.2±0.01
* - Total phenolic substances expressed as gallic acid equivalents					
Note - Values represent the mean ± standard error, n=3					

α -Tocopherol content in grape seed extracts ranged from 45.8-97.4 mg/100g, while pumpkin seed extracts contained 0.72-0.88 mg/100g β -carotene. Phenolic substance content in grape seed extracts was 45.4-97.3 mg gallic acid equiv/100g. The mixed white and red grape seed extract showed the highest linoleic acid content (70.2%), while pumpkin seed extracts contained 40.4-41.7% linoleic acid. Linolenic acid comprised 0.2-0.4% of total fatty acids.

Modern technology for manufacturing bioactive substance supplements from grape and pumpkin seeds in gelatin capsule form was implemented at "Oil Eco Grup" LLC (Figure 8).

The technological equipment operates on 220V AC power with encapsulator capacity up to 15,000 capsules/hour. The production cycle from encapsulation to packaging requires 2-3 days. The production facility, employing 5 personnel, can produce 1.5 million capsules monthly.



Figure 8. Technological equipment for food supplement production

CONCLUSIONS

The present study showed that technological regimes for supercritical CO₂ extraction of biologically active substances from selected native raw materials ('Chardonnay' grape seeds, 'Cabernet Sauvignon' grape seeds, and pumpkin seeds) were successfully developed. Production documentation under experimental conditions was established.

Also, comprehensive analysis determined the content of key bioactive substances in concentrated post-extraction extracts: linoleic and linolenic acids, α -tocopherol, and β -carotene.

Gelatin capsule samples were produced, and documentation for bioactive substance supplement production under experimental conditions was established through collaboration between IP NIARAVM and "Oil Eco Grup" LLC.

The technology demonstrates successful scale-up potential with an established production capacity of 1.5 million capsules monthly.

ACKNOWLEDGEMENTS

The study is part of the Technology Transfer Project "Development and implementation of encapsulation technology for bioactive compounds from local plant raw materials" (2023). We are grateful to the "Agro.Biolab Laboratory" LLC, Italy, and the Food Quality Control Laboratory of IP NIARAVM for researching various quality indicators of samples. We are

also grateful to "Oil Eco Grup" LLC for the opportunity to conduct research in industrial conditions.

REFERENCES

1. Aziz, M. et al. (2020). Biochemical profile of milk thistle (*Silybum Marianum* L.) with special reference to silymarin content. *Food Science & Nutrition Journal*, 9 (1):244-250. doi: 10.1002/fsn3.1990
2. Batool, M., Ranjha, M., Roobab, U, Manzoor, M. et al. (2022). Nutritional Value, Phytochemical Potential, and Therapeutic Benefits of Pumpkin (*Cucurbita* sp.). *Plants* (Basel), 11(11):1394. doi: 10.3390/plants11111394.
3. Liviu, I. (2017). Changes in the antioxidant properties of food oils subjected to microwave irradiation, Biophysical study. "Carol Davila" University of Medicine and Pharmacy, Bucharest. https://umfcd.ro/wp-content/uploads/2017/09/SUSTINERE_TEZA/IOSIF_LIVIU/Liviu-Iosif-REZUMAT-TEZA.pdf
4. Cengiz, N., Guclu, G., Kelebek, H., Capanoglu, E., Selli, S. (2022). Application of Molecularly Imprinted Polymers for the Detection of Volatile and Off-Odor Compounds in Food Matrices. *ACS Omega* 7(18):15258-15266. <https://doi.org/10.1021/acsomega.1c07288>
5. Zhou. D.-D., Li, J., Xiong R.-G., Saimaiti, A. et al. (2022). Bioactive Compounds, Health Benefits and Food Applications of Grape. *Foods*. 11(18):2755. doi: 10.3390/foods11182755
6. Zueva, T. (2004). Development of low-waste technology for processing grape seeds and obtaining medicinal and cosmetic products based on them. Medical Dissertations. Pyatigorsk. <http://medical-diss.com/farmakologiya/razrabotka-maloothodnoy-tehnologii-pererabotki-semyan-vinograda-i-poluchenie-na-ih-osnove-lekarstvennyh-i-kosmeticheskikh-#ixzz6nIQntyVN>

Supercritical CO₂ extraction: Methodological approaches and practical applications for vaporizing agro-industrial secondary products

L. Yushan^{1*}, T. Andrusenko², N. Taran¹ and E. Zyryanova¹

¹ National Institute for Applied Research in Agriculture and Veterinary Medicine, Department Food Technology and Microbiology, Kishinev, Republic of Moldova

