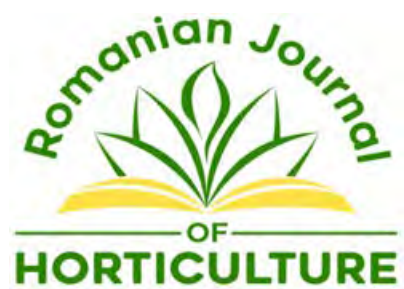




Vol. 5
2024

Romanian Journal of Horticulture





**ROMANIAN JOURNAL
OF HORTICULTURE**

Vol. 5



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



Romania Journal of Horticulture

Vol. 5

Editura PIM
- 2024 -

Publisher

Academy of Agricultural and Forestry Sciences – “Gheorghe Ionescu-Şișești”

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-Argeş - NRDIBH; member of the Academy of Agricultural and Forestry Sciences

"Gheorghe Ionescu-Şișești"

(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, Čacak, 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-Argeş

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

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)

Ioan ZAGRAI - Research & Development Station for Fruit Growing, Bistrita, Romania

ISSN-L 2734 – 7656

Print ISSN 2734 – 7656

On line ISSN 2734 – 8083

CD-ROM ISSN 2734 – 7664

CONTENT

I. BIOTECHNOLOGY	7
1 EFFECTS OF NUTRIENT SOLUTION CONCENTRATION DURING THE NURSERY PERIOD ON THE GROWTH OF STEVIA CUTTINGS IN HYDROPONICS	9
<i>S. Mizushima</i>	
2. EVALUATION OF VIRUS-FREE POTATO MINITUBERS DERIVED FROM “IN VITRO” MERISTEM CULTURES FOR HIGH-QUALITY SEED POTATO PRODUCTION	15
<i>D M. Cioloca, A. Tican and M. Popa</i>	
3. CABBAGE BREEDING TOOLS FOR BIOTIC AND ABIOTIC RESISTANCE – REVIEW	23
<i>N.K. Prajapati*, Anjali, R.C. Meena, Razauddin, P. Kumar, K. Chand, P. Kumar and S. Kumar</i>	
II. VEGETABLE, AROMATIC AND MEDICINAL PLANTS	33
4. THE IMPACT OF FOLIAR TREATMENTS ON YIELD AND QUALITY OF ROUND PEPPER (CAPSICUM ANNUUM L.) AT ASTEROID 204 CV.	35
<i>D. C. Constantin, M. Paraschiv and G. Sbîrciog</i>	
5. INTEGRATED NUTRIENT MANAGEMENT FOR SUSTAINABLE CULTIVATION OF OKRA (ABELMOSCHUS ESCULENTUS L.) IN NORTHERN GANGES RIVER FLOODPLAIN SOILS OF BANGLADESH	43
<i>M.H. Sohel, T.T. Tithi, M.F. Ali, M. Abdullah-Al-Mamun and M.M. Rahman</i>	
6. IMPROVEMENT OF THE URBAN MICROCLIMATE WITH AROMATIC AND MEDICINAL PLANTS	51
<i>K. I. Makne, N. Thymakis, J. N. Tzortzi and A. C. Asănică</i>	
III. POTATO GROWING	59
7. TUBER YIELD COMPONENTS OF POTATO (SOLANUM TUBEROSUM L.) AS INFLUENCED BY EARTHING UP AND INFLORESCENCE REMOVAL	61
<i>D. Mosneh Wudu, M. Workineh Kassa and B. Negash Demissie</i>	
IV. FRUIT GROWING	69
8. BEHAVIOUR OF SOME BULGARIAN PLUM CULTIVARS IN THE PEDO-CLIMATIC CONDITIONS OF THE PITESTI – ROMANIA AREA	71
<i>M. Butac, M. Nesheva, M. Chivu, A. Stan, E. Mareși and S. Nicolae</i>	
9. RESULTS OF SOME APPLE HYBRID POPULATIONS TO THE ATTACK OF SCAB AND POWDERY MILDEW UNDER NATURAL CONDITIONS	77
<i>G. M. Guzu, S.D. Roșu-Mareș, C. Moldovan, I. Zagrai, L.A. Zagrai and M.I. Cordea</i>	
10. THE EFFICACY OF SOME ECOLOGICAL INSECTICIDES AGAINST THE DEFOLIATING LARVAE OF LEPIDOPTERA SPP. ON EUROPEAN PLUM UNDER CONTROLLED CONDITIONS	85
<i>C. Moldovan, S.D. Roșu-Mare, G.M. Guzu, I. Zagrai, L.A. Zagrai, Z. Jakab-Ilyefalvi and A.M. Chiorean</i>	
11. SPREAD AND DEVELOPMENT OF VERTICILLUM WILT OR COTTON WILT IN THE WESTERN PART OF AZERBAIJAN	93
<i>L. Huseynova</i>	
12. EFFECTIVENESS OF ACTIVE INGREDIENTS IN CONTROLLING FIREBLIGHT (ERWINIA AMYLOVORA) IN MODERN APPLE ORCHARDS	99
<i>F.C. Marin, M. Călinescu, M. Sumedrea and E. Chițu</i>	

V. VITICULTURE AND OENOLOGY	107
13. THE INFLUENCE OF CLIMATIC FACTORS ON THE QUALITY OF GRAPES IN THE VINEYARDS MURFATLAR AND IASI	109
<i>V. Artem, A. Ranca, C. Ciobanu, I. Dina, A. Nechita and G. Zaldea</i>	
14. THE <i>IN VITRO</i> EVALUATION OF THE ANTIFUNGAL POTENTIAL OF NATURAL <i>CAPSICUM ANNUUM</i> L. EXTRACT AND SYNTHETIC COMPOUNDS AGAINST THE PATHOGENS <i>ALTERNARIA SPP.</i> AND <i>FUSARIUM OXYSPORUM</i>	117
<i>A. Din, I.D. Sărdărescu, D.E. Vizitiu, D.I. Sumedrea, D. Vilcoci, G. Cirstea and C. Mihăescu</i>	
15. THE EVOLUTION OF THE DEGREE OF ATTACK BY GREY ROT (<i>BOTRYTIS CINEREA</i>) ON FOUR WINE GRAPE CULTIVARS UNDER CONVENTIONAL AND ORGANIC CONTROL TREATMENTS, IN THE MURFATLAR VITICULTURAL CENTER	125
<i>I. Dina, A. Ranca, A.S. Ene and A. Tanase</i>	
16. ISSUES WHICH PERTAIN TO THE VINE PLANTS INTRINSIC FEATURES AS WELL AS TO THE PROGRESSIVE AND FURTHER CIVILIZING TOUCH HELD BY ITS CULTURE AS IT HAS BEEN CURRENTLY PRACTICED UPON THE ACTUAL TERRITORY OF ROMANIA	133
<i>A. Popa and F. Tuțulescu</i>	
17. CLIMATE CHANGE A NECESSITY IN THE PROCESS OF IMPROVEMENT AND CULTIVATION OF THE GRAPEVINE	149
<i>E. Alexandrov</i>	
18. THE EFFECT OF NITROGEN ON THE GROWTH AND DEVELOPMENT OF GRAFTED CUTTINGS FROM THE NURSERY	157
<i>D.Dinu, D.I. Sumedrea, A. Florea and C. Ciobotea</i>	
VI. FLORICULTURE, ORNAMENTAL PLANTS, LANDSCAPE ARCHITECTURE	165
19. THE EFFECT OF CONTROLLED RELEASE FERTILIZER ON GROWTH, FLOWERING, AND LEAF MORPHOLOGY OF <i>PULSATILLA</i> SPECIES	167
<i>H.M. Kim, M.S. Roh, G. Burchi and Y.H. Joung</i>	
20. STUDIES ON THE BEHAVIOR OF SOME CULTIVARS OF <i>DAHLIA</i> IN THE BOTANICAL GARDEN CLUJ-NAPOCA	175
<i>M. Cantor, A. Pui, I. Moldovan, A.J. Kozel, Zs. Szekely-Varga and L. Draghia</i>	
21. PASTURE WITH SCATTERED TREES	185
<i>R. M. Tăslăvan, Zs. Szekely-Varga, I. Lihăt and E. Kentelky</i>	
22. THE EFFECT OF ALTERNATING TEMPERATURE TREATMENT ON SEED GERMINATION OF <i>PULSATILLA TONGKANGENSIS</i>	193
<i>H.M. Kim, M. S. Roh, G. Burchi and Y.H. Joung</i>	
23. INTEGRATING ROMANIAN TRADITIONAL MOTIFS WITH SUSTAINABLE SUCCULENT LANDSCAPES	197
<i>A.P. Cotoz, V.S. Dan and M. Cantor</i>	



Biotechnology

Effects of nutrient solution concentration during the nursery period on the growth of *Stevia* cuttings in hydroponics

S. Mizushima*

Department of Agriculture, Fukui prefectural Wakasa-higashi High School,
Japan

*Corresponding author email: uwabamisou.mizu@gmail.com

ABSTRACT

The effects of nutrient solution concentration on the growth of stevia cuttings during the nursery period in hydroponics were investigated. Cuttings were inserted in continuously aerated water, nutrient solution of 0.3 dS m⁻¹, and nutrient solution of 0.7 dS m⁻¹. Rooting of cuttings began on the 10 days after cutting. The rooting rate of cuttings under water and EC 0.3 dS m⁻¹ reached 100% on day 13. The nutrient solution concentration during the nursery period did not affect rooting rate. At transplanting, best stem length and number of leaves were observed 0.7 dS m⁻¹. The maximum root length was not affected nutrient solution concentration. At harvest, the shoot dry weight of 0.7 dS m⁻¹ was significantly heavier than that of water. Survival rates after transplanting of 0.3 dS m⁻¹ and 0.7 dS m⁻¹ were lower than that of water. Despite some issues with the survival rate, the use of a nutrient solution during the nursery period in hydroponics of stevia could lead to more efficient production.

Keywords: electric conductivity, medicinal plant, rooting, survival rate, vegetative propagation

INTRODUCTION

Stevia (*Stevia rebaudiana* Bertoni) is a perennial plant of the Asteraceae family. It has been introduced in Japan, Brazil, Korea, Mexico, USA, Indonesia, Tanzania, and Canada (Megeji *et al.*, 2005). The leaves of this plant are mainly used as a sweetener, because it is with compounds that 200-300 times sweeter than sugar (Hossain *et al.*, 2017). Sweetness of stevia comes from steviol glycosides. The four major steviol glycosides in stevia are stevioside, rebaudioside A, rebaudioside C, and dulcside A (Singh and Rao, 2005). *Stevia* plants included wide ranges of genetic variation in content and in composition of the glycosides (Nakamura and Tamura, 1985). Hata and Fujita (1996) bred tetraploid plants of stevia. Hata *et al.* (2001) bred a triploid plant of stevia with high rebaudioside A content. Morita Kagaku Kogyo Co., Ltd. selected and bred stevia that produces a higher ratio of rebaudioside A (Ohta *et al.*, 2010). In this way, the breeding of stevia is gradually being reported. However, transplants of stevia are propagated often using wild stevia as a propagation source. The main propagation methods of stevia are seeds, cutting (Miyazaki and Watanabe, 1974), and tissue culture (Miyagawa *et al.*, 1984). Since stevia is allogamous plant, various phenotypes appear in seed propagation (Nakamura and Tamura, 1985). Vegetative propagation such as cutting propagation and tissue culture can produce transplants of the same phenotype. Especially, cutting propagation are a feasible technique in the field. Effects of cutting position (Abdullateef and Osman, 2012; Kassahun

and Mekonnen, 2012; Kassahun *et al.*, 2013; Rakibuzzaman *et al.*, 2018), node number of cuttings (Kassahun *et al.*, 2013), plant growth regulator (Abdullateef and Osman, 2012; Kassahun and Mekonnen, 2012; Rakibuzzaman *et al.*, 2018; Manohar *et al.*, 2022), and growing media (Miyazaki and Watanabe, 1974; Ogao-Ogao *et al.*, 2017; Manohar *et al.*, 2022) on rooting of cuttings were investigated in stevia. Thus, cutting propagation are a major propagation method in stevia. Stevia was cultivated in soil, but in recent years it has also been cultivated in hydroponics. Yoneda (2021) reported on the cultivation of stevia in hydroponics at a closed-type plant factory. In cutting propagation, cuttings are inserted in the media such as vermiculite, sand, and rock wool. However, when cultivating by hydroponics, the media in the root zone of the transplants needs to be removed. In hydroponics of tomato, cuttings were inserted in the nutrient solution when raising transplants (Namiki *et al.*, 1977). Mizushima (2016) reported that nutrient solution concentration to insert cuttings during the nursery period by hydroponics affected the growth of cuttings in *Elatostema involucratum* Franch. & Sav. With this method, removal of the media in the root zone of the transplants not required. However, in stevia, the effect of nutrient solution concentration by hydroponics is unclear. Therefore, this paper aims to clarify the effects of nutrient solution concentration on stevia cuttings during the nursery period in hydroponics.

MATERIALS AND METHODS

This experiment was conducted at Wakasa-higashi High School during the year 2016. Cuttings were taken from mother plants of stevia maintained at a glasshouse in Wakasa-higashi High School experimental farm. Stem were cut from the tip of the plant including apical bud. Stem length and leaves number of each cutting were prepared in the form of 5 cm and 6 to 8, respectively. Cuttings were inserted in continuously aerated water or nutrient solution (SA-prescription; OAT Agrio Co., Ltd, Tokyo, Japan) with different electrical conductivity (EC) levels on 2, May. The experimental plot was following three; water, EC 0.3 dS m⁻¹, and EC 0.7 dS m⁻¹. The cuttings were grown in containers (length; 37.0 cm, width; 25.5 cm, height; 13.5 cm) containing 5 L of each solution in the growth chamber (Preset temperature; 22°C). One container grew 15 cuttings at a spacing of 5 cm×5 cm (Fig. 1 A). An LED unit (Valore Corp., Kyoto, Japan) with alternating red and white LED chips was used as the light source. Cuttings were grown under a 12 h photoperiod and a total photosynthetic photon flux density of 100 μmol m⁻² s⁻¹ on the tip of the cutting. The pH value of each solution was 6.0 to 7.0 during the nursery period. The following parameters were recorded: number of rooted cuttings until 15 days after inserted in water or nutrient solution and stem length, number of leaves, and maximum root length at the transplanting. The transplants (rooted cutting) were planted in a hydroponic system at a spacing of 16 cm×16 cm in the glasshouse on 30, May (Fig. 1 B and C). The nutrient solution concentration after transplanting was controlled from 1.3 to 1.7 dS m⁻¹ in all experimental plots. Stevia plants were harvested at 39 days after transplanting and recorded shoot dry weight. The shoot dry weight was taken after drying the samples in an electric oven for 72 h at 85°C. The data were analysed using Tukey-Kramer test.

RESULTS AND DISCUSSIONS

The rooted cuttings were not observed in all experimental plots at seven days after cuttings (Fig. 2). The number of rooted cuttings in 10 days after cutting was 11 (rooting rate; 73%) or more in all experimental plots. Miyazaki and Watanabe (1974) reported that rooting of cuttings inserted in Kanuma soil, sand, and sandy soil begins around 10 days

and ends around 2 to 3 weeks after cutting. Ogao-Ogao *et al.* (2017) reported that rooting of cuttings in soil culture began between 8 and 12 days after cutting..

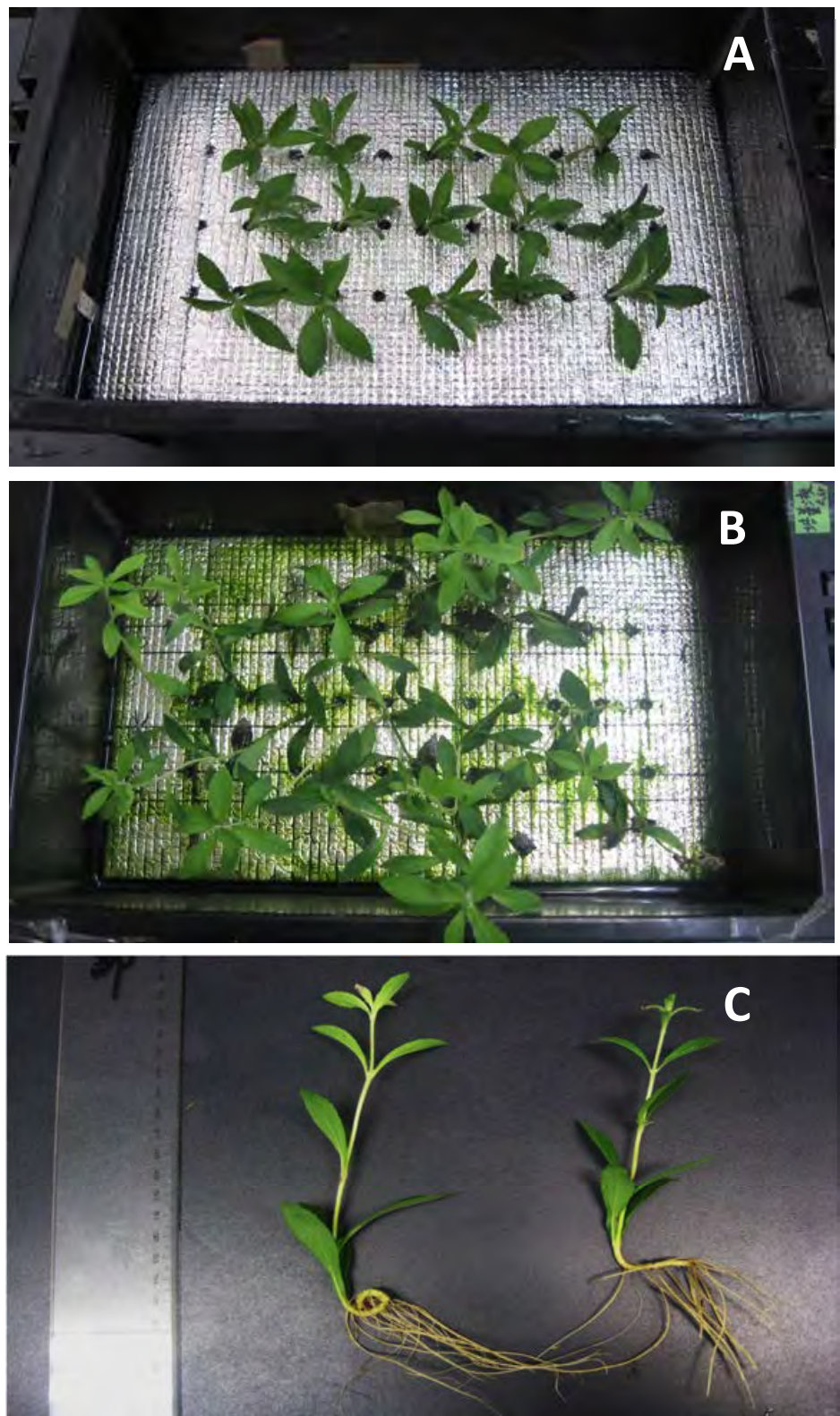


Figure 1. Stevia at cutting (A), at transplanting (B), and transplanted rooted stevia cuttings (C).

Similarly, rooting of cuttings in this study began 10 days after cutting. In 13 days after cutting, a number of rooted cuttings was 14 (rooting rate; 93%) or more in all experimental plots. The rooting rate of cuttings under water and EC 0.3 dS m⁻¹ reached 100% on day 13 after cutting. The nutrient solution concentration during the nursery period did not affect rooting initiation and rooting rate. In soil culture, the rooting rate of stevia cutting under good conditions was close to 100% (Miyazaki and Watanabe, 1974; Ogao-Ogao *et al.*, 2017).

Since the rooting rate in this study was 93% or more, it was considered that cuttings rooted normally even by inserted into water or nutrient solution. At transplanting, nutrient solution concentration during the nursery period affected stem length and number of leaves (Table 1). The best stem length and number of leaves were observed 0.7 dS m⁻¹.

The maximum root length was not affected by nutrient solution concentration. The effect of nutrient solution concentration during the nursery period appeared on the shoot growth. Mizushima (2016) reported the effect of the nutrient solution concentration during the nursery period in *Elatostema involucratum* Franch. & Sav. appeared on the shoot rather than on the roots. This phenomenon was observed in this study too.

The nutrient solution concentration during the nursery period significantly affects shoot dry weight at harvest. The shoot dry weight of 0.7 dS m⁻¹ was significantly higher than that of water. Survival rates after transplanting tended to be lower with 0.3 dS m⁻¹ and 0.7 dS m⁻¹ compared to water.

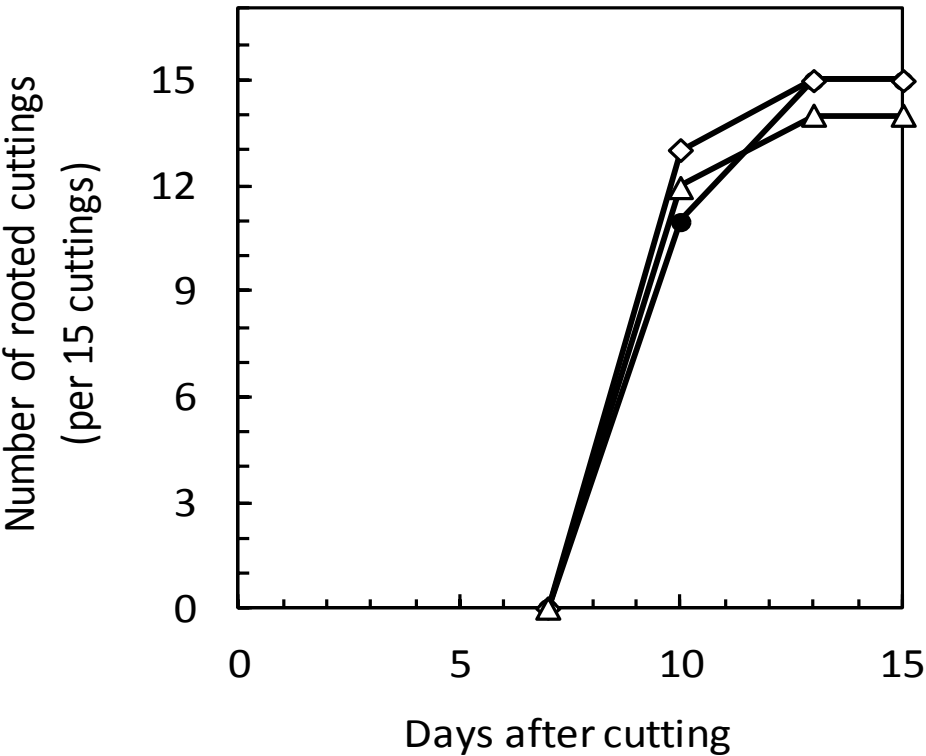


Figure 2. Changes in a number of rooted cuttings of stevia after cutting. Black circle, white rhombus, and white triangle in the figure indicate water, 0.3 dS m⁻¹, and 0.7 dS m⁻¹, respectively.

Table 1. Effects of nutrient solution concentration during the nursery period on the growth at transplanting and at harvest.

Experimental plot	At transplanting			At harvest	
	Stem length (cm)	Number of leaves	Maximum root length (cm)	Shoot dry weight (g plant ⁻¹)	Survival rate (%)
Water	12.7 b ^z	13.2 b	9.4 a	1.43 b	100
0.3 dS m ⁻¹	14.4 ab	15.6 ab	11.3 a	1.69 ab	86
0.7 dS m ⁻¹	16.3 a	16.6 a	9.1 a	2.22 a	71

^z Means with different letters indicate a significant difference at the 5% level by the Tukey-Kramer test in each column.

CONCLUSIONS

In this study, despite some issues with the survival rate, the use of a nutrient solution during the nursery period in hydroponic stevia could lead to more efficient production.

REFERENCES

1. Abdullateef R.A. and Osman M. (2012). Effects of stem cutting types, position and hormonal factors on rooting in *Stevia rebaudiana* Bertoni. J. Agric. Sci. 4: 49-57.

2. Hata S. and Fujita S. (1996). Artificially induced tetraploid plants in stevia (*Stevia rebaudiana* Bertoni) with high rebaudioside A content. Res. Bull.: Published by Dept. Hort., Keisen Junior College. 28:21-27.

3. Hata S., Yomo T., and Fujita S. (2001). Breeding of triploid plants of stevia (*Stevia rebaudiana* Bertoni) with high rebaudioside A content. Japan. J. Trop. Agr., 45: 281-289.

4. Hossain M.F., Islam M.T., Islam M.A., and Akhtar S. (2017). Cultivation and uses of stevia (*Stevia rebaudiana* Bertoni): A review. Afr. J. Food Agric. Nutr. Dev. 17: 12745-12757.

5. Kassahun B.M. and Mekonnen S.A. (2012). Effect of cutting position and rooting hormone on propagation ability of stevia (*Stevia rebaudiana* Bertoni). Afr. J. Plant Sci. Biotechnol. 6: 5-8.

6. Kassahun B.M., Nurhusain H., Gebremeskel H., Mekonnen S.A., Damtew Z., Philipos, M., Melka B., and Niide T. (2013). Participatory development of quality seedlings for stevia (*Stevia rebaudiana* Bertoni) using stem cuttings. World J. Agric. Sci. 9: 148-154.

7. Manohar K.A., Shukla G., Roy, B., and Chakravarty S. (2022). Effects of plant growth regulators and growing media on propagation and field establishment of *Stevia rebaudiana*: a medicinal plant of commerce. CABI Agric. Biosci. 3: 4.

8. Megeji N.W., Kuma, J.K., Singh V., Kaul, V.K., and Ahuja P.S. (2005). Introducing *Stevia rebaudiana*, a natural zero-calorie sweetener. Curr. Sci. 88: 801-804.

9. Miyagawa H., Fujita, Y., Fujioka N., Kohda H., and Yamasaki K. (1984). Studies on the tissue culture of *Stevia rebaudiana* Bertoni and its components (I). Shoyakugaku Zasshi. 38: 12-18.

10. Miyazaki Y. and Watanabe H. (1974). Studies on the cultivation of *Stevia rebaudiana* Bertoni: I. On the propagation of the plant. Japan. J. Trop. Agr. 17: 154-157.

11. Mizushima S. (2016). Effects of nutrient solution concentration on rooting and growth of cuttings in *Elatostema involucratum* Franch. & Sav. cultured by hydroponics. Hort. Res. (Japan). 15: 371–376.

12. Nakamura S. and Tamura Y. (1985). Variation in the main glycosides of *Stevia (Stevia rebaudiana bertoni)*. Japan. J. Trop. Agr. 29: 109-115.

13. Namiki T., Oda M., Kudo,Y., and Takashima S. 1977. Studies on production of vegetable crops in water culture XVI: Use of cuttings from young seedlings of tomatoes. Sci. rep. Kyoto Pref. Univ. Agric. 29: 17-22.

14. Ogao-Ogao R.J.A., Nital P.S., and Claveria F.G. (2017). Vegetative propagation of stevia (*Stevia rebaudiana* Bertoni Hemsl) using stem tip cuttings in different growing media. Philipp. J. Sci. 146: 437-443.

15. Ohta M., Sasa, S., Inoue A., Tamai T., Fujita I., Morita K., and Matsuura F. (2010). Characterization of novel steviol glycosides from leaves of *Stevia rebaudiana* Morita. J. Appl. Glycosci. 57: 199-209.
16. Rakibuzzaman M., Shimasaki K., and Jamal Uddin A.F.M. (2018). Influence of cutting position and rooting hormones on rooting of Stevia (*Stevia rebaudiana*) stem cutting. Int. J. Bus. Soc. Sci. Res. 6: 122-125.
17. Singh S.D. and Rao G.P. (2005). Stevia: The herbal sugar of 21 st Century. Sugar Tech. 7: 17-24.
18. Yoneda Y. (2021). Optimization process of plant growth environment for improving content compounds using physiological and genetic information in a closed-type plant factory. JARQ. 55: 201-208.

Evaluation of virus-free potato minitubers derived from “*in vitro*” meristem cultures for high-quality seed potato production

M. Cioloca*, A. Tican and M. Popa

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

*Corresponding author email: mihaela.cioloca@potato.ro

ABSTRACT

At present, the base for seed potato production is the potato minitubers produced in “protected” insect-proof spaces. For seed potato production it is essential to use a healthy and high-quality biological material, which contributes to obtaining superior production, both quantitatively and qualitatively. In potatoes, to eradicate viruses and obtain a healthy initial material, we have at our disposal the technique of sampling and “*in vitro*” cultivation of meristems. In 2024, 11 potato cultivars grown in greenhouses were evaluated. The mini-tubers are derived from “*in vitro*” cultivated plants. Before micropropagation, the plantlets have been subjected to viral testing using the ELISA technique. Potato microplants used for “*in vitro*” multiplication were obtained from meristem cultures. Regarding the average number of minitubers/pot, the best results were obtained in 'Castrum' (11.50), 'Ervant' (10.80) and 'Sevastia' (10.10) cultivars. The best results in terms of the average weight of minitubers/plant were obtained in 'Braşovia' (271.62 g), 'Darilena' (270.13 g) and 'Ervant' (258.87 g) cultivars. The 'Braşovia' and 'Darilena' cultivars also recorded the highest values of the average weight of minitubers (53.2 g and 50.92 g, respectively). The highest percentage of minitubers >30 mm (60%) was found for 'Braşovia' and 'Darilena' cultivars. Also, for 'Asinaria', 'Cosiana', 'Ervant', 'Foresta', 'Marvis' and 'Sarmis' cultivars, the highest rate was recorded for minitubers in the size fraction 25-30 mm and >30 mm. By selecting appropriate cultivars and managing them according to their specific needs, potato growers can enhance the efficiency of seed production systems and achieve higher yields in field multiplication stages.

Keywords: potato, meristem cultures, micropropagation, minitubers, seed potato production

INTRODUCTION

High-quality seed is a crucial investment in both low-input and high-input farming systems. The availability of a sufficient supply of healthy tubers is an essential requirement for effective potato production. The quality of seed potato tubers is the most significant factor influencing yield (Bus and Wustman, 2007). Vegetative propagation of potatoes leads to the transmission of viruses from one generation to the next, with virus levels increasing due to repeated propagation (Thomas-Sharma *et al.*, 2016; Priegnitz *et al.*, 2020). Viral diseases not only increase susceptibility to other pathogens but also lead to economic losses by negatively affecting tuber yield and quality (Lin *et al.*, 2014; Adolf *et al.*, 2020). Obtaining virus-free plants is necessary for successful viral disease management and sustainable propagation activities, including potato germplasm conservation and global exchange of genetic resources (Naik and Khurana, 2003; Volmer *et al.*, 2017; Ellis *et al.*, 2020). Because

potatoes are vegetative propagated, viral infections worsen with each successive generation, making the production of healthy material crucial (Faccioli, 2001). Preventive measures can help limit the spread of potato viruses. In seed potato production, a key step is obtaining high-quality phytosanitary initial material. Using superior planting material is fundamental not only for achieving high yields and quality but also for supporting a sustainable production system. While it does not ensure a successful outcome, it provides the crop with a strong start, as the negative impact of using poor-quality initial material cannot be corrected during the growing season (Morrenhof, 1998).

To eliminate viruses and secure healthy initial material in potatoes, we can utilize the technique of sampling and "*in vitro*" cultivation of meristems. Meristems are tissues composed of young cells that retain their ability to proliferate throughout the plant's life. The meristems used for initiating an "*in vitro*" culture are found at the plant's growth tip, in the apical buds, within the leaf axils, in the axillary buds, or the dormant buds of the tubers. Meristem culture serves as the foundation for obtaining healthy material and is the first step in starting "*in vitro*" cultures. This technique enables the intensive propagation of selected individuals with a higher multiplication potential than traditional methods while preserving the genetic uniformity of the biological material. In meristem culture methods, the size of the explant significantly influences the effectiveness of virus eradication. Typically, excising 0.2 mm shoot tips that include the apical dome along with one or two leaf primordia is necessary (Wang *et al.*, 2006; Zhang *et al.*, 2019). To regenerate the meristematic explant, the apical tip must include at least 1–2 leaf primordia, which are essential for the production of auxins and cytokinins (Bhojwani and Dantu, 2013). The excision of such small shoot tips is a labor-intensive, time-consuming process that requires a high level of skill. Furthermore, the outcomes can vary significantly regarding shoot growth and the effectiveness of virus eradication (Bettoni *et al.*, 2016; Magyar-Tábori *et al.*, 2021). A notable limitation of these meristem culture-based techniques is the inability to ensure complete removal of viral particles, particularly in cases of mixed infections (Faccioli and Marani, 1998; Zhang *et al.*, 2019). The successful elimination of potato viruses relies on both the type of virus targeted for eradication and the size of the meristematic explant being inoculated, which is the primary factor influencing the ability to produce healthy plants. Generally, the success of disease eradication, particularly for viruses, is inversely related to the size of the meristem. Smaller explants tend to have a higher rate of virus elimination, but their regenerative capacity significantly diminishes, resulting in a lower chance of survival for the explant. Typically, the regenerative potential of these meristems is very high. They adapt well to "*in vitro*" conditions, readily recover from the trauma of excision, and resume their activity after being transferred to aseptic media with appropriate nutrient composition and optimal cultivation conditions, ultimately generating plants. This process involves both the growth of the bud axis and its primordia, leading to leaf development and the formation of roots. Ultimately, the terminal, apical, or axial meristem produces plantlets, depending on the hormonal balance present in the culture medium (Cachiță, 1984).