² "Viorica-Cosmetic" JSC, Chisinau, Republic of Moldova

* Corresponding author: email: iushan.larisa76@gmail.com

ABSTRACT

Secondary products from the agro-industrial complex in the Republic of Moldova are potential resources for biorefineries, that is, which process secondary bioproducts into raw materials for the production of value-added products. Rich in multifunctional biologically active compounds—including fatty and essential oils, phenolic substances, dietary fibers and other metabolites with demonstrated health benefits—they offer significant potential for the development of value-added ingredients. Supercritical CO₂ extraction (SFE-CO₂) provides a sustainable, selective and solvent-free method for isolating such compounds, particularly from plant- and animal-derived residues. This research presents a methodological overview and practical case applications of SFE-CO₂ for the valorization of agro-industrial by-products. Emphasis is placed on extraction principles, selective programming strategies and the technological advantages of supercritical processing. Examples from grape pomace, *Ginkgo biloba* leaves, insect biomass and other secondary materials illustrate the capacity of SFE-CO₂ to obtain high-quality extracts suitable for use in dietary supplements, food formulations, cosmetic products and pharmaceutical applications. The analysis demonstrates that SFE-CO₂ is a key technology for implementing innovative, waste-free biorefinery processes.

Keywords: agro-industrial complex, bioactive additives, dietary supplements, raw materials, secondary bio products, SFE technologies, waste-free production.

Principles of Supercritical CO₂ Extraction

Supercritical fluid extraction (SFE) of natural materials, both of plant and animal origin, using solvents such as carbon dioxide is attracting growing scientific and industrial interest, as it represents a promising direction for advanced processing technologies and waste-free production. Carbon dioxide (CO₂) is the most commonly used solvent in SFE due to its safety, non-toxicity, non-flammability, affordability and complete removability from the final product. Moreover, CO₂ possesses low critical parameters and behaves as a hydrophobic, non-polar solvent, which facilitates the solubilization of small and low-polarity molecules (Reverchon, 2006; Agostini *et al.*, 2012).

The critical temperature of CO₂, close to ambient temperature, makes it particularly suitable for extracting thermolabile compounds without inducing chemical degradation. As a result, SFE allows processing of plant materials at relatively low temperatures, which minimizes microbiological contamination and prevents thermal decomposition of native substances,

while simultaneously eliminating the use of toxic organic solvents. For these reasons, SFE technologies are now firmly positioned within the family of green chemistry methods. Beyond its environmental advantages, SFE is fundamentally a **separation technology**, capable of selectively isolating a broad range of compounds present in natural substrates. Consequently, SFE has been increasingly applied in the food, cosmetic and pharmaceutical industries for the extraction and purification of natural active ingredients at both laboratory and industrial scales. Applications include the isolation of bioactive and herbal preparations, vitamins, essential and fatty oils, phytoncides, waxes, lipids and phytosterols from plant and animal raw materials—making it highly suitable for the processing of secondary products originating from numerous industrial sectors.

Key qualitative features of SFE driving its adoption include: complete absence of solvent residues in the final products; higher extraction efficiency compared to traditional liquid-liquid extraction; the ability to fine-tune the solvent power of supercritical CO₂ over a wide range of pressures and temperatures; compatibility with co-solvents when processing moderately polar or polar compounds (Reverchon *et al.*, 2006; Agostini *et al.*, 2012).

From a scientific standpoint, the most distinctive feature of supercritical fluids is their **programmable dissolving ability**, a universal property absent in traditional solvents and liquefied subcritical gases. This characteristic has stimulated extensive global research on the applied use of controlled solubility and other programmable properties of supercritical fluids in modern separation processes (Sovová, 1994; McHugh *et al.*, 1994). the laboratory-scale CO₂ SFE extractor (1-liter capacity) presented in this study, together with a schematic representation of the extraction workflow (Figure 1).

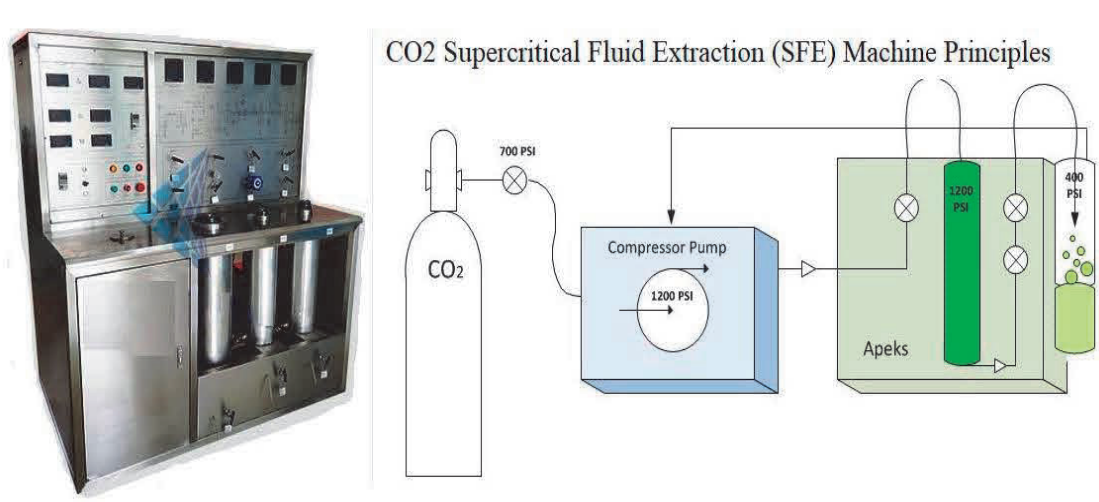


Figure 1. Photo of a laboratory SCF CO₂ extractor machine with a volume of 1 liter of loading source and schematic diagram of the technological process of supercritical fluid extraction.

Methodological Approaches for Selective SFE

A distinctive advantage of supercritical fluid extraction (SFE) is the ability to program the dissolving capacity of the supercritical solvent, enabling selective removal of undesired compounds while isolating target molecules in concentrated fractions (Pourmortazavi *et al.*, 2007; Díaz-Reinoso *et al.*, 2006). Through precise adjustment of pressure and temperature, it is possible to suppress the solubility of heavy, non-volatile constituents and simultaneously enhance the solubilization of desired low-molecular-weight compounds.



Figure 2a. Representatives of modern equipment for separating SCF extractor machines.



Figure 2b. Final products of SCF separation of various components of plant raw materials.

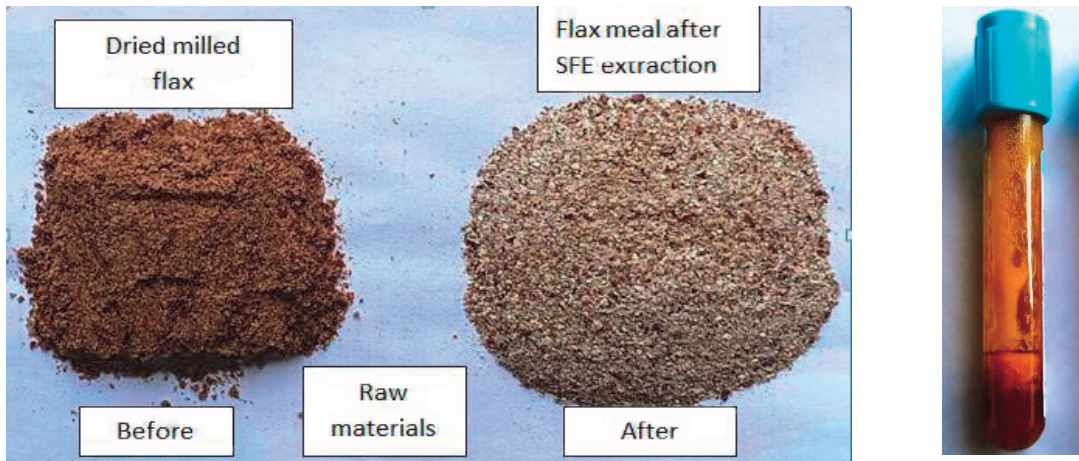


Figure 3. Vegetable raw materials before and after CO₂-SFE extraction and the final product

For example, during the extraction of essential-oil-bearing plants, SFE parameters can be programmed so that volatile terpenoids, responsible for the aromatic and functional profile of the extracts—are selectively dissolved in SCF-CO₂. Meanwhile, high-molecular-weight lipids, waxes, carotenoids and other heavy components remain insoluble and are excluded from the final product. This selective behavior is illustrated by the modern SCF extraction equipment (Figure 2a), which enables precise control of extraction parameters, and by the fractionated plant-derived products obtained through SCF separation (Figure 2b).

An important methodological development in SFE technology is the stepwise or fractionated separation of molecules with different physicochemical characteristics. Using multi-stage separators, substances contained in a single raw material can be separated into multiple distinct fractions during one extraction cycle (Arumugham *et al.*, 2021; Jokić *et al.*, 2016). This strategy greatly enhances the efficiency and economic value of SFE in biorefinery applications.

In addition to solubility programming, the selectivity of SFE can be improved through the use of co-solvents, such as ethanol or diethyl ether. These enhance the extraction of moderately polar or nonionic compounds from hydrophobic matrices. In applications requiring highly clean analytical samples—such as chromatographic-mass spectrometric analysis of pharmaceutical creams—non-polar supercritical solvents (e.g., low-molecular-weight fluoroalkanes) may replace CO₂ to minimize co-extraction of fatty impurities (Taylor, 1996; Clifford, 1998).

Moreover, selective extraction can be further advanced by incorporating chemical modifying agents into the supercritical phase. A well-documented example is the extraction of dicarboxylic acids, which cannot dissolve in SCF-CO₂ due to their high polarity and strong hydrogen bonding. However, when tertiary alkylamines are introduced during the SFE process, the solubility of these acids increases significantly, allowing their selective isolation in pure form after removal of the modifier (Chiu *et al.*, 2002; Wrona *et al.*, 2017; Capuzzo *et al.*, 2013).

Together, these approaches, pressure-temperature programming, co-solvent integration and chemical modification—form a versatile methodological framework that enables precise, selective and clean extraction of a wide spectrum of bioactive compounds from plant and animal biomass.

RESULTS AND DISCUSSIONS

Supercritical CO₂ extraction was applied in the laboratory using a 1-L SFE installation to evaluate the technology's capacity to process diverse natural secondary materials.