Due to their high vigor and exceptional health status, "*in vitro*" derived plantlets are essential for producing top-quality, true-to-type, disease-free seed material (Pruski, 2007). One of the most common methods for producing pre-basic seed is cultivating minitubers in greenhouses from "*in vitro*" plantlets obtained from nodal cuttings. These nodal cuttings can be aseptically produced in large quantities by "*in vitro*" laboratories that specialize in rapid multiplication. Minitubers are typically defined as the progeny tubers produced from "*in vitro*" derived plantlets. The term refers to their size, which is smaller than conventional seed tubers but larger than "*in vitro*" tubers (microtubers) produced in aseptic conditions on artificial media. Minituber production has become an integral part of the global seed production system, serving as a link between the rapid "*in vitro*" multiplication using nodal cuttings and the field multiplication of seed tubers (Struik,

2007). The health standard of minitubers is high because they are generated under controlled conditions from aseptically produced “*in vitro*” plantlets. Although minitubers are small, they contain a significant number of eyes, allowing for the potential to produce many sprouts per individual tuber if treated appropriately. However, the vigor of these sprouts will be limited by the resources available from the mother tuber.

MATERIALS AND METHODS

The potato cultivars used in this study were created at the National Institute of Research and Development for Potato and Sugar Beet, Brasov, Romania. The potato minitubers are produced in a protected “*insect-proof*” space belonging to the Plant Tissue Culture Research Laboratory. Potato microplants regenerated from viable meristematic tissues were subjected to viral testing using the ELISA technique. Healthy clones of each cultivar are then used in the micropropagation process to obtain planting material. The potato plantlets were transplanted from “*in vitro*” conditions to “*ex vitro*” between May 14-16, 2024. At the beginning of April, the substrate necessary for the production of minitubers was prepared, which consists of a mixture of red peat with bentonite, black peat and perlite (4:2:1). In order for the culture substrate to be richer in nutrients, and complex fertilizer NPK 15:15:15 + 6% sulfur was applied before planting, in the form of granules, after which the pots with the mixture were watered daily (2 hours/day), to facilitate the dissolution of complex granules. During the growing season, preventive treatments against potato late blight (*Phytophthora infestans*) and treatments with foliar fertilizers were applied (Table 1).

Table 1. Application schedule for disease control treatments and foliar fertilization during the growing season

Date of treatment application	Product applied	Dose
May 27	Cropmax	20 ml/10 l water
	Razormin	20 ml/10 l water
	Agroleaf Power	30 g/10 l water
June 4th	Cropmax	20 ml/10 l water
	Razormin	20 ml/10 l water
	Agroleaf Power	30 g/10 l water
June 10	Cropmax Zetanil	20 ml/10 l water 0.45 kg/ha
June 21	Cropmax Cimbal	20 ml/10 l water 0.25 kg/ha
July 5th	Carial	0.6 l/ha
	Cropmax	20 ml/10 l water
	Agroleaf Power	30 g/10 l water
July 19	Banjo	0.3 l/ha
	Cropmax	20 ml/10 l water
	Agroleaf Power	30 g/10 l water
August 2	Zetanil	0.45 kg/ha
August 14	Infinito	1.4 l/ha
August 30	Shirlan	0.3 l/ha

The pots used for transplanting are made of plastic with a diameter of 17 cm, a height of 12.9 cm and a volume of 2 l (manufacturer Desch Plantpak BV, Netherlands). During the vegetation period (Figure 1), to ensure the nutrients necessary for the growth and development of potato plants, foliar fertilizers treatments were applied. Their activity is based on the combination of microelements, amino acids, vitamins and polysaccharides.

The application of these products ensures a rapid development of the root system and activates the development of the leaf mass, helping the plants to realize their biological potential.



Figure 1. Potato plants obtained from "*in vitro*" cultures, in vegetation

Starting from August 30, the plants were no longer watered, and after 2 weeks after the interruption of watering, the haulms were removed. This activity of removing the potato stems helps to suberization of minitubers skin, and this aspect is important for a proper preservation of the biological material in the warehouse.

The potato minitubers were harvested between October 1-8 (Figure 2). After harvesting, minitubers of the 11 potato cultivars were evaluated in terms of the following parameters: average number of minitubers/pot, the average weight of minitubers/pot, the average weight of minitubers. The calibration of the minitubers by size classes was also carried out.



Figure 2. Aspects from the harvesting of minitubers

RESULTS AND DISCUSSIONS

In this study, 11 potato cultivars were grown in protected areas in order to obtain a high-quality pre-basic seed from "*in vitro*" plantlets. After 19 weeks from planting, the minitubers were harvested and evaluated in terms of number, weight, and size.

The influence of the cultivars on the number of minitubers/plant

The number of "*in vitro*" plantlets typically range from 2 to 5, but this can vary significantly depending on the specific cultivar and how the crop is managed. The number of minitubers per individual "*in vitro*" microplants is an important yield component. The results presented in Figure 3 highlight the fact that the highest number of minitubers/plant was obtained in 'Castrum' (11.50), 'Ervant' (10.80) and 'Sevastia' (10.10) cultivars. This aspect has a major importance on the production capacity of the cultivars. If the potato plants produce more minitubers, the productivity of cultivars is higher.

The lowest values, in terms of the average number of minitubers/plant, were obtained in the 'Cosiana' (4.7), 'Braşovia' (5.3) and 'Darilena' (5.6) cultivars.

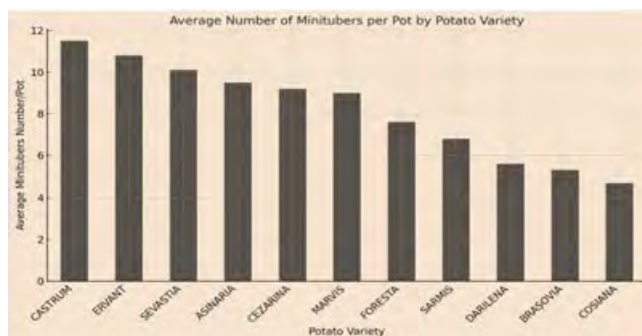


Figure 3. Effect of potato cultivars on the average number of minitubers/plant

The influence of the cultivar on the average weight of minitubers/plant

The Figure 4 shows the evolution of average weight of minituber depending on the analyzed potato cultivars.

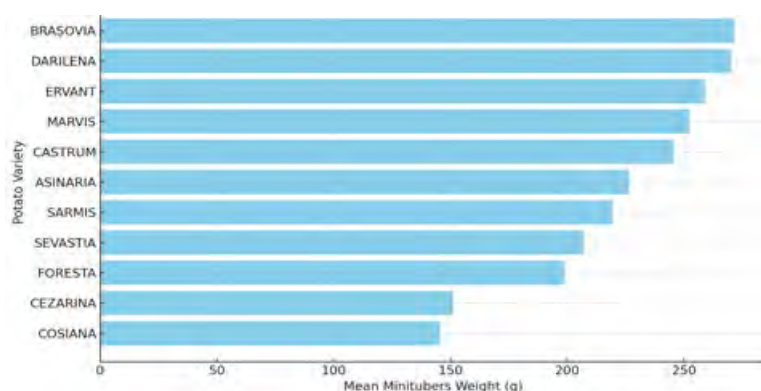


Figure 4. Comparison of mean minituber weight/plant across potato cultivars

The best results in terms of the average weight of minitubers/plant were obtained in Brașovia (271.62 g), Darilena (270.13 g) and Ervant (258.87 g).

The small number of minituberc recorded in the 'Brașovia' and 'Darilena' cultivars. (Figure 3) attracted their greater weight (Figure 4). It should be noted that the number of minitubers (Figure 3) did not influence their weight (Figure 4) for the 'Ervant' cv. This cultivar recorded a number of minitubers of 10.80 (Figure 3) and an average weight of 258.87 g (Figure 4).

Minitubes weighing less than 60 g, depending on the cultivars

The cultivar plays a critical role in determining the average weight of minitubers, which directly impacts the overall production efficiency. Different cultivars have unique genetic traits that influence tuber size, growth patterns, and yield potential. These genetic characteristics are essential for understanding how a particular cultivar will perform under specific conditions and how it contributes to the final output.

Figure 5 shows the results regarding the average weight of minitubers for the 11 evaluated potato cultivars. For this analyzed parameter, the 'Brașovia' and 'Darilena' cvs. stood out with an average weight of the minitubers of 53.20 g and 50.92 g, respectively. The two cultivars obtained the highest values also in the case of the average weight of minitubers/plant.

The lowest values were obtained for the 'Cezarina' (20.14 g) and 'Sevastia' (20.84 g) cultivars.

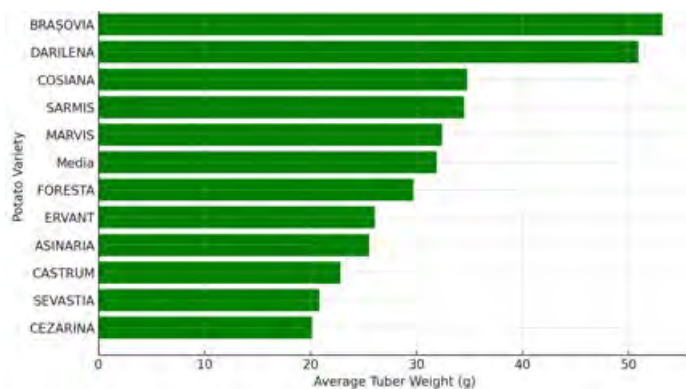
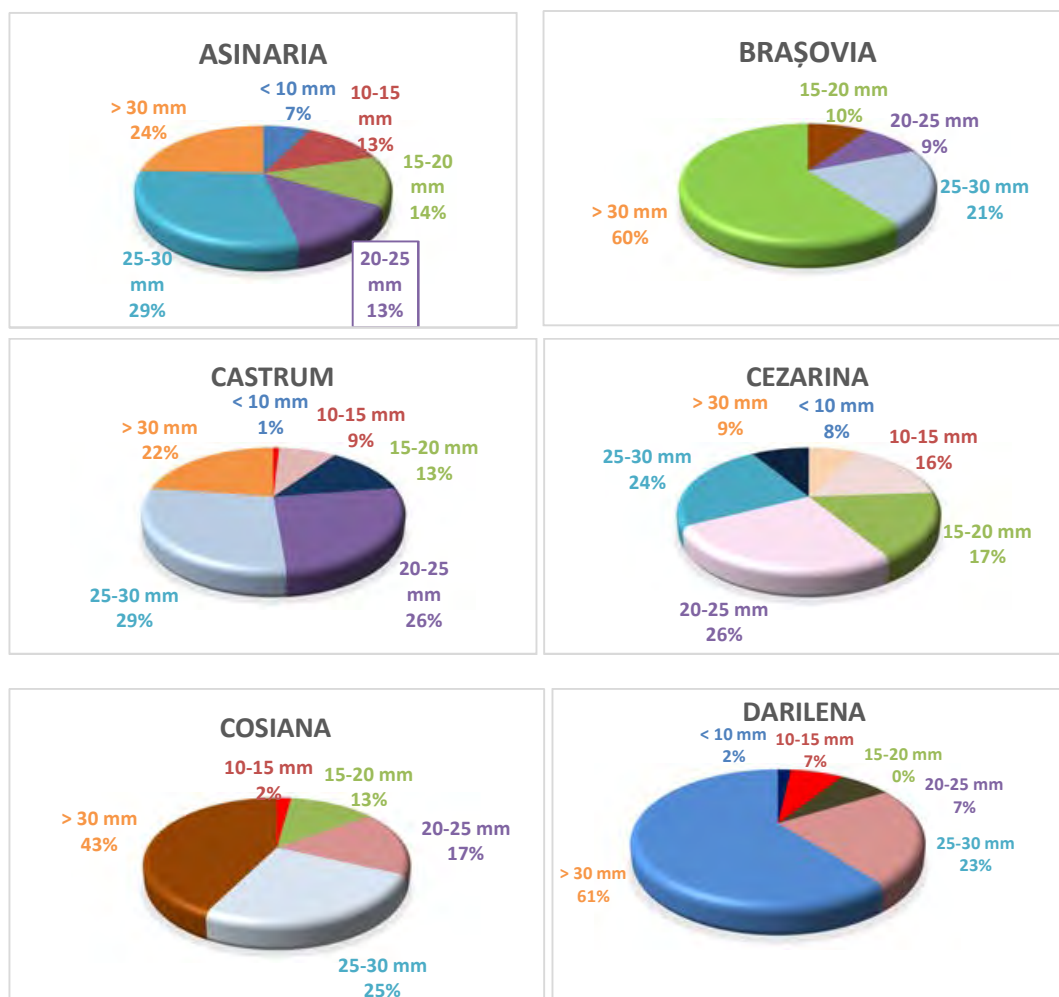


Figure 5. Average minituber weight of potato cultivars

Minitubers calibration by size classes

The size of the minituber plays a crucial role, as it influences the dormancy period, the vigor of the seed tuber, the number of stems that can be successfully generated, the speed of emergence, the survival rate of plants and stems, and the overall yield potential. Although minitubers are small, they still contain a significant number of eyes and, with proper treatment, can generate numerous sprouts from each tuber.

Figure 6 shows the percentage distribution of mini tubers according to the calibration classes and the percentages.



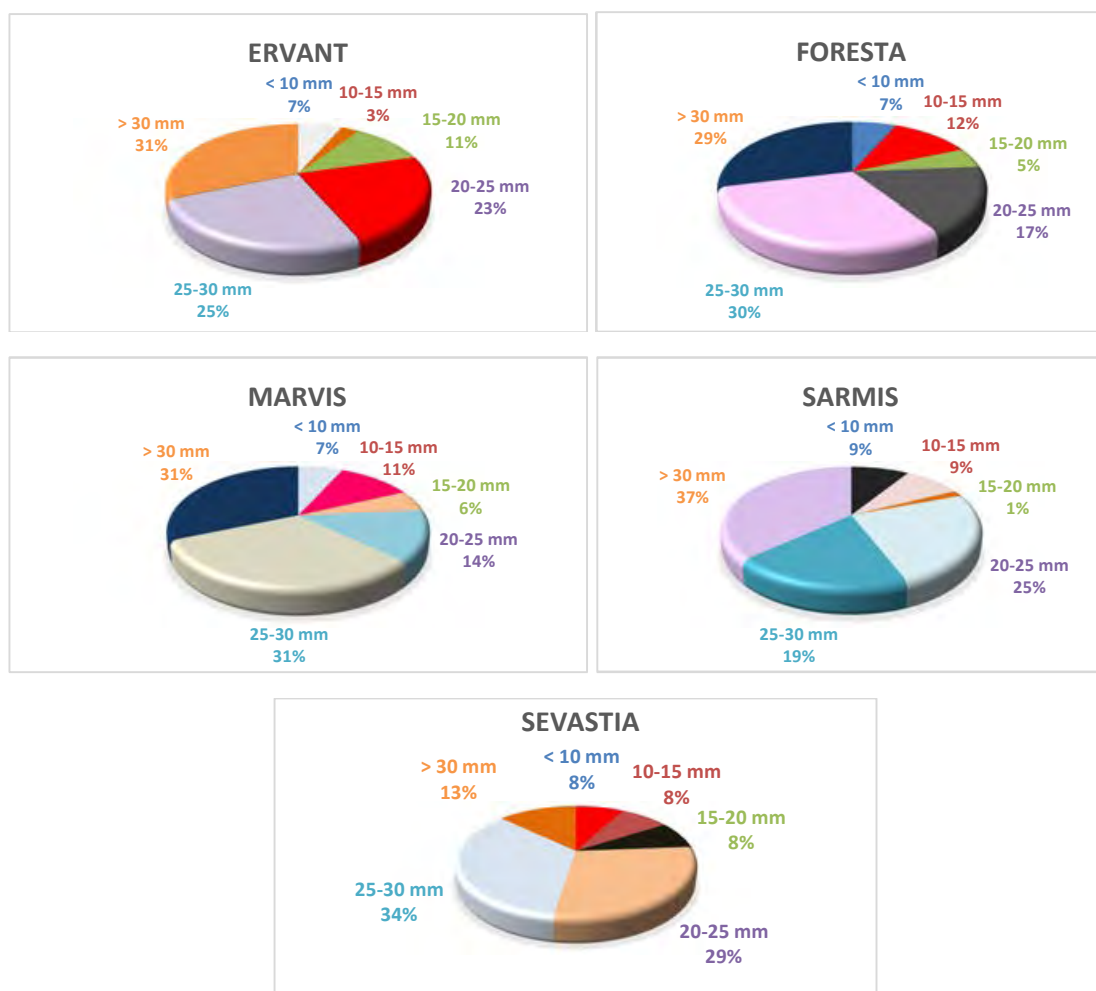


Figure 6. Percentage distribution of minitubers on calibration classes according to cultivar

Among the 11 potato cultivars evaluated, in 8 of them ('Asinaria', 'Braşovia', 'Cosiana', 'Darilena', 'Ervant', 'Foresta', 'Marvis' and 'Sarmis'), following the percentage distribution of minitubers by calibration classes, it can be seen that the minitubers were mainly in the size fraction 25-30 mm and >30 mm. For 'Braşovia' and 'Darilena' cvs., the percentage of >30 mm minitubers was 60% and 61%, respectively.