The results obtained confirm that SFE-CO₂ provides efficient and selective extraction of valuable bioactive compounds from a wide spectrum of plant- and animal-derived residues. The extracts obtained under the applied operating conditions were clean, free of solvent residues, and characterized by a broad biochemical profile corresponding to the composition of the original raw materials.

A variety of secondary products were processed, including amaranth, *Ginkgo biloba* leaves, dried maggots (fly larvae), plankton/krill, grape seeds and grape pomace, cannabis seeds, flax, corn and other grains.

The chromatogram of the *Ginkgo biloba* extract (Figure 4) illustrates the presence of multiple bioactive components and demonstrates the ability of CO₂-SFE to recover thermolabile compounds without degradation.

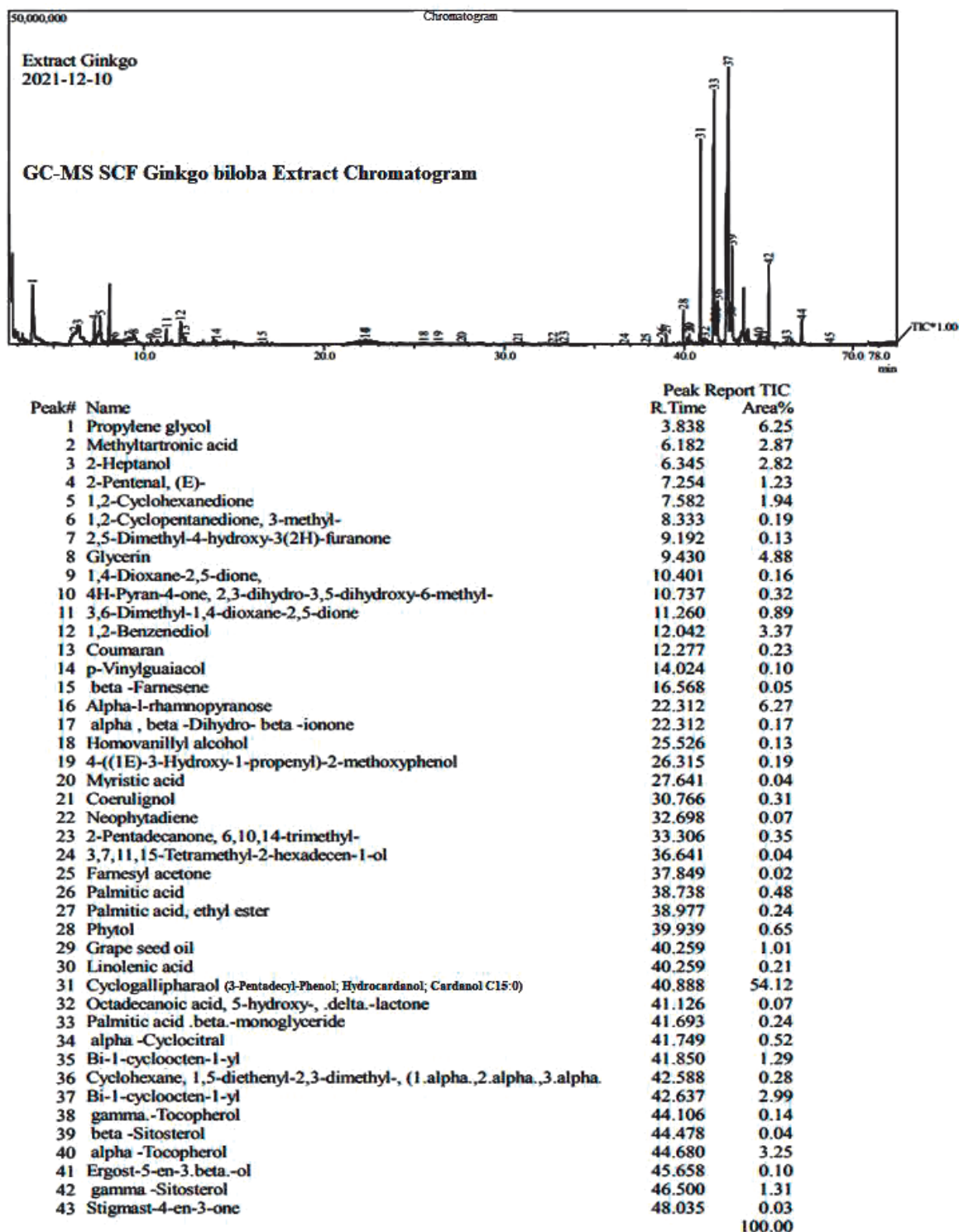


Figure 4. Ginkgo biloba leaves SCF CO₂-extract Chromatogram

Extraction conducted at 35 MPa and 50 °C for 2 hours yielded >15% fatty-wax fraction from the grape pomace of 'Cabernet Sauvignon', 'Saperavi', 'Fetească albă' and 'Fetească neagră' cultivars. The resulting extracts contained a wide range of biologically active compounds, including fatty acids, fatty alcohols and esters, vitamins, phytosterols and other lipidic constituents. Representative chromatograms of grape-derived extracts confirming the extraction selectivity and chemical diversity of these fractions (Figures 5a and 5b).