For 'Castrum', 'Cezarina', and 'Sevastia' cvs., the harvested minitubers mainly fell into the 20-25 mm and 25-30 mm size classes (24-34%).

CONCLUSIONS

This study highlights the diverse potential of potato cultivars in minituber production. Generally, larger minitubers contain more stored nutrients, which promotes early growth, quicker emergence, and overall healthier plants. This increased vigor contributes to stronger stem and root development, enabling better nutrient and water uptake, and potentially boosting yields. Conversely, cultivars that naturally produce smaller minitubers can still perform well but may require more careful management to optimize their growth.

The use of "*in vitro*" multiplication technologies ensures the production of biologically healthy material from genotypes suited to sustainable agriculture, yielding clonal material that is from a higher phytosanitary category. By selecting the appropriate cultivars and

tailoring their management to specific needs, potato growers can enhance seed production system efficiency and improve yields during field multiplication stages.

ACKNOWLEDGEMENTS

This research work is part of the 2023-2026 ADER Project 4.1.1. "Research on the identification of effective methods of *"in vitro"* conservation to ensure the maintenance of the biodiversity of potato, sweet potato and medicinal plant germplasm" which is supported by the Ministry of Agriculture and Rural Development.

REFERENCES

1. Adolf B., Andrade-Piedra J., Molina F.R., Przetakiewicz J., Hausladen H., Kromann P., Lees A., Lindqvist-Kreuzw H., Perez W. and Secor A.G. (2020). Fungal, oomycete, plasmodiophorid diseases of potato, in The Potato Crop. eds. H. Campos and O. Ortiz (Cham: Springer), 307–350.
2. Bettoni J.C., Costa M.D., Gardin J.P.P., Kretschmar A.A. and Pathirana R. (2016). Cryotherapy: a new technique to obtain grapevine plants free of viruses. Rev. Bras. Frutic. 38:e-833. doi: 10.1590/0100-29452016833.
3. Bhojwani S.S. and Dantu P.K. (2013). Production of virus-free plants. In Plant Tissue Culture: An Introductory Text; Bhojwani, S.S., Dantu, P.K., Eds.; Springer: India, New Delhi, 2013; pp. 227–243.
4. Bus C.B. and Wustman R. (2007). The Canon of Potato Science: Seed Tubers. Potato Research, Volume 50, Numbers 3/4, p. 319-322; ISSN: 0014-3065.
5. Cachiță D. (1984). Culturi de celule și țesuturi vegetale, aplicații în agricultură, Capitolul I: Tehnica cultivării "in vitro" a celulelor și țesuturilor vegetale, Editura Ceres, București.
6. Ellis D., Sala, S A., Chavez O., Gomez R. and Anglin N. (2020). Ex situ conservation of potato [Solanum section Petota (Solanaceae)] genetic resources in genebanks," in The Potato Crop. eds. H. Campos and O. Ortiz (Cham: Springer), 109–138.
7. Faccioli G. and Marani F. (1998). Virus elimination by meristem tip culture and tip micrografting, in Plant Virus Diseases Control. eds. A. Hadidi, A. R. K. Khetarpal and H. Koganezawa (St. Paul, MN: APS Press), 346–380.
8. Lin Y.H., Johnson D.A. and Pappu H.R. (2014). Effect of potato virus S infection on late blight resistance in potato. Am. J. Potato Res. 91, 642–648. doi: 10.1007/s12230-014-9394-8. 13.
9. Magyar-Tábori K., Mandler-Drienyovszki, N., Hanász A., Zsombik L. și Dobránszki J. (2021). Phytotoxicity and other adverse effects on the "in vitro" shoot cultures caused by virus elimination treatments: reasons and solutions. Plan. Theory 10:670. doi: 10.3390/plants10040670.
10. Morrenhof J. (1998). The Road to Seed Potato Production (broșură). Netherlands Potato Consultative Institute (NIVAA), Haga, Olanda.
11. Nai k P.S and Khurana SP. (2003). Micropropagation in potato seed production: need to revise seed certification standards. J. Indian Potato Assoc. 30, 267–273.
12. Priegnitz U., Lommen W.J.M., van der Vlugt R.A.A. and Struik, P.C. (2020). Potato yield and yield components as affected by positive selection during several generations of seed multiplication in southwestern Uganda. Potato Res. 63, 507–543. doi: 10.1007/s11540-020-09455-z. 15.
13. Pruski K. (2007). The Cannon of Potato Science: "in vitro" multiplication through Nodal Cuttings. Potato Research, Volume 50, Numbers 3/4, p. 293-296; ISSN: 0014-3065.
14. Struik P.C. (2007). The Cannon of Potato Science: Minitubers. Potato Research, Volume 50, Numbers 3/4, p. 305-308; ISSN: 0014-3065.
15. Thomas-Sharma S., Abdurahman, A., Ali S., Andrade-Piedra J.L., Bao S., Charkowski A. O., Crook D., Kadian M., Kromann P., Struik P.C., Torrance L., Garrett K.A. and Forbes G.A. (2016). Seed degeneration in potato: the need for an integrated seed health strategy to mitigate the problem in developing countries. Plant Pathol. 65, 3–16. doi: 10.1111/ppa.12439.
16. Volmer R., Villagaray R., Cárdenas J., Castro M., Chávez, O., Anglin, N. and Ellis D. (2017). A large-scale viability assessment of the potato cryobanks at the international potato center (CIP). In vitro cell. Dev. Biol. Plant 53, 309–317. doi: 10.1007/s11627-017-9846-1.
17. Wang Q., Liu Y., Xie Y. and You M. (2006). Cryotherapy of potato shoot tips for efficient elimination of potato leafroll virus (PLRV) and potato virus Y (PVY). Potato Res. 49, 119–129. doi: 10.1007/s11540-006-9011-4.
18. Zhang Z., Wang Q. C., Spetz C. și Blystay D.R. (2019). "in vitro" therapies for virus elimination of potato-valuable germplasm in Norway. Sci. Hortic. 249, 7–14. doi: 10.1016/j.scienta.2019.01.027.

Cabbage breeding tools for biotic and abiotic resistance

N.K. Prajapati*, Anjali, R.C. Meena, Razauddin, P. Kumar,
K. Chand, P. Kumar and S. Kumar

Department of Horticulture, School of Agricultural Sciences and Technology,
Babasaheb Bhimrao Ambedkar University (A Central University), India

*Corresponding author email: nkp.ofcl@gmail.com

ABSTRACT

Cabbage (*Brassica oleracea* var. *capitata*) is an important vegetable crop grown globally for its nutritional value and economic importance. However, cabbage production faces significant challenges from various biotic and abiotic stresses, including pests, diseases, and environmental factors such as drought, heat, and salinity. Developing cabbage cultivars with improved resistance to these stresses is crucial for sustainable and productive agriculture. This review article examines the latest breeding tools and approaches used to enhance biotic and abiotic stress resistance in cabbage. It explores traditional breeding methods, marker-assisted selection, genetic engineering, genome editing techniques like CRISPR/Cas9, and emerging technologies such as genomic selection and speed breeding. Furthermore, the article discusses the integration of -omics approaches, including genomics, transcriptomics, proteomics, and metabolomics, to accelerate the development of stress-resistant cabbage cultivars. The study also highlights the importance of incorporating farmer preferences and participatory breeding strategies to ensure the adoption and success of these improved cabbage cultivars.

Keywords: crop improvement, cole crops, breeding tools, stress tolerance, breeding approaches.

INTRODUCTION

Cabbage (*Brassica oleracea* var. *capitata*) is a cool-season vegetable crop belonging to the Brassicaceae family. It is widely cultivated and consumed around the world for its nutritional value, as it is a rich source of vitamins, minerals, and fiber (Sarıkamış *et. Al*, 2009). Cabbage production plays a significant role in the global economy, with an annual production of over 70 million tons (<http://www.fao.org/faostat/en/#data/QC>). However, cabbage cultivation faces various biotic and abiotic stresses that can significantly reduce yield and quality. Biotic stresses, such as insect pests, fungal diseases, bacterial diseases, and viral diseases, pose major threats to cabbage production (Juroszek, P., and Tsai, 2009). Some of the most common insect pests affecting cabbage include cabbage loopers, cabbage root flies, cabbage aphids, and diamondback moths (Shelton, A. M., and Badenes-Perez, 2006). Fungal diseases like clubroot, black rot, and downy mildew can cause severe damage to cabbage crops (Rimmer *et. al.*, 2007). Bacterial diseases, such as black rot and bacterial soft rot, and viral diseases like cauliflower mosaic virus and turnip mosaic virus, also contribute to yield losses (. Bhat, K. A., and Kolanjakkaren, 2014; Shukla and Tenzer, 2017). Abiotic stresses, including drought, heat, salinity, and nutrient deficiencies, can also

have detrimental effects on cabbage growth, development, and productivity (Farooq *et al.*, 2014). Climate change is exacerbating these abiotic stresses, making it increasingly challenging for farmers to maintain stable cabbage yields (Fahad *et al.*, 2017). Developing cabbage cultivars with improved resistance to biotic and abiotic stresses is crucial for sustainable and productive agriculture.

TRADITIONAL BREEDING METHODS

Traditional breeding methods have been extensively used in cabbage improvement programs for decades. These methods involve selecting and crossing parent lines with desirable traits, followed by multiple generations of selfing and selection to develop improved cultivars (Bradshaw, 2016).

Pedigree breeding: Pedigree breeding is a commonly used method in cabbage breeding programs. It involves selecting superior individuals from segregating populations and self-pollinating them for several generations to develop pure lines. These pure lines can then be crossed to combine desirable traits, such as pest resistance or improved yield (Prakas *et al.*, 2014).

Recurrent selection: Recurrent selection is another traditional breeding approach used in cabbage breeding. It involves cyclically selecting and intermating superior individuals from a population to gradually increase the frequency of favourable alleles for target traits, such as disease resistance or stress tolerance (. Shukla and Sundaram, 2004).

Hybridization and heterosis breeding: Hybridization involves crossing two genetically diverse parent lines to produce F₁ hybrids that exhibit heterosis or hybrid vigour. Heterosis breeding in cabbage has been successful in developing high-yielding and stress-tolerant hybrids by exploiting the phenomenon of hybrid vigour (Shukla, S., Naik, A. K., and Sundaram, 2014).

Mutation breeding: Mutation breeding involves inducing genetic variations through physical or chemical mutagens, such as gamma rays, X-rays, or ethyl methane sulfonate (EMS). These mutations can lead to new trait variations, including enhanced biotic or abiotic stress resistance, which can be selected and incorporated into breeding programs (Ahloowalia *et al.*, 2004).

While traditional breeding methods have contributed significantly to cabbage improvement, they have limitations, such as long breeding cycles, the polygenic nature of many traits, and the difficulty in pyramiding multiple resistance genes (Tester, and Langridge, 2010). To overcome these challenges, modern breeding approaches, including marker-assisted selection, genetic engineering, and genome editing, have been developed and integrated into cabbage breeding programs.

MARKER-ASSISTED SELECTION (MAS)

Marker-assisted selection (MAS) is a breeding approach that utilizes molecular markers linked to specific traits or genes of interest to facilitate the selection of desirable genotypes (Collard, and Mackill, 2008), MAS has been widely applied in cabbage breeding for various traits, including disease resistance, insect resistance, and abiotic stress tolerance.

Resistance gene mapping and MAS: Molecular markers have been developed and used for marker-assisted selection of resistance genes against various cabbage diseases, such as black rot, fusarium wilt, and clubroot (Sharma, *et al.*, 2017; Ren *et al.*, 2001; Chandra *et al.*, 2021). For example, the Crr1 gene conferring resistance to clubroot disease has been

mapped and validated, enabling the development of molecular markers for MAS in cabbage breeding programs (Hirai, 2006).

QTL mapping and MAS for complex traits: Quantitative trait loci (QTL) mapping has been employed to identify genomic regions associated with complex traits, such as yield, quality, and abiotic stress tolerance in cabbage. Molecular markers linked to these QTLs have been used for marker-assisted selection to improve these traits (Lee *et. al.*, 2021; Zhang *et. al.*, 2020).

Genomic selection (GS): Genomic selection is an advanced form of MAS that utilizes genome-wide marker data and statistical models to predict the breeding values of individuals for complex traits (Meuwissen *et. al.*, 2001). GS has shown promising results in cabbage breeding for traits like drought tolerance, where it has been used to accelerate the breeding cycle and improve selection accuracy (Bhandari *et. al.*, 2022).

While MAS has facilitated more efficient and precise selection in cabbage breeding, it has limitations, such as the need for extensive marker development and validation, and the potential for linkage drag (Bernardo *et. al.*, 2008). Additionally, MAS may not be effective for traits controlled by many minor-effect QTLs or epistatic interactions.

GENETIC ENGINEERING

Genetic engineering involves the direct transfer and integration of specific genes from various sources into the cabbage genome to introduce desirable traits (Bawa, and Anilakumar, 2013). This approach has been explored in cabbage breeding for enhancing biotic and abiotic stress resistance.

Insect resistance: Genes encoding insecticidal proteins, such as *Bacillus thuringiensis* (Bt) toxins, have been introduced into cabbage to confer resistance against lepidopteran pests like cabbage loopers and diamondback moths (Cao *et. al.*, 2002). These *Bt* cabbage lines have shown improved protection against insect damage and reduced the need for insecticide applications.

Disease resistance: Transgenic approaches have been used to develop cabbage cultivars with resistance against various fungal, bacterial, and viral diseases. For example, the introduction of antifungal proteins, like chitinases and glucanases, has been explored for enhancing resistance against fungal pathogens like clubroot and downy mildew (Muzzarelli, *et. al.*, 2001; Mora, and Earle, 2001).

Abiotic stress tolerance: Genetic engineering has also been employed to improve abiotic stress tolerance in cabbage. Genes involved in stress response pathways, such as those encoding transcription factors, Osmo protectants, or antioxidant enzymes, have been introduced into cabbage to enhance drought, heat, and salinity tolerance (Wang, *et. al.*, 2003; Bajji *et. al.*, 2001).

While genetic engineering has shown promising results in cabbage improvement, concerns over the potential risks associated with genetically modified (GM) crops have led to strict regulations and public acceptance challenges in some regions (Nicolia, *et.al.*, 2014). Additionally, the complexity of many stress tolerance mechanisms and potential unintended effects remains challenges in genetic engineering approaches.

GENOME EDITING

Genome editing technologies, particularly CRISPR/Cas9, have emerged as powerful tools for precise and targeted modifications of the cabbage genome (Boetesi and Fischer, 2015).

These techniques offer advantages over traditional genetic engineering by enabling site-specific modifications without the introduction of foreign DNA.