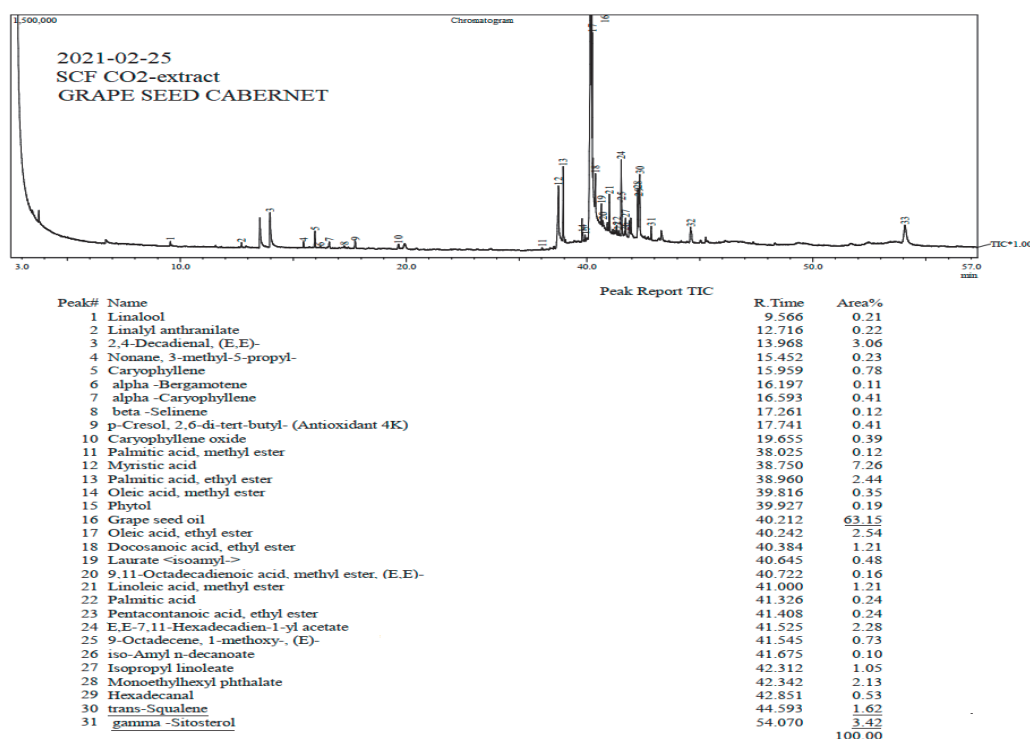


Figure 5a. SCF CO₂ extract chromatogram of Cabernet grape seeds

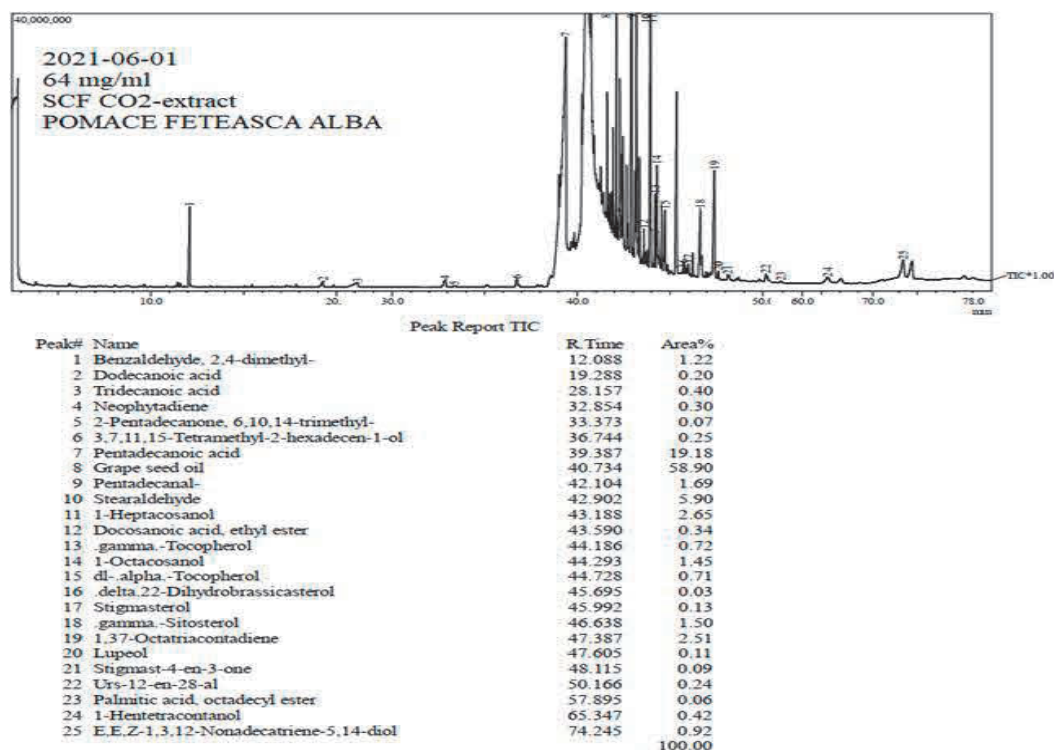


Figure 5b. SCF CO₂ extract chromatogram of 'Fetească albă' grape seeds

Across all processed materials, SFE-CO₂ produced fractions rich in bioactive molecules, primarily concentrated in the fatty-wax portion of the extracts.