Disease resistance: CRISPR/Cas9 has been used to knockout or modify susceptibility genes in cabbage to enhance resistance against various diseases. For example, editing the BraA.FR.a gene, which encodes a susceptibility factor for the *Fusarium oxysporum* fungus, has been shown to confer resistance against Fusarium wilt disease in cabbage (Chaturvedi *et. al.*, 2012).

Insect resistance: CRISPR/Cas9 has also been employed to modify endogenous cabbage genes involved in defence pathways or plant-insect interactions to enhance insect resistance. For instance, editing the BrPDF2.1 gene, which encodes a plant defensin protein, has been reported to improve resistance against diamondback moths (Hu *et. al.*, 2019).

Abiotic stress tolerance: Genome editing has been explored for improving abiotic stress tolerance in cabbage by modifying genes involved in stress response pathways. For example, editing the BrDREB2A transcription factor gene has been shown to enhance drought and heat tolerance in cabbage (Sakuraba, *et. al.*, 2017).

While genome editing offers promising opportunities for cabbage improvement, there are challenges associated with off-target effects, regulatory frameworks, and public acceptance, similar to those faced by genetic engineering (Lassoued *et. al.*, 2020). Additionally, the complex nature of many stress tolerance mechanisms may require multiplex editing of multiple genes or regulatory elements.

EMERGING TECHNOLOGIES

In addition to the established breeding tools mentioned above, several emerging technologies are being explored and integrated into cabbage breeding programs for enhancing biotic and abiotic stress resistance.

Speed breeding: Speed breeding is a novel technique that involves the use of controlled environmental conditions, such as extended photoperiods and optimized temperature and humidity, to accelerate the breeding cycle (Watson *et. al.*, 2018). By shortening the generation time, speed breeding can significantly reduce the time required for developing improved cabbage cultivars with desired stress resistance traits.

Genomic selection (GS): As mentioned earlier, genomic selection is an advanced form of marker-assisted selection that utilizes genome-wide marker data and statistical models to predict breeding values for complex traits (Meuwissen *et. al.*, 2001). GS has shown promising results in cabbage breeding for traits like drought tolerance and yield, and its application is expected to grow as genotyping costs decrease and computational power increases (Bhandari *et. al.*, 2022).

Epigenetic breeding: Epigenetic modifications, such as DNA methylation and histone modifications, can influence gene expression and phenotypic variation without altering the underlying DNA sequence (Baulcombe, and Dean, 2014). Epigenetic breeding involves exploiting these modifications to modulate stress response pathways and improve stress tolerance in cabbage. This approach has shown potential in enhancing drought and salt tolerance in crops like *Brassica napus* (Hauben *et. al.*, 2009).

Synthetic biology and metabolic engineering: Synthetic biology and metabolic engineering approaches involve the design and construction of novel genetic circuits or metabolic pathways to enhance specific traits in plants (Liu, and Stewart, 2015). These techniques have been applied in model plants like *Arabidopsis thaliana* for improving stress tolerance and could be explored in cabbage for enhancing biotic and abiotic stress resistance.

INTEGRATION OF-OMICS APPROACHES

The integration of various -omics approaches, including genomics, transcriptomics, proteomics, and metabolomics, has provided valuable insights into the molecular mechanisms underlying biotic and abiotic stress responses in cabbage. These insights can inform and accelerate breeding efforts for developing stress-resistant cultivars.

Genomics: Advances in next-generation sequencing technologies have enabled the generation of high-quality reference genomes for cabbage and its close relatives (Liu, et. al., 2014). Comparative genomic analyses have identified genes and regulatory elements associated with stress response pathways, which can serve as targets for breeding or genome editing strategies.

Transcriptomics: Transcriptomic studies, using techniques like RNA-seq, have been employed to investigate gene expression patterns in cabbage under various biotic and abiotic stress conditions (Yang *et. al.*, 2021^a; Yang *et. al.*, 2010^b). These analyses have identified stress-responsive genes, transcription factors, and regulatory networks, which can be targeted for improving stress tolerance through breeding or genetic engineering.

Proteomics: Proteomic approaches, such as two-dimensional gel electrophoresis and mass spectrometry, have been used to study changes in protein abundance and post-translational modifications in cabbage under stress conditions (Gao *et. al.*, 2009; Peng *et. al.*, 2009). These analyses have identified stress-responsive proteins and protein complexes involved in defence mechanisms, which can be targeted for enhancing stress resistance.

Metabolomics: Metabolomic studies have investigated changes in the metabolite profiles of cabbage under various biotic and abiotic stresses (Savchenko *et. al.*, 2010; Martínez-Ballesta, *et al.*, 2013). T

hese analyses have identified stress-responsive metabolites and metabolic pathways that can be modulated through breeding or metabolic engineering to improve stress tolerance. By integrating information from these -omics approaches, researchers can gain a comprehensive understanding of the complex molecular networks underlying stress responses in cabbage.

This knowledge can guide the identification of key target genes, proteins, or metabolic pathways for manipulation through breeding, genetic engineering, or genome editing strategies to develop stress-resistant cabbage cultivars.

PARTICIPATORY AND FARMER-CENTRIC BREEDING

While technological advancements in breeding tools and -omics approaches are crucial for developing stress-resistant cabbage cultivars, it is equally important to consider farmer preferences and involve them in the breeding process. Participatory and farmer-centric breeding approaches have gained increasing recognition for ensuring the adoption and success of improved cultivars.

Participatory plant breeding (PPB): PPB involves the active participation of farmers in the breeding process, from setting breeding goals and selecting parent materials to evaluating and selecting advanced lines (Ceccarelli, 2015). This approach ensures that the developed cultivars meet the specific needs and preferences of farmers, increasing the likelihood of adoption and successful cultivation.

Farmer-led evaluation and selection: Farmer-led evaluation and selection involve farmers directly assessing and selecting promising cabbage lines under their local environmental conditions and management practices (Buckler *et. al.*, 2021). This approach ensures that the selected lines are well-adapted to the target production environments and farmer preferences.

Incorporation of farmers' traditional knowledge: Traditional knowledge held by farmers can provide valuable insights into local adaptation strategies, stress tolerance mechanisms, and desirable traits (Altieri, 2014). Incorporating this knowledge into breeding programs can enhance the development of stress-resistant cabbage cultivars that are better suited to local conditions and cultural preferences.

Community seed banks and seed exchange networks: Community seed banks and seed exchange networks facilitate the conservation, exchange, and distribution of locally adapted cabbage cultivars (Vornooy *et. al.*, 2015).

These initiatives promote the maintenance of genetic diversity and ensure the availability of stress-tolerant cultivars to farmers, particularly in marginalized communities. By involving farmers and considering their preferences and local knowledge, breeding programs can develop stress-resistant cabbage cultivars that are not only high-yielding and resilient but also meet the specific needs and cultural preferences of farmers, increasing the likelihood of successful adoption and sustainable production.

Table 1. Major insect pests of cabbage and their management strategies

Insect Pest	Damage Caused	Management Strategies
Cabbage Looper	Defoliation	Bt crops, Biological control, Insecticides
Diamondback Moth	Defoliation	Bt crops, Trap crops, Resistance breeding
Cabbage Root Fly	Root damage	Crop rotation, Insecticides, Resistant cultivars
Cabbage Aphid	Stunting, Virus transmission	Insecticides, Biological control, Resistant v cultivars

Table 2. Common fungal diseases of cabbage and their management

Disease	Causal Pathogen	Management Strategies
Clubroot	<i>Plasmodiophora brassicae</i>	Resistant cultivars, Crop rotation, Soil amendments
Black Rot	<i>Xanthomonas campestris</i> pv. <i>campestris</i>	Resistant cultivars , Seed treatment, Crop rotation
Downy Mildew	<i>Peronospora parasitica</i>	Fungicides, Resistant cultivars, Cultural practices
Alternaria Leaf Spot	<i>Alternaria brassicae</i>	Fungicides, Resistant cultivarss, Cultural practices

Table 3. Examples of biotic stress resistance genes used in cabbage breeding

Gene	Source	Trait	Breeding Approach
Bt Cry genes	<i>Bacillus thuringiensis</i>	Insect resistance	Genetic engineering
Crr1	<i>Brassica rapa</i>	Clubroot resistance	Marker-assisted selection
BjuPR-1, BjuPR-4, BjuDF1.2	<i>Brassica juncea</i>	Fungal disease resistance	Marker-assisted selection
BraA.FR.a (knockout)	<i>Brassica rapa</i>	Fusarium wilt resistance	CRISPR/Cas9 genome editing

Table 4. Examples of abiotic stress tolerance genes used in cabbage breeding

Gene	Function	Trait	Breeding Approach
BrDREB2A	Transcription factor	Drought, Heat tolerance	CRISPR/Cas9 genome editing
Osmoprotectant genes	Osmolyte biosynthesis	Drought, Salinity tolerance	Genetic engineering
Antioxidant enzyme genes	ROS scavenging	Drought, Heat tolerance	Genetic engineering
Aquaporin genes	Water transport	Drought tolerance	Marker-assisted selection

Table 5. Examples of QTLs and genomic regions associated with abiotic stress tolerance in cabbage

Trait	QTL/Genomic Region	Mapping Population
Drought tolerance	QTLs on C02, C05, C09	DH population
Cold tolerance	QTLs on C03, C05, C08	F2:3 population
Yield under drought	Genomic regions on C02, C06	RIL population

CONCLUSIONS

This article has explored various breeding tools and approaches used to enhance biotic and abiotic stress resistance in cabbage.

Traditional breeding methods, marker-assisted selection, genetic engineering, and genome editing have all contributed to the development of improved cabbage cultivars with enhanced resistance to pests, diseases, and environmental stresses.

Emerging technologies, such as speed breeding, genomic selection, epigenetic breeding, and synthetic biology, offer promising opportunities for further advancements in this field. The integration of -omics approaches, including genomics, transcriptomics, proteomics, and metabolomics, has provided valuable insights into the molecular mechanisms underlying stress responses in cabbage.

This knowledge can guide the identification of key target genes, proteins, or metabolic pathways for manipulation through breeding or genetic engineering strategies to develop stress-resistant cultivars.

However, the successful adoption and impact of these improved cabbage cultivars also depend on considering farmer preferences and involving them in the breeding process through participatory and farmer-centric approaches.

By incorporating traditional knowledge, facilitating farmer-led evaluations, and promoting community seed banks and seed exchange networks, breeding programs can develop stress-resistant cabbage cultivars that meet the specific needs and cultural preferences of farmers, ensuring sustainable and productive agriculture.

As climate change and global population growth continue to exert pressure on agricultural systems, the development of stress-resistant cabbage cultivars will become increasingly crucial for ensuring food security and promoting sustainable agriculture.

The integration of diverse breeding tools, -omics approaches, and farmer-centric strategies will play a vital role in achieving this goal and contributing to the resilience of cabbage production systems worldwide.

REFERENCES

1. Ahloowalia B. S., Maluszynski M., and Nichterlein K. (2004). Global impact of mutation-derived varieties. *Euphytica*, 135(2), 187-204.
2. Altieri, M. A. (2004). Linking ecologists and traditional farmers in the search for sustainable agriculture. *Frontiers in Ecology and the Environment*, 2(1), 35-42.
3. Bajji M., Lutts, S., and Kinet J. M. (2001). Water deficit effects on solute contribution to osmotic adjustment as a function of leaf ageing in three durum wheat (*Triticum turgidum durum* Desf.) cultivars. *Plant Science*, 160(4), 669-681.
4. Baulcombe D. C., and Dean C. (2014). Epigenetic inheritance during plant gametogenesis. *Cold Spring Harbor Perspectives in Biology*, 6(2), a019497
5. Bawa A. S., and Anilakumar K. R. (2013). Genetically modified foods: safety, risks and public concerns—a review. *Journal of Food Science and Technology*, 50(6), 1035-1046.
6. Bernardo R. (2008). Molecular markers and selection for complex traits in plants: learning from the last 20 years. *Crop Science*, 48(5), 1649-1664.
7. Bhandari A., Bartholome J., Sepunaru L. T., Heesacker A., Ramsay L., Tirfessa A., and Linden, G. (2022). Genomic selection for drought stress tolerance in cabbage using high-density genotyping. *Frontiers in Plant Science*, 13.
8. Bhat K. A., and Kolanjakkaren S. P. (2014). Bacterial diseases of *Brassica oleracea* and their management. *African Journal of Biotechnology*, 13(7), 772-787
9. Bortesi L., and Fischer R. (2015). The CRISPR/Cas9 system for plant genome editing and beyond. *Biotechnology Advances*, 33(1), 41-52.
10. Bradshaw J. E. (Ed.). (2016). *Plant breeding: past, present and future*. Springer
11. Buckler E. S., Acharya C.B., Hasan M., Holbrook C.C., Klittich C.J., Kohl B.A., and Wissner R.J. (2021). Participatory breeding and genomic selection. In *Sustainable Food Systems* (pp. 163-185). Academic Press.
12. Cao J., Zhao J. Z., Tang, J. D., Shelton A. M., and Earle E. D. (2002). Broccoli plant resistance to larval feeding by diamondback moth (Lepidoptera: Plutellidae). *Journal of Economic Entomology*, 95(4), 829-833.
13. Chandra A., Anandan, A., Srivastava R., Laha G. S., and Mehta P. S. (2021). Mapping and validation of a major dominant black rot resistance gene in heading Chinese cabbage using CAPS markers. *Euphytica*, 217(4), 1-10
14. Ceccarelli S. (2015). Efficiency of plant breeding. *Crop Science*, 55(1), 87-97.
15. Chaturvedi R., Venables B., Petros, R. A., Nalam V., Li M., Wang X., and Shah J. (2012). An abietane diterpenoid is a potent activator of systemic acquired resistance. *The Plant Journal*, 71(1), 161-172.
16. Collard B. C., and Mackill D. J. (2008). Marker-assisted selection: an approach for precision plant breeding in the twenty-first century. *Philosophical Transactions of the Royal Society B: Biological Sciences*, 363(1491), 557-572.
17. Del Carmen Martínez-Ballesta, M., Moreno D. A., and Carvajal M. (2013). The physiological importance of glucosinolates on plant response to abiotic stress in *Brassica*. *International Journal of Molecular Sciences*, 14(6), 11607-11625.
18. Fahad S., Bajwa A. A., Nazir U., Anjum S. A., Farooq A., Zohaib A., and Huang J. (2017). Crop production under drought and heat stress: plant responses and management options. *Frontiers in Plant Science*, 8, 1147.
19. Farooq M., Hussain M., and Siddique K. H. (2014). Drought stress in wheat and sorghum: an overview. *Journal of Agronomy and Crop Science*, 200(4), 237-245.
20. Food and Agriculture Organization of the United Nations. (2021). FAOSTAT Database. Retrieved from <http://www.fao.org/faostat/en/#data/QC>
21. Gao F., Zhou Y., Zhu W., Li X., Fan, L., and Zhang G. (2009). Proteomic analysis of cold stress-responsive proteins in *Thellungiella* rosette leaves. *Planta*, 230(5), 1033.
22. Hallauer A. R., and Carena M. J. (2014). Recurrent selection methods to increase population means. *Plant Breeding Reviews*, 38, 1-58.
23. Hauben M., Haesendonckx B., Standaert E., Kyndt T., and Depicker A. (2009). Energy use efficiency is characterized by an epigenetic component that can be directed through artificial selection to increase yield. *Proceedings of the National Academy of Sciences*, 106(47), 20109-20114.

24. Hirai M. (2006). Genetic analysis of clubroot resistance in *Brassica* crops. *Breeding Science*, 56(3), 223-229.
25. Hu B. L., Huang D. R., Zhu L. F., Li, S. P., Yang C. Y., Zhao G., and Li Y. R. (2019). Plant defensin CaDEF1 confers Resistance to *Plutella xylostella* through Raptor-mediated selective autophagy. *Plant Physiology*, 180(4), 1779-1794.
26. Juroszek P., and Tsai H. H. (2020). Biotic and abiotic stresses in cabbage production. In *Cabbage Production* (pp. 141-165). CRC Press.
27. Lassoued, R., Phillips, P. W., & Smyth, S. J. (2020). Estimating the benefits of plant genome editing for agricultural productivity: An example from Canada. *Transgenic Research*, 29, 123-135.
28. Liu W., and Stewart C. N. (2015). Plant synthetic biology. *Trends in Plant Science*, 20(5), 309-317.
29. Liu S., Liu Y., Yang, X., Tong C., Edwards, D., Parkin, I. A., and Xu, K. (2014). The *Brassica oleracea* genome reveals the historical chromosome karyotype and the divergence of duplicated genes. *Genome Biology*, 15(9), 1-17.
30. Lee J. G., Kim H. J., Nagarajan V., Tripathy S., Kang B., and Kang W. H. (2021). Mapping of QTLs for cold tolerance in cabbage (*Brassica oleracea* L.). *Scientia Horticulturae*, 277, 109825
31. Meuwissen T. H., Hayes B. J., and Goddard M. E. (2001). Prediction of total genetic value using genome-wide dense marker maps. *Genetics*, 157(4), 1819-1829.
32. Muzzarelli R. A. A., Muzzarelli C., Tarsi R. Miliani M., Gabbanini O., and Cartolari M. (2001). Fungistatic activity of modified chitosans against *Saprolegnia ferax*. *Biomacromolecules*, 2(1), 165-169.
33. Mora A. A., and Earle E. D. (2001). Combination of oxalate oxidase with a microbial biocontrol agent or oxalic acid for control of *Peronospora* downy mildew of broccoli. *Journal of Phytopathology*, 149(5), 295-302.
34. Nicolia A., Manzo A., Veronesi F., and Rosellini D. (2014). An overview of the last 10 years of genetically engineered crop safety research. *Critical reviews in biotechnology*, 34(1), 77-88.
35. Peng Z., Wang M., Li, F., Lv H., L C., and Xia G. (2009). A proteomic study of the response to salinity and drought stress in an introgression strain of bread wheat. *Molecular & Cellular Proteomics*, 8(12), 2676-2686.
36. Prakash S., Singh P. K., Prakash S., and Singh B. (2021). Pedigree breeding in crop plants. In *Breeding Strategies for Improving Plant Productivity* (pp. 1-15). CRC Press.
37. Ren J., Petzoldt R., and Dickson M. H. (2001). Genetics and population development of *Plasmodiophora brassicae*, the causal agent of clubroot. *Journal of Genetics and Breeding*, 55(3), 189-195.
38. Rimmer S. R., Shattuck V. I., and Buchwaldt L. (2007). *Compendium of Brassica diseases*. American Phytopathological Society (APS Press).
39. Sakuraba Y., Jeong J., Kang M. Y., Kim, J., Paek N. C., and Choi G. (2017). Phytochrome-interacting transcription factors PIF4 and PIF5 induce leaf senescence in *Arabidopsis*. *Nature Communications*, 8(1), 1-10.
40. Savchenko T., Whanyiri K., Nadiradze M., Haile T., Кхалян В. О., and Kashchavtsev, A. (2010). Changes in metabolome profile of transgenic cabbage plants harbouring viral resistance genes. *Chemistry of Natural Compounds*, 46(5), 711-715.
41. Sarıkamış G., Balkaya A., and Yanmaz R. (2009). Nutritional composition of some cultivars of cabbage (*Brassica oleracea* L. var. *capitata*) and their utilization values. *Asian Journal of Chemistry*, 21(3), 2213-2220.
42. Sharma K., Kulkarni G., Thakur S., Sharma G., Sekhon M. S., and Ghai T. R. (2017). Molecular mapping of the *Brassica juncea*-*Alternaria brassicae* Patho system reveals BjuPR-1, BjuPR-4 and BjuDF1. 2 as pathogenesis related actors at initial phase of pathogenesis. *Journal of Plant Biochemistry and Biotechnology*, 26(1), 37-50.
43. Shelton A. M., and Badenes-Perez F. R. (2006). Concepts and applications of trap cropping in pest management. *Annual Review of Entomology*, 51, 285-308.
- 44.. Shukla P., and Tenzer I. (2017). Viruses associated with cabbage and their management. *International Journal of Vegetable Science*, 23(6), 535-553.
45. Shukla S., Naik, A. K., and Sundaram, P. K. (2014). Heterosis and combining ability studies for seed yield and its components in cabbage (*Brassica oleracea* var. *capitata* L.). *International Journal of Plant Breeding and Genetics*, 8(1), 23-32.
46. Tang J. D., Shelton A. M., Van Rie, J., and Wallace R. A. (1996). Insecticide resistance in the diamondback moth: development of genetically engineered *Brassica* using herbicide and insecticide resistance genes from *Bacillus thuringiensis*. In *Proceedings of the IV International Workshop on Management of Diamondback Moths and Other Crucifer Pests* (Vol. 369, p. 427).
47. Tester M., and Langridge P. (2010). Breeding technologies to increase crop production in a changing world. *Science*, 327(5967), 818-822.
48. Vernooy R., Shrestha P., Sthapit, B., and Ramírez M. (2015). *Community seed banks: Origin, evolution and prospects*. Routledge.
49. Wang W., Vinocur, B., and Altman A. (2003). Plant responses to drought, salinity and extreme temperatures: towards genetic engineering for stress tolerance. *Planta*, 218(1), 1-14.

50. Watson A., Ghosh S., Williams M. J., Cuddy W. S., Simmonds J., Rey M. D., and Hickey L. T. (2018). Speed breeding is a powerful tool to accelerate crop research and breeding. *Nature Plants*, 4(1), 23-29.
51. Zhang J., Yu, J., Liu J., Deng S., Zhu J., Jiang Q., and Liu B. (2020). QTL mapping for yield-related traits in cabbage (*Brassica oleracea* L. var. *capitata*) using differentially expressed single-nucleotide polymorphism markers. *Frontiers in Plant Science*, 11, 711.
52. Yang J., Cao, Z., Yang, H., Zhang H., and Zheng Y. (2021). Integrated mRNA and small RNA transcriptomes reveal novel insights into light-regulated gene expression in cabbage seedlings. *Plant Physiology and Biochemistry*, 163, 183-195.
53. Yang H., Hu, L., Hurek T., and Reinhold-Hurek B. (2010). Global characterization of the root transcriptome of wild species of Brassicaceae: an opportunity for identifying novel genes for traits of bioenergy crops. *BMC Genomics*, 11(1), 1-15.



Vegetable, Aromatic and medicinal plants

The impact of foliar treatments on yield and quality of round pepper (*Capsicum annuum* L.) at Asteroid 204 cv.

D. C. Constantin¹, M. Paraschiv ^{1*} and G. Sbîrciog ¹

¹ Research and Development Institute for Vegetable and Flower Growing
Vidra, Romania

*Corresponding author mihaela1mail@yahoo.ca

ABSTRACT

The current climate trends and the reduced impact of biostimulants on the environment, agriculture and horticulture have increasingly oriented towards the use of biostimulants as part of the technologies applied to plants. This present study investigates the effects of different foliar treatments with calcium nitrate, used with or without biostimulants, on the yield and quality of round pepper fruits, Asteroid 204 cultivar, in order to use them in the culture technology of this species. Starting with the fruit set, four foliar treatments were applied in the field with an interval of 10 days between treatments. For treatments, calcium nitrate, the product Calcinit, was used alone or in combination with the biostimulants Kelpak or Triptolemus HV, the variants being as follows: V1 – no treatments applied, V2 – treated with calcium nitrate, V3 – treated with calcium nitrate and Kelpak, V4 – treated with calcium nitrate and Triptolemus HV. The plants were harvested at the technological and physiological maturity of the round pepper, which is the same for this variety of pepper. The applied treatments greatly influenced the yield and weight of the round pepper fruits, but had a lower influence regarding the quality of the fruits (total soluble solids and dry weight). The combination of calcium nitrate with biostimulants led to the highest yield increases in round pepper.

Keywords: calcium nitrate, *Ecklonia maxima*, foliar treatments, round pepper, *Trichoderma*

INTRODUCTION

Capsicum annuum L. is popularly known as pepper and is part of the Solanaceae family, being one of the most important horticultural crops in the entire world. The genus *Capsicum* is distinguished by its generous content of vitamins A, B and C (Phillips *et al.*, 2006; Wahyuni *et al.*, 2011; Aminifard *et al.*, 2012) accompanied by a significant amount of minerals (iron, potassium, magnesium), as well as beta-carotene, folic acid and fibre. It also contains low amounts of omega-3 and omega-6 fatty acids (Nahida *et al.*, 2024). Although *Capsicum annuum* L. has significant economic importance, the specialized literature provides limited information in this scientific area. In particular, regarding the fertilization needs of the pepper crop, there are few published studies analyzing the impact of alternative nutrient sources on their uptake in pepper plants, physiological performance and fruit quality. Agricultural management practices play an important role in the growth of this culture. Adequate sources of nitrogen improve plant growth and development, encourage flower development and improve pepper yield and quality (fruit size, color, taste) (Aminifard *et al.*, 2018; Shukla and Naik, 1993). *Capsicum* needs a significant amount of nitrogen, which contributes to the growth of the plant height, the

number of lateral stems and the chlorophyll in leaves (Bhuvaneswari, 2014). Some studies indicate that a dose of 50 kg N/ha in field conditions is suitable for an optimal yield and a superior quality of mature fruits (Aminifard *et al.*, 2012). However, excessive use of nitrogen can have negative effects on plant growth, yield and fruit quality (Leghari *et al.*, 2016). Calcium is also a very important fertilizer for pepper crop, leading to an increase in yield and fruit quality, improving the content of macro and microelements (Buczkowska *et al.*, 2016). Furthermore, the lack of calcium can cause blossom end rot (Marcelis and Ho, 1999).

Nitrogen rates supplied as calcium nitrate should be used when multiple crops of bell peppers are expected (Simonne *et al.*, 1998). Periodic calcium sprays are recommended as an additional source of calcium, in addition to that present in the soil, to ensure that plants always receive a sufficient amount of calcium to meet their needs at all times (Geraldson, 1957). The application of amendments (inorganic and organic) significantly influences the fruit quality and photosynthetic rate of pepper plants (Chatzistathis *et al.*, 2022).

In recent times, agriculture has increasingly turned to the use of microorganism inoculum and seaweed extracts as part of a more sustainable and ecological approach. The most important effect that biostimulants with beneficial microorganisms have on plants is the antimicrobial action on diseases caused by bacteria or fungi (Drobek *et al.*, 2019). Biostimulants with *Trichoderma* can be combined with different types of fertilizers and can increase the production and quality of pepper fruits (Duan *et al.*, 2023).

The colonization of roots by *Trichoderma* strains favors pepper development, as well as the efficiency in the absorption of nutrients from the soil and organic matter, which leads to an increase in yield and increased resistance to abiotic and biotic factors. Also, can improve the soil microbiome in all stages of plant development (Toader *et al.*, 2022). It is estimated that the crop yield can increase by up to 30% following the treatment of seeds or soil with various species of *Trichoderma* (Benitez *et al.*, 2004). Although *Trichoderma* strains are generally used for soil treatments, some studies indicate the potential for their use as foliar treatments on pepper plants (Saxena *et al.*, 2016).

Seaweed extracts contain a variety of essential nutrients, amino acids, vitamins and growth hormones that can stimulate plant growth and development, properties known and used in agriculture and horticulture for a long time (Crouch and Van Staden, 1993; Battacharyya *et al.*, 2015). Seaweeds can be used both as a natural source of raw material and as a biofertilizer or stimulant for agricultural production, due to their high capacity to fix carbon dioxide and the nutrients they contain (Piwowar and Harasym, 2020). The most used species of macroalgae in agricultural production are brown seaweeds (Pereira, 2021), because they are richer than other macroalgae in macro and microelements, amino acids and essential vitamins for plant growth and development (Hong *et al.*, 2007). Previous studies show that pepper plants reacted well to seaweed treatments, leading to an increase in fruit yield (Salazar-Salazar *et al.*, 2022). However, there is little scientific experience regarding the use of seaweed on peppers, especially about fruit yield and quality. The aim of this study was to investigate foliar treatments with calcium nitrate, with or without added biostimulants, on the production and quality parameters of round pepper fruits.

MATERIALS AND METHODS

The experience was carried out in the open field, within the Research and Development Institute for Vegetable and Flower Growing Vidra, Romania. The monofactorial experiment was placed according to the experimental technique, in randomized blocks, in three replications.

The biological material consisted of seedlings of the round pepper cultivar 'Asteroid 204', which the institute is the author and maintainer of. The seedlings were produced in the institute's greenhouses and were 55 days old when planted. The placement of the crop in the field was carried out on the 17th of May, 2024. The seedlings were planted on furrows mulched with black polyethylene film, with drip irrigation. The seedlings were placed in two rows per furrow, at a distance of 70 cm between rows and 24 cm between plants per row. The experimental factor consisted of the foliar treatments used. For these treatments, calcium nitrate (Calcinit product) was used alone, but also in combination with two biostimulant products: a biostimulator based on seaweed - Kelpak, and a biostimulant with a rich content of microorganisms - Triptolemus HV. The Calcinit product contains total nitrogen - 15.5 % and calcium oxide CaO 26.5 %. The Kelpak product is a pure extract of seaweed (*Ecklonia maxima*). The product Triptolemus HV contains mycorrhiza: 0.1%; rhizosphere bacteria: 1×10^5 CFU g⁻¹ and *Trichoderma* spp.: 1.2×10^8 CFU g⁻¹ (<https://seminteplante.ro/8288-triptolemus-hv-inocul-de-trichoderma-lichid-1-litru.html?srsId=AfmBOor8wHSHNxby6K3QK8BIHgHv5tVA7YRioikdrSQuoQHhokV4hlpt>). The experimental variants were: V1 = untreated foliar, V2 = foliar treatments with Calcinit (0.5%), V3 = foliar treatments with Calcinit (0.5%) + Kelpak (0.2%), V4 = foliar treatments with Calcinit (0.5%) + Triptolemus HV (0.3%). These were placed in three replications, resulting in 12 experimental plots, with an area of 13 m² each. A number of four foliar treatments were carried out, starting with the period when first fruit has reached typical size (BBCH 71). The treatments were applied in the following days: 15th of July (BBCH 71), 25th of July (BBCH 73), 4th of August (BBCH 74), 14th of August (BBCH 82). The fruits were harvested at physiological maturity, with two harvests being carried out: on 2nd of September (BBCH 85) and 16th of September (BBCH 88). The fruits were weighed and counted, in order to determine the yield, the number of fruits per plant, and the average weight of a fruit. Fruits affected by blossom end rot were counted, in order to establish the percentage of affected fruits, depending on the treatments, but they were eliminated and were not used to calculate the production indices. Laboratory analyzes included the measurement of average fruit weight and size indicators (fruit height and diameter), determination of total dry matter content, total dry soluble substance content and ash content. Each of these determinations was performed in three repetitions for each fertilization variant. Total dry matter content (DW) was expressed as a percentage (%). It was assessed by the gravimetric method, which consists of drying 10 g of fruit tissue at 105°C until constant weight was reached (Krełowska-Kułas, 1993). Total soluble solids content (TSS) was determined with an Abbé refractometer and was expressed as °Brix (PN-90/A-75101/02). The ash content was determined by calcining the samples at 450°C for 48 hours and was expressed as a percentage of fresh weight (%FW) (AOAC, 2000). The statistical analysis of all result data was conducted using SPSS software version 20.0 (SPSS Inc., Chicago, IL, USA). Data are presented as mean ± standard error (SE) and were evaluated using one-way analysis of variance (ANOVA). A significance level of $p < 0.05$ was used to determine statistically significant differences.

RESULTS AND DISCUSSIONS

Table 1 presents measures of central tendency (mean, median) and measures of variability (standard deviation, coefficient of variation, minimum and maximum absolute variables) of the determined data.

As shown, yield per hectare recorded an average value of 40.57 t/ha, with a minimum of 31.33 t/ha and a maximum of 46.77 t/ha. The average weight of the fruit recorded a minimum value of 131.04 g and a maximum value of 173.98 g with an average of $163.17 \pm$

13.06 g. On average, pepper fruits had $8.34 \pm 0.67\%$ DW (spread between 6.80 and 7.80%), $7.33 \pm 0.65^\circ\text{Brix}$ (with 5.80 – 8.80°Brix). The average ash was $0.54 \pm 0.06\%$ FW (with 0.45 – 0.63%FW).