Compared to conventional pressing, SFE-CO₂ generated significantly higher yields and a broader spectrum of extractable substances. The difference is illustrated by comparing the extract composition obtained by classical pressing of dried maggots with that achieved through CO₂-SFE, clearly demonstrating the superior extraction efficiency and selectivity of the SFE method (Figure 6).

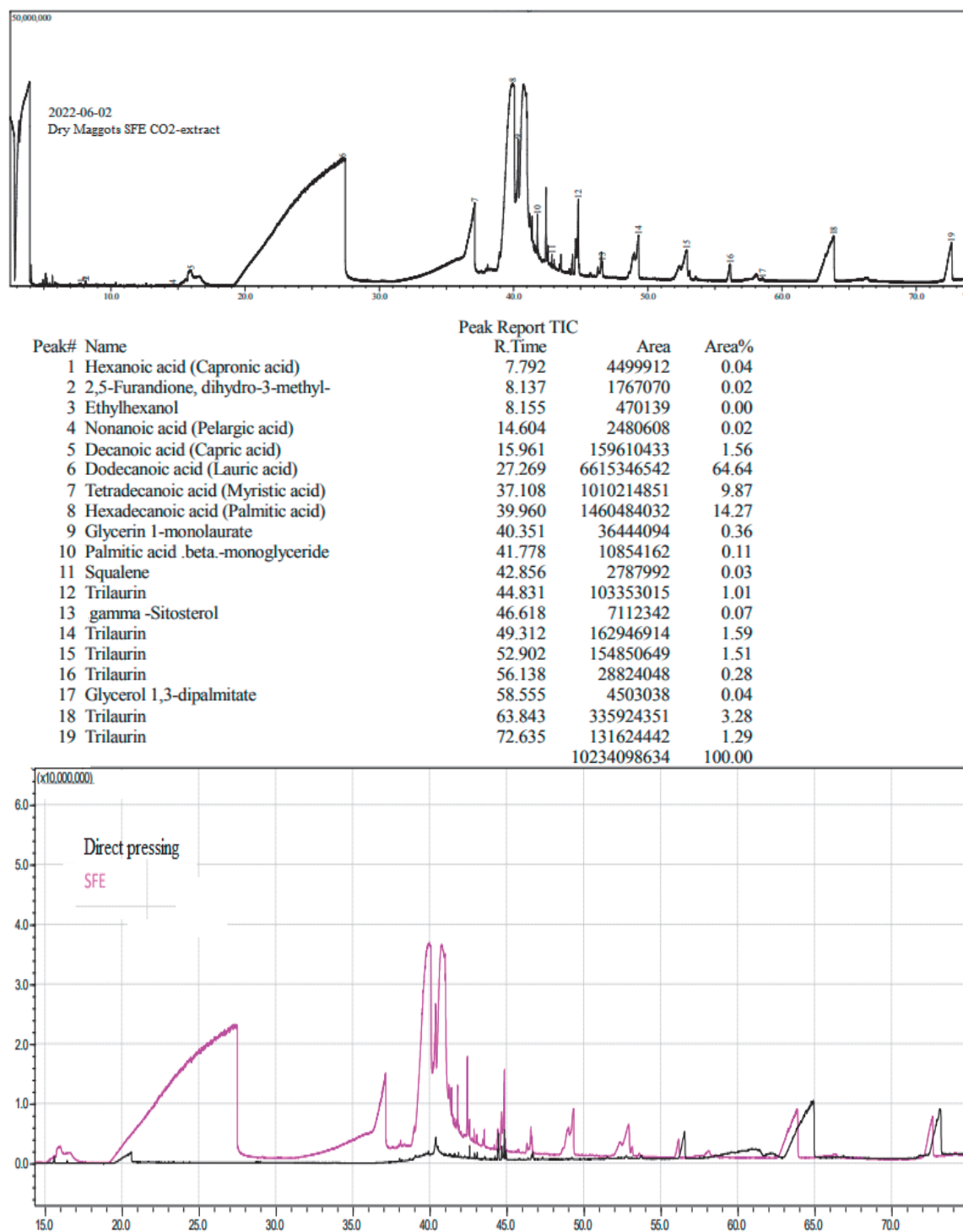


Figure 6. Comparison of extract yield and composition obtained from dried maggots by classical pressing and SFE-CO₂.

To summarize the practical applications demonstrated in this study, provides an overview of the raw materials processed, extract types, representative compounds identified (figures 4–6), extraction outcomes, and application fields of interest (Table 1).