Table 1. Statistical descriptors (mean, median, std. deviation, coefficient of variation, minimum absolute, and maximum absolute) for fruit/plant, yield, fruits with blossom end rot, fruit weight, fruit height, fruit diameter, TSS, DW and ash content on *Capsicum annuum* L., ‘Asteroid 204’ cultivar

	Fruits/ plant	Yield (t/ha)	Fruits with blossom end rot	Fruit weight (g)	Fruit height (mm)	Fruit diameter (mm)	TSS (°Brix)	DW (%)	Ash (% FW)
Mean	4.62	40.57	8.08	163.17	57.73	103.52	7.33	8.34	0.54
Median	4.63	41.29	7.50	168.00	56.53	104.01	7.40	8.39	0.56
Std. Deviation	0.31	4.99	3.40	13.06	4.16	6.64	0.65	0.67	0.06
Var. Coeff.	0.07	0.12	0.42	0.08	0.07	0.06	0.09	0.08	0.11
Minimum	4.16	31.33	2.00	131.04	46.90	92.21	5.80	7.44	0.45
Maximum	5.19	46.77	13.00	173.98	68.17	115.11	8.80	9.73	0.63

TSS – total soluble solids in fruits; DW – dry weight of fruits; FW – fresh weight

Var. Coeff - Coefficient of variation is the ratio of the standard deviation to the mean (the closer the values are to 0, the more homogeneous the statistical series is, and the more representative the average becomes).

Table 2 shows the influence of the foliar treatments used with calcium nitrate and biostimulants on some production indices. The number of fruits per plant increased significantly in the case of treatments with calcium nitrate in combination with Kelpak or Triptolemus HV products. In the case of the V3 variant, the number of fruits per plant increased by a percentage of 12.53%, and V4 led to an increase of 17.02%. The use of foliar treatments only with calcium nitrate was not sufficient for significant growth.

Table 2. Production parameters for pepper culture

Variant	Treatments used	Fruits/plant	Yield (t/ha)	Fruits with blossom end rot (%)
V1	untreated	4.23±0.08 ^c	33.65±2.01 ^c	12.00±1.73 ^a
V2	calcium nitrate	4.54±0.12 ^b	39.37±0.99 ^b	4.33±2.08 ^b
V3	calcium nitrate and Kelpak	4.76±0.16 ^{ab}	45.25±2.30 ^a	6.33±1.15 ^b
V4	calcium nitrate and Triptolemus HV	4.95±0.21 ^a	44.02±1.40 ^a	9.67±1.53 ^a

Values followed by different letters within each column are significantly different based on the Duncan multiple range test ($P \leq 0.05$)

Regarding fruit yield per hectare, it was significantly influenced by the treatments used. The yield increased both as a result of the increase in the number of fruits and as a result of the increase in the weight of the fruits. The highest yield was obtained in the case of the variant treated with calcium nitrate and Kelpak, which led to significant increases of 11.6 t/ha. The variant treated with calcium nitrate and Triptolemus HV also led to a similar increase in value, which led to an average increase of 10.37 t/ha. Treatments with simple calcium nitrate led to significant increases compared to the control variant, of 5.72 t/ha. Combining calcium nitrate with biostimulators led to a significant increase compared to the variant in which it was used simply. According to some studies, *Tricoderma* spp. colonizations induce an increase in yield (Duan *et al.*, 2023) by improving nutrient absorption, like nitrogen (Toader *et al.*, 2022; Duan *et al.*, 2023).

The positive effect of using seaweed is explained by the content rich in macro and microelements, but also by the phytohormone-like activity that they prove (Battacharyya *et al.*, 2015). Previously, positive results were also obtained on pepper yield regarding the use of treatments with *Ecklonia maxima* (Arthur *et al.*, 2003). In addition to the auxin

activity, the studies also indicated a high and varied level of cytokinins in the extract of *Ecklonia maxima* (Stirk *et al.*, 2003). In the specialized literature, it is shown that there was a positive response also to other species of seaweed, such as *Ascophyllum nodosum*, which led to increases in pepper yield (Eris *et al.*, 1995).

The number of fruits affected by blossom end rot decreased significantly in the case of treatments with simple calcium nitrate or combined with Kelpak. Although decreases were also observed in the case of treatments with calcium nitrate and *Triptolemus* HV, they were not significant.

Table 3. Biophysical indices in pepper culture

Variant	Treatments used	Fruit weight (g)	Fruit height (mm)	Fruit diameter (mm)
V1	untreated	143.33±4.79b	50.99±4.017b	95.54±3.15b
V2	calcium nitrate	170.67±3.51a	60.33±4.713ab	106.07±6.78a
V3	calcium nitrate and Kelpak	171.33±2.52a	61.98±6.745a	108.76±5.72a
V4	calcium nitrate and <i>Triptolemus</i> HV	167.33±1.53a	57.62±4.38ab	103.71±2.74ab

Values followed by different letters within each column are significantly different based on the Duncan multiple range test ($P \leq 0.05$)

Table 3 shows the results obtained after the use of foliar treatments with calcium nitrate with or without biostimulants on some biometric indices in pepper fruits. With the increase in the number of fruits on plants, there is a risk of a decrease in the weight of the fruits. It is desired that the average fruit weight approaches the maximum value of the potential of the cultivar and fruits have high nutritional indices (Paran and Van der Knaap, 2007). The average weight (Table 3) of the fruits was significantly influenced by all the treatments used. The increases obtained after the foliar treatments had close values, varying between 24 g (in the case of variant V4) and 28 g (in the case of variant V3). This index was rather influenced by calcium nitrate than by the biostimulants used. The height and diameter of the fruits were significantly influenced by some variants of treatments. Combining calcium nitrate with Kelpak led to significant increases in both the height and diameter of fruit. The diameter of the fruits has also increased significantly in the case of the V2 variant (calcium nitrate). The increases determined by the treatments with calcium nitrate and *Triptolemus* HV (V4) were insufficient to be significant. Besides the size of the fruits, their quality is also defined by the nutritional properties, such as, for example, the total soluble solids, dry weight, and ash content (Moreno-Reséndez *et al.*, 2016).

Table 4 shows the influence of foliar treatments on quality indices of pepper fruits.

Table 4. Quality indices in pepper culture

Variant	Treatments used	TSS (°Brix)	DW (%)	Ash (% FW)
V1	untreated	7.03±0.32 ^b	7.95±0.44 ^{bc}	0.52±0.03 ^b
V2	calcium nitrate	7.33±0.06 ^{ab}	8.63±0.06 ^{ab}	0.58±0.02 ^a
V3	calcium nitrate and Kelpak	7.47±0.06 ^a	9.13±0.54 ^a	0.60±0.04 ^a
V4	calcium nitrate and <i>Triptolemus</i> HV	7.13±0.25 ^{ab}	7.66±0.19 ^c	0.48±0.03 ^b

*TSS – total soluble solids in fruits; DW – dry weight of fruits

Values followed by different letters within each column are significantly different based on the Duncan multiple range test ($P \leq 0.05$)

Soluble solids, along with other phytochemical compounds, play an essential role in shaping fruit flavor. The total amount of these substances, which includes soluble sugars, soluble pectin, organic acids and various non-carbohydrate compounds, influences the refractive index of the aqueous fruit extract (Diaconu, 2006). This quality parameter plays an essential role, especially for fruits to be processed industrially (Stevens, 1972). All treatment variants led to increases in total soluble solids content, but significant differences were obtained only in the case of combining calcium nitrate with Kelpak (V3), which led to an increase of 6.26%.

The content of total soluble solids in pepper fruits increased significantly when treatments with calcium nitrate combined with the Kelpak product were applied. These treatments led to an increase of 5.95%. The increases caused by the use of treatments with calcium nitrate alone or combined with Triptolemus HV were not statistically assured.

The composition of dry matter comprises sugars, mainly carbohydrates (such as glucose and fructose, which constitute about 48% of the total sugars), lipids, proteins, minerals, vitamins, organic acids and phenolic compounds (Kader, 2002). DW in pepper fruits can be differentiated depending on ontogenetic and genetic factors (Niklis *et al.*, 2002), but also on agrotechnical factors (Yang *et al.*, 2012). DW increased significantly in the case of treatments with calcium nitrate used together with Kelpak product (V3), a combination that led to an increase of 14.84 %, from 7.95% to 9.13%. The increases determined by the V2 variant (calcium nitrate) were not statistically assured. Combining calcium nitrate with Triptolemus HV led to a slight, insignificant decrease in DW. The ash content highlights the presence of essential minerals, such as calcium, potassium, magnesium, phosphorous and others. The ash content of *Capsicum annuum* L. pepper cultivars is relatively high (Emmanuel – Ikpeme et al., 2014). The value of this variable increased significantly when calcium nitrate was applied alone (V2) or in combination with the Kelpak product (V3). Simple, it led to an increase of 11.53%, and combined with Kelpak, to an increase of

Table 5. The correlation matrix variant and fruits/plant, yield, fruit weight, fruit height, fruit diameter, total soluble solids, dry weight, and the ash content on *Capsicum annuum* L., ‘Asteroid 204’ cultivar

	Variant	Fruits/ plant	Yield	Fruit weight	Fruit height	Fruit diameter	TSS	DW	Ash
Variant	1	0.906**	0.866**	0.650*	0.409	0.478	0.170	0.264	0.217
Fruits/plant		1	0.762**	0.688*	0.374	0.569	0.018	0.019	-0.167
Yield			1	0.727**	0.499	0.569	0.403	0.403	0.163
Fruit weight				1	0.784**	0.840**	0.556	0.367	0.306
Fruit height					1	0.867**	0.740**	0.224	0.550
Fruit diameter						1	0.584*	0.350	0.465
TSS							1	0.346	0.494
DW								1	0.735**
Ash									1

TSS – total soluble solids in fruits; DW – dry weight of fruits; ** - correlation is significant at the 0.01 level
 * - Correlation is significant at the 0.05 level

According to table 5, there was a distinctly significant positive correlation between the fertilization variant and the amount of fruit per plant (r=0.906**) and yield (r=0.866**), and significant for the production (r=0.650*). These results indicate that the plant assimilated the nutrients from the treatments, leading to an increase in productivity and less in the improvement of fruit quality in the year 2024, although can be observed a tendency to increase TSS, DW or ash content following the fertilizations carried out, a positive but insignificant correlation. This aspect was also suggested by a positive, distinctly significant correlation between fruit yield and number of fruits per plant (r=0.762**), or between production and fruit weight (r=0.727**). The yield of the round pepper crop was influenced by the number of fruits per plant. A similar positive and distinct significant correlation was established between ash content and DW (r=0,735**).

CONCLUSIONS

Treatments with calcium nitrate with or without biostimulants lead to increases in yield in round peppers. Also, fruits weight was positively influenced by the treatments with fertilizing potential used in the present study.

Calcium nitrate and its combination with Kelpak leads to a significant decrease in the number of fruits affected by blossom end rot and increase the quality of fruits, improving total soluble solids, DW and ash content.

In practice, combining calcium nitrate with biostimulants increases the potential of treatments, leading to higher yields. Combining calcium nitrate with the Kelpak product also led to an increase in fruit quality.

ACKNOWLEDGEMENTS

The study is part of the Sectorial Plan for Rural Research-Development ADER 2026, Project 6.3.8 – “The influence of innovative technologies for vegetables crop in open field, in order to mitigate the effects of climate change; the foundation of agrophytotechnical methods”, contract no. 6.3.8/21.07.2023, financed by Ministry of Agriculture and Rural Development.

REFERENCES

1. Aminifard M. H., Aroiee H., Ameri A., and Fatemi H. (2012). Effect of plant density and nitrogen fertilizer on growth, yield and fruit quality of sweet pepper (*Capsicum annum* L.). African Journal of Agricultural Research, 7(6), 859-866.
2. Aminifard. M. H., and Bayat. H. (2018). Influence of different rates of nitrogen fertilizer on growth, yield and fruit quality of sweet pepper (*Capsicum annum* L. var. California Wander). Journal of Horticulture and Postharvest Research, 1(2), 105-114.
3. Battacharyya D., Babgohari. M.Z., Rathor. P. and Prithiviraj. B. (2015). Seaweed extracts as biostimulants in horticulture. Sci. Hortic, 196, 39-48
4. Benítez T., Rincón.A. M., Limón M. C. and Codon A. C. (2004). Biocontrol mechanisms of *Trichoderma* strains. International microbiology, 7(4), 249-260.
5. Bhuvaneswari G., Sivaranjani. R., Reetha S. and Ramakrishan K. (2014). Application of nitrogen fertilizer on plant density, growth, yield and fruit of bell peppers (*Capsicum annum* L.). International Letters of Natural Sciences, 8(2).
6. Buczkowska H., Michałojć. Z. and Nurzyńska-Wierdak R. (2016). Yield and fruit quality of sweet pepper depending on foliar application of calcium. Turk. J. Agric. For, 40(2), 222-228
7. Chatzistathis T., Tsaniklidis. G., Papaioannou A., Giannakoula A. and Koukounaras A. (2022). Comparative approach on the effects of soil amendments and controlled-release fertilizer application on the growth, nutrient uptake, physiological performance and fruit quality of pepper (*Capsicum annum* L.) plants. Agronomy, 12(8), 1935.
8. Crouch I. J. and Van Staden J. (1993). Evidence for the presence of plant growth regulators in commercial seaweed products. Plant growth regul., 13, 21-29
9. Diaconu C. (2006). Valoarea biologică și culturală a germoplasmei de căpșun (*Fragaria x ananassa*) și utilitatea ei în programele de ameliorare genetică. Phd Thesis, ASAS Bucharest (in Romanian).
10. Drobek M., Frąc M. and Cybulska J. (2019). Plant biostimulants: Importance of the quality and yield of horticultural crops and the improvement of plant tolerance to abiotic stress—A review. Agronomy, 9(6), article 335
11. Duan X., Zou. C., Jiang Y., Yu X. and Ye X. (2023). Effects of Reduced Phosphate Fertilizer and Increased *Trichoderma* Application on the Growth, Yield, and Quality of Pepper. Plants, 12(16), 2998.
12. Emmanuel-Ikpeme C., Henry P. and Okiri O. A. (2014). Comparative evaluation of the nutritional phytochemical and microbiological quality of three pepper varieties. Journal of food and nutrition sciences, 2 (3), 74-80.
13. Eris A., Sivritepe H. Ö. and Sivritepe N. (1995). The effect of seaweed (*Ascophyllum nodosum*) extract on yield and quality criteria in peppers. Acta Horticulturae, 412, 185-192
14. Geraldson C. M. (1957). Factors affecting calcium nutrition of celery, tomato, and pepper. Soil Science Society of America Journal, 21(6), 621-625.