Table 1. Practical applications of SFE-CO₂ on various secondary raw materials

Raw material	Type of extract obtained	Key compounds identified	Extraction outcome mentioned in text	Application potential	Reference
<i>Ginkgo biloba</i> leaves	CO ₂ -SFE extract	Terpenoids, bioactive plant metabolites	Chromatogram shown in Figure 4	Nutraceuticals, phytotherapeutics	Figure 4
Grape pomace ('Cabernet', 'Saperavi', 'Fetească')	Fatty-wax fraction	Fatty acids, fatty alcohols, esters, vitamins, phytosterols	>15% yield at 35 MPa, 50°C, 2 h; Figures 5a–5b	Cosmetics, supplements, food formulations	Figures 5a–5b
Grape seeds	Lipid-based fraction	Fatty acids, vitamin E, sterols	Wide biochemical profile	Cosmetic oils, nutraceuticals	Figures 5a–5b
Dried maggots (fly larvae)	Lipid extract	Fatty acids, nutrients	SFE yield much higher vs. pressing (Figure 6)	Pet nutrition, protein-lipid concentrates	Figure 6
Plankton / krill	Marine lipid fraction	Omega fatty acids, wax esters	Obtained by SFE-CO ₂ in laboratory tests	Marine nutraceuticals	Author's data
Amaranth, cannabis, flax, corn seeds	Seed oil fractions	Fatty acids, sterols, vitamins	Extracts obtained successfully by SFE-CO ₂	Functional food, cosmetic oils	Author's data

Technological considerations and practical outcomes

The practical applications presented in this study demonstrate that supercritical CO₂ extraction (SFE-CO₂) is not only a selective and clean technology, but also one that provides significant technological advantages over conventional extraction methods. The ability to adjust pressure and temperature enables highly controlled extraction of bioactive compounds from a wide variety of secondary raw materials, including *Ginkgo biloba* leaves, grape pomace, grape seeds, dried insect biomass (maggots), plankton/krill and numerous seed materials.

One of the primary technological benefits of SFE-CO₂ is its high extraction efficiency, illustrated by the >15% yield of fatty-wax fractions obtained from grape pomace under optimized laboratory conditions (35 MPa, 50 °C, 2 hours). These extracts contain a wide spectrum of biologically active compounds, including fatty acids, fatty alcohols, their esters, vitamins and phytosterols. The chromatographic profiles shown in Figures 4, 5a and 5b confirm the selectivity of the process and the chemical diversity of the extracted fractions.

Another important technological outcome is the superior quality of extracts, which are free of solvent residues and require minimal or no post-processing. Compared to classical pressing, SFE-CO₂ produced both a higher yield and a broader range of extractable substances, as illustrated in Figure 6 for dried maggots. This highlights the technological advantage of SFE-CO₂ for processing lipid-rich insect biomass and other unconventional protein-fat sources.

The method also enables comprehensive valorization of raw materials, as the programmable solubility of supercritical CO₂ allows recovery of multiple fractions from a single substrate

through stepwise separation. This contributes to waste minimization and increases the economic value of secondary products, aligning with modern biorefinery principles.

In practical terms, the extracts obtained by SFE-CO₂ in this study exhibit potential applications in the nutraceutical, food, cosmetic and pharmaceutical sectors. The ability to process thermolabile materials without degradation, combined with the absence of residual solvents, positions SFE-CO₂ as a technologically sound and environmentally compatible extraction method.

Overall, the laboratory results confirm that SFE-CO₂ is a robust, efficient and scalable technology capable of transforming diverse secondary materials into high-value bioactive products suitable for multiple industrial uses.

CONCLUSIONS

The methods of fine programming and customization of selective supercritical CO₂ extraction described in this work represent only a portion of the strategies currently implemented in the rapidly developing field of SFE technology. Owing to its highly tunable physicochemical properties, SFE-CO₂ offers virtually unlimited potential for the efficient isolation of valuable components from both plant and animal raw materials, as well as from secondary products and accumulated biowaste generated across various industrial sectors.

The expanding commercial applicability of SFE-CO₂ confirms its technological relevance. It is increasingly employed in the production of dietary supplements, nutraceuticals, specialized cosmetic ingredients, pharmaceutical preparations, flavoring substances and food-grade extracts, including highly purified individual compounds. The technology places no inherent limitations on the type of raw material that can be processed, and current industrial applications already include residues from fruit and vegetable processing, berry seeds, and other agro-industrial by-products.

Overall, CO₂-based supercritical extraction has emerged as a modern, environmentally compatible and highly efficient technology. Its selectivity, solvent-free processing, and capacity to valorize secondary materials position SFE-CO₂ as a key enabling technology for sustainable biorefinery models and for the development of high-value bioactive products.

ACKNOWLEDGEMENTS

We are grateful to the Food technology and microbiology Laboratory of IP NIARAVM for conducting research on various quality indicators of samples.

REFERENCES

1. Agostini, F., Bertussi, R.A., Agostini, G., Atti dos Santos, A.C., Rossato, M., Vanderlinde, R. (2012) Supercritical extraction from vinification residues: fatty acids, α -tocopherol, and phenolic compounds in the oil seeds from different varieties of grape. *Scientific World Journal*, <https://doi.org/10.1100/2012/790486>
2. Aizpurua-Olaizola, O., Ormazabal, M., Vallejo, A., Olivares, M., Navarro, P., Etxebarria, N., Usobiaga, A. (2015) Optimization of supercritical fluid consecutive extractions of fatty acids and polyphenols from *Vitis vinifera* grape wastes. *Journal of Food Science*, 80 (1), pp. E101–E107. doi:10.1111/1750-3841.12715
3. Arumugham, Th., Rambabu, K., Hasan, Sh. W., Show, P. L. (2021) Supercritical carbon dioxide extraction of plant phytochemicals for biological and environmental applications – A review. *Chemosphere*, 271, 129525. <https://doi.org/10.1016/j.chemosphere.2020.129525>
4. Capuzzo, A., Maffei, M.E., Occhipinti, A. (2013) Supercritical fluid extraction of plant flavors and fragrances. *Molecules*, 18, pp. 7194–7238. <https://doi.org/10.3390/molecules18067194>
5. Chiu, K.-L., Cheng, Y.-C., Chen, J.-H., Chang, C. J., Yang, P.-W. (2002) Supercritical fluids extraction of *Ginkgo* ginkgolides and flavonoids. *The Journal of Supercritical Fluids*, 24 (1), pp. 77–87. [https://doi.org/10.1016/S0896-8446\(02\)00014-1](https://doi.org/10.1016/S0896-8446(02)00014-1)
6. Clifford, T. (1999) *Fundamentals of supercritical fluids*. Oxford: Oxford Science Publications. ISBN 978-0-19-850137-4.

7. Díaz-Reinoso, B., Moure, A., Domínguez, H., Parajó, J. C. (2006) Supercritical CO₂ extraction and purification of compounds with antioxidant activity. *Journal of Agricultural and Food Chemistry*, 54 (7), pp. 2441–2469. <https://doi.org/10.1021/jf052858j>
8. Duba, K.S., Fiori, L. (2015) Supercritical CO₂ extraction of grape seed oil: effect of process parameters on the extraction kinetics. *The journal of supercritical fluids*, 98, pp. 33-43. <https://doi.org/10.1016/j.supflu.2014.12.021>
9. Fiori, L., Lavelli V., Duba K. S. *et al.* (2014) Supercritical CO₂ extraction of oil from seeds of six grape cultivars: modeling of mass transfer kinetics and evaluation of lipid profiles and tocol contents. *The journal of supercritical fluids*, 94, pp. 71-80. <https://doi.org/10.1016/j.supflu.2014.06.021>
10. Jokić, S., Bijuk, M., Aladić, K., Bilić, M., Molnar, M. (2016) Optimisation of supercritical CO₂ extraction of grape seed oil using response surface methodology. *International Journal of Food Science and Technology*, 51, p. 403. <https://doi.org/10.1111/ijfs.12986>
11. McHugh, M.A., Krukonis, V.J. (1994) *Supercritical fluid extraction – principles and practice*. Butterworth-Heinemann Series In Chemical Engineering (2nd Edition). ISBN 0-7506-9244-8
12. Pourmortazavi, S.M., Hajimirsadeghi, S.S. (2007) Supercritical fluid extraction in plant essential and volatile oil analysis. *Journal of Chromatography A*, 1163 (1-2), pp. 2-24. <https://doi.org/10.1016/j.chroma.2007.06.021>
13. Reverchon, E., De Marco, I. (2006) Supercritical fluid extraction and fractionation of natural matter. *The Journal of Supercritical Fluids*, 38 (2), pp. 146-166. <https://doi.org/10.1016/j.supflu.2006.03.020>
14. Sovová, H. (1994) Rate of the vegetable oil extraction with supercritical CO₂—I. Modelling of extraction curves. *Chemical Engineering Science*, 49 (3), pp. 409-414. [https://doi.org/10.1016/0009-2509\(94\)87012-8](https://doi.org/10.1016/0009-2509(94)87012-8)
15. Taylor, L. T. (1996) *Supercritical fluid extraction*. John Wiley and Sons, Inc. ISBN 978-0-471-11990-6. <https://www.wiley.com/en-us/Supercritical+Fluid+Extraction-p-9780471119906#aboutauthors-section>
16. Turner Ch., Eskilsson C. S., Björklund E. (2002) Collection in analytical-scale supercritical fluid extraction. *Journal of chromatography A*, 947 (1), pp. 1-22. [https://doi.org/10.1016/s0021-9673\(01\)01592-8](https://doi.org/10.1016/s0021-9673(01)01592-8)
17. Wrona, O., Rafińska, K., Możejński, C., Buszewski, B. (2017) Supercritical fluid extraction of bioactive compounds from plant materials. *Journal of AOAC International*, 100 (6). <https://doi.org/10.5740/jaoacint.17-0232>



Magazine financially supported by
the Romanian Society of Horticulturist