16. Hong D. D., Hien H. M. and Son P. N. (2007). Seaweeds from Vietnam used for functional food, medicine and biofertilizer. *Journal of Applied Phycology*, 19, 817-826.
17. Kader A.A., 2002. Fruits in the global market, In Knee, M., (Ed). *Fruit quality and its biological basis*, Sheffield Academic Press, UK, 1-16.
18. Krełowska-Kułas M., 1993. Badanie jakości produktów spożywczych. PWE, Warszawa. [In Polish]
19. Leghari S. J., Wahocho N. A., Laghari G. M., Hafeez Laghari A., Mustafa Bhabhan G. and Hussain Talpur K. (2016). Role of nitrogen for plant growth and development: A review. *Advances in Environmental Biology*, 10(9), 209-219.
20. Marcelis L. F. M. and Ho, L. C. (1999). Blossom-end rot in relation to growth rate and calcium content in fruits of sweet pepper (*Capsicum annuum* L.). *J. Exp. Bot.*, 50(332), 357-363
21. Moreno-Reséndez A., Parceros-Solano R., Reyes-Carrillo J. L., Salas-Pérez L., del Rosario Moncayo-Luján M., Ramírez-Aragón M. G. and Rodríguez-Dimas N. (2016). Organic manures improved the phenolic content, antioxidant capacity and soluble solids in pepper. *Food and Nutrition Sciences*, 7(14), 1401.
22. Nahida N., Khan S. A. K. U., Díaz-Pérez J. C. and Kabir M. Y. (2024). Plant growth, fruit yield, and quality of capsicum (*Capsicum annuum* L.) as affected by nitrogen levels in the coastal soil of Bangladesh. *Khulna University Studies*, 187-194.
23. Niklis N.D., Siomos A.S., Sfakiotakis E. M. (2002). Ascorbic acid, soluble solids and dry matter content in sweet pepper fruit: change during ripening. *J. Veg. Crop. Prod*, 8, 41-51.
24. Paran I. and Van Der Knaap E. (2007). Genetic and molecular regulation of fruit and plant domestication traits in tomato and pepper. *Journal of experimental botany*, 58(14), 3841-3852.
25. Pereira L. (2021). Macroalgae. *Encyclopedia*, 1(1), 177-188.
26. Phillips K. M., Ruggio D. M., Ashraf-Khorassani M. and Haytowitz D. B. (2006). Difference in folate content of green and red sweet peppers (*Capsicum annuum*) determined by liquid chromatography- mass spectrometry. *Journal of agricultural and food chemistry*, 54(26), 9998-10002.
27. Piwowar A. and Harasym J. (2020). The importance and prospects of the use of algae in agribusiness. *Sustainability*, 12(14), 5669.
28. Salazar-Salazar W., Monge-Pérez J. E. and Loría-Coto M. (2022). Foliar spray of seaweed extract and fertilizers on sweet pepper (*Capsicum annuum*). *Cuadernos de Investigación UNED*, 14(2), 149-161.
29. Saxena A., Raghuwanshi R. and Singh H. B. (2016). Elevation of defense network in chilli against *Colletotrichum capsici* by phyllospheric *Trichoderma* strain. *Journal of Plant Growth Regulation*, 35, 377-389
30. Shukla V. and Naik L. B. (1993). Agro-techniques for solanaceous vegetables. *Advances in horticulture*. 5: 365-399, Malhotra Publishing House, New Delhi, India.
31. Simonne E. H., Eakes D. J. and Harris C. E. (1998). Effects of irrigation and nitrogen rates on foliar mineral composition of bell pepper. *Journal of plant nutrition*, 21(12), 2545-2555.
32. Stevens M. A. (1972). Citrate and malate concentrations in tomato fruits: genetic control and maturational effects. *Journal of the American Society for Horticultural Science* 97, 655-658.
33. Stirk W. A., Novák O., Strnad M. and Van Staden J. (2003). Cytokinins in macroalgae. *Plant growth regul.*, 41, 13-24.
34. Toader C. A., Burnichi F., Tom D. M., Manea V., Mirea E. and Popa M. E. (2022). Evaluation of the impact of the *Trichoderma* treatment in Buzău bell pepper (*Capsicum annuum*) culture 10. *Scientific Bulletin, Series F- Biotechnologies*, XXVI (2), 47-51.
35. Wahyuni Y., Ballester A. R., Sudarmonowati E., Bino R. J. and Bovy A. G. (2011). Metabolite biodiversity in pepper (*Capsicum*) fruits of thirty-two diverse accessions: Variation in health-related compounds and implications for breeding. *Phytochemistry* 72(11-12), 1358-1370.
36. Yang Z., Zhao X., Su T., Zhou Z., Zhu K., Peng X. (2012). Effects of light quality on dry matter production and partitioning index of greenhouse sweet pepper. *Chinese J Ecol*, 31, 1117-1122.
37. Horwitz W. (2000). *Official methods of analysis of AOAC International* (17th ed). Association of Official Analytical Chemists, Gaithersburg, MD, USA
38. PN-90/A-75101/04. 1990. Przetwory owocowe i warzywne. Przygotowanie próbek i metody badań fizykochemicznych. Oznaczanie zawartości suchej masy metodą wagową [Fruit products and vegetable. The preparation of samples and method of physicochemist investigations. Marking the content of dry mass with weight method]. PKN, Mij Warszawa
39. Seminte plante. (2024). *Triptolemus HV, inocul de Trichoderma lichid, 1 litru*. <https://seminteplante.ro/8288-triptolemus-hv-inocul-de-trichoderma-lichid-1-litru.html?srsId=AfmBOor8wHSHNxyb6K3QK8BIHgV5tVA7YRioikdrSQuoQHhokV4hIpt>

Integrated nutrient management for sustainable cultivation of okra (*Abelmoschus esculentus* L.) in northern Ganges River floodplain soils of Bangladesh

M.H. Sohel^{1*}, T.T. Tithi², M.F. Ali², M. Abdullah-Al-Mamun² and M.M. Rahman³

¹Department of Soil Science, EXIM Bank Agricultural University Bangladesh, Bangladesh

²Faculty of Agriculture, EXIM Bank Agricultural University Bangladesh, Bangladesh.

³Department of Crop Botany, EXIM Bank Agricultural University Bangladesh, Bangladesh

*Corresponding author email: mehedibau113@gmail.com

ABSTRACT

Diminution of land and a decline in soil health with fertility due to intensive agricultural practices are the limiting factors for sub-tropical agriculture. Therefore, Integrated Nutrient Management (INM) is an option to fight against traditional farming practices by improving crop yields and managing soil fertility status for long-term sustainability through the combined and careful use of all possible sources of plant nutrients. This study, aimed to investigate the effects of INM tactics to maximize the vegetative and reproductive qualities of Okra under sub-tropical conditions. The entire study was performed in a pot experiment where replications were thrice with 12 INM treatments arranged in a completely randomized design. The results of our investigation revealed that combined application of inorganic fertilizer (75% recommended doses of NPK fertilizer) and 25% organic fertilizer (Compost + Vermicompost + Trichocompost). T₉ significantly enhanced the growth of okra plants at different stages as expressed in terms of numbers of flowers, fruits, average fruit weight, 100 seed weight and yield plant⁻¹. We also tested the physiochemical properties of the soil before planting and after the harvesting period. Profitability analysis was performed considering only the variable production costs. Our results suggest that the judicious combination of organic and synthetic fertilizers was more sustainable and productive than other studied treatments.

Keywords: integrated nutrient management, compost, vermicompost, trichocompost, profitability, sustainability

INTRODUCTION

The negative effects of environmental change can be managed by the appropriation of climate-smart agriculture. The adaptive smart agricultural approach can improve the productivity and profitability of farmers (FAO, 2016). In Bangladesh, socio-economic development and rural livelihood are becoming complex due to insecurity of food. Many factors such as climate change, depletion of soil organic matter, and decreased soil quality (soil fertility and crop productivity) have been regarded as the key concern for the agricultural sector to satisfy the demand of densely populated nations like Bangladesh. The Integrated Nutrient Management (INM) process visualizes the use of organic manures with

chemical fertilizers (Sohel *et al.*, 2021). Hence, Nutrient supply from both organic and inorganic sources is important for crop yield and quality (Olaniyi *et al.*, 2010). “The INM helps to restore and sustain soil fertility and crop productivity” (Sachan *et al.*, 2017; Wagh *et al.*, 2014). Besides chemical fertilizers, the use of organic fertilizers like compost, vermicompost, rich compost helps to improve soil health and in turn, enhances the yield per unit of nutrient applied and thereby saving energy (Sohel and Ghosh, 2021; Kumar *et al.*, 2017; Sanwal *et al.*, 2007). Nath *et al.*, (2011) and Naidu *et al.*, (2002) suggested that sustainable and eco-friendly agriculture, which minimizes the use of harmful energy-intensive inputs is achievable through the use of organic and biofertilizers. Thus, INM involves chemical fertilizers along with organic manure, compost, and vermicompost are quite imperative for the maintenance of long-term soil health and productivity of crops (Sohel *et al.*, 2021; Bairwa *et al.*, 2013; Behera *et al.*, 2007). Nutrient use efficiency and soil fertility status may be accelerated due to the use of integrated nutrient sources which also helps to increase productivity and product quality simultaneously (Mondal *et al.*, 2016; Singh *et al.*, 2004; Nanjappa *et al.*, 2001). However, the most suitable combination of chemical fertilizers, organic manures, and biofertilizers for okra cultivation can enhance the growth and yield of studied crops (Wagh *et al.*, 2014; Haque *et al.*, 2012; El-Kader, 2010). Considering these contemporary researches, the current study was designed to screen out a suitable combination of chemical fertilizers and organic manures for sustainable okra cultivation in the northern Ganges floodplain soils of Bangladesh. The findings of our study may act as a guide for the management of soil health as well as for maintaining the optimum growth of okra without any yield penalty.

MATERIALS AND METHODS

The field laboratory of EXIM Bank Agricultural University Bangladesh was the experimental site for conducting the study. The geographical position of the study area was placed between the latitude 24'22 to 24'57 and longitude 87'23 to 88'23 (Wikipedia). The study site was dominated by sub-tropical climates and situated in the northwestern part of Bangladesh. The dry weather with moderate rainfalls was the predominant climatic feature of the study site. Generally, Chapainawabganj is dominated by the tropical wet and dry climate. The monsoon high temperature with high humidity and moderate rainfall is the predominant feature of the climate of Nawabganj. However, our studied okra plant was tolerant to moderately high temperatures (26.3°C to 29.2°C). The month with the highest relative humidity was June (79.50%). The month with the lowest relative humidity was February (37.40%).

Table 1. The treatments applied in the experiment

T ₁ = Control (without fertilizer)	T ₇ = Trichocompost + 75% RFD
T ₂ =100% Recommended Fertilizer Dose (RFD)	T ₈ = Trichocompost + 100% RFD
T ₃ = Compost + 75% RFD	T ₉ = Compost + Vermicompost + Trichocompost + 75% RFD
T ₄ = Compost + 100% RFD	T ₁₀ = Compost + Vermicompost + Trichocompost + 100% RFD
T ₅ = Vermicompost + 75% RFD	T ₁₁ = Compost + Vermicompost + Trichocompost
T ₆ = Vermicompost + 100% RFD	T ₁₂ = Farmer’s practice

Management of field activities and plant protection

Suitable fertilizer doses were used following the Fertilizer Recommendation Guide (BARC, 2012). Proper Integrated Weed Management (IWM) was done to control the weed infestation in the pots. The modified and adapted weed control practice from the early

observation was scheduled in our present study according to Swanton and Murphy (1996).

The physical and yield parameters; plant height (cm), number of leaves, leaf length (cm), flower plant⁻¹, fruit plant⁻¹, average fruit length (cm), average fruit weight (g), 100 seed weight (g), yield plant⁻¹ (g), yield (t ha⁻¹) was obtained during vegetative and reproductive stages, accordingly following the standard methods (Sayed *et al.*, 2020).The physical and chemical attributes of soil were tested in two different stages, 1. Before planting and 2 after the harvesting period of the crop the soil sample was collected from 0-15 cm depth of soil profile presented in Table 2 and Table 3. “The analysis of soil was accomplished following the standard analytical methods” (Page *et al.*, 1982). The soil was collected from the 2 different AEZ (Agro-Ecological Zone) of the country: AEZ 10- Active Ganges Floodplain and AEZ 11- High Ganges River Floodplain for further testing. Net Returns (NR) were calculated by deducting the overall total variable cost (TVC) of cultivation from gross returns (GR) (NR = GR-TVC) (Parihar *et al.*, 2015; Das *et al.*, 2018). The benefit-cost ratio (BCR) is another fundamental indicator for measuring profitability. The equation used for calculating the benefit-cost ratio is $BCR = GR/TVC$.“Mean comparisons between treatments were performed by the least significant difference (LSD) test. All statistical calculations were performed using the F-variance test, statistical significance was indicated at 1% & 5% level of probability” (SAS, 1993).

RESULTS AND DISCUSSION

Nutrient management practices demonstrated a significant effect on the fertility status of the test soil and a very significant effect on the soil's physical and chemical properties.

Table 2. Soil physical properties before planting and after harvesting of okra						
Soil physical properties						
Treatmen ts	Paramet ers	BD (g/cm ³)	PD (g/cm ³)	Porosity (%)	FC (%)	Texture
T ₁	BP	1.50	2.00	24	29	Silty Clay Loam
	AH	1.75	1.90	26	27	
T ₂	BP	1.45	2.20	38	33	
	AH	1.68	2.10	35	30	
T ₃	BP	1.24	2.30	42	35	
	AH	1.55	2.10	36	29	
T ₄	BP	1.59	1.90	39	32	
	AH	1.52	2.00	34	31	
T ₅	BP	1.50	1.90	39	36	
	AH	1.52	2.00	33	34	
T ₆	BP	1.55	1.98	29	28	
	AH	1.59	2.12	33	29	
T ₇	BP	1.40	2.20	32	35	
	AH	1.55	2.18	35	28	
T ₈	BP	1.18	2.40	40	36	
	AH	1.50	2.35	38	34	
T ₉	BP	1.20	2.65	46	38	
	AH	1.00	2.75	48	40	
T ₁₀	BP	1.50	2.50	40	35	
	AH	1.41	2.45	38	36	
T ₁₁	BP	1.35	2.20	28	34	
	AH	1.32	2.30	29	33	
T ₁₂	BP	1.56	1.88	29	25	
	AH	1.60	1.85	27	25	

Notes: BD- Bulk Density; PD- Particle Density; FC- Field Capacity; BP-Before Planting; AH- After Harvesting.

Soil physical properties like soil density, especially, the bulk density of surface soil decreased with the depth and organic matter present in soil and varied from 1.00 to 1.50 g/cm³ in mineral soil. The highest bulk density (1.75 g/cm³) was observed in T₁ (after harvesting) due to the compaction of the soil (Table 1) as compared to initial values (before planting) and the lowest bulk density was observed in T₉ due to high organic matter content of the soil. The highest field capacity (40 %) and porosity (48 %) were observed in T₉ and the lowest field capacity (25 %) and porosity (24 %) were found in T₁₂ (Farmer's practice) and T₁ (control), respectively. The textural class of experimental soil was silty clay loam (Table 1). Similar findings were found in Sayed *et al.*, (2020).

Table 3. Soil chemical properties before planting and after harvesting of okra

Treatments	Parameters	Soil chemical properties								
		Soil pH	OM (%)	EC (µs/m)	Available N (%)	K	P	S	B	Zn
							meq /100 ml		µg/g	
T ₁	BP	6.50	0.30	126	0.08	0.04	04	10	0.02	0.60
	AH	6.40	0.20	116	0.05	0.03	02	08	0.01	0.50
T ₂	BP	6.50	0.80	135	0.30	0.16	10	25	0.10	1.80
	AH	6.20	0.65	129	0.12	0.10	09	25	0.10	1.65
T ₃	BP	6.50	0.80	134	0.25	0.12	11	21	0.11	1.50
	AH	6.70	1.00	127	0.20	0.12	08	20	0.10	1.37
T ₄	BP	6.50	1.20	133	0.21	0.13	15	19	0.11	1.90
	AH	6.60	1.20	127	0.10	0.12	12	14	0.10	1.70
T ₅	BP	6.50	1.10	134	0.32	0.15	13	25	0.10	2.00
	AH	6.50	1.20	138	0.22	0.11	11	25	0.10	1.90
T ₆	BP	6.50	1.50	135	0.33	0.14	12	23	0.09	2.20
	AH	6.60	1.60	135	0.17	0.13	10	19	0.07	2.00
T ₇	BP	6.50	1.50	154	0.28	0.10	13	20	0.08	2.10
	AH	6.70	1.60	145	0.23	0.10	12	15	0.08	1.90
T ₈	BP	6.50	1.45	139	0.20	0.12	13	24	0.10	2.25
	AH	6.40	1.50	132	0.12	0.12	11	22	0.10	2.20
T ₉	BP	6.72	2.20	164	0.41	0.18	18	28	0.30	2.65
	AH	6.98	2.50	165	0.48	0.20	19	30	0.29	2.63
T ₁₀	BP	6.50	2.20	135	0.40	0.12	14	25	0.21	2.60
	AH	6.76	2.20	132	0.20	0.12	16	25	0.10	2.70
T ₁₁	BP	6.50	2.00	133	0.22	0.10	10	20	0.08	2.44
	AH	6.60	2.50	128	0.22	0.09	09	19	0.06	2.47
T ₁₂	BP	6.50	0.70	120	0.12	0.05	07	18	0.04	1.00
	AH	6.30	0.60	111	0.12	0.05	06	12	0.02	0.80
Critical level					0.12	0.20	14	14	0.20	2.0

Notes: OM: Organic Matter, EC: Electrical Conductivity, BP: Before Planting, AH: After Harvesting

The study demonstrated that chemical fertilizers along with organic manure and biofertilizer increased the soil pH cancelling the acidifying effect of chemicals in the soil (Table 2). Chemical fertilizers alone could not help much in improving the pH and fertility status of the experimental soil. Moreover, it marginally reduced the soil pH. After the end of the experiment, INM with compost + vermicompost + rich compost and chemical fertilizers significantly increased organic matter, available N, P, K, and S contents in soil over their initial values. The highest organic matter and available N, P, K, and S contents in soil were recorded from the pot having compost + vermicompost + rich compost and 75% chemical fertilizers (T₉) under the study. The electrical conductivity (EC), Zinc (Zn), and Boron (B) content before planting and after harvesting of soil were not significantly changed (Table 2).

Effect of treatment combinations on okra vegetative and reproductive growth

Vegetative parameters

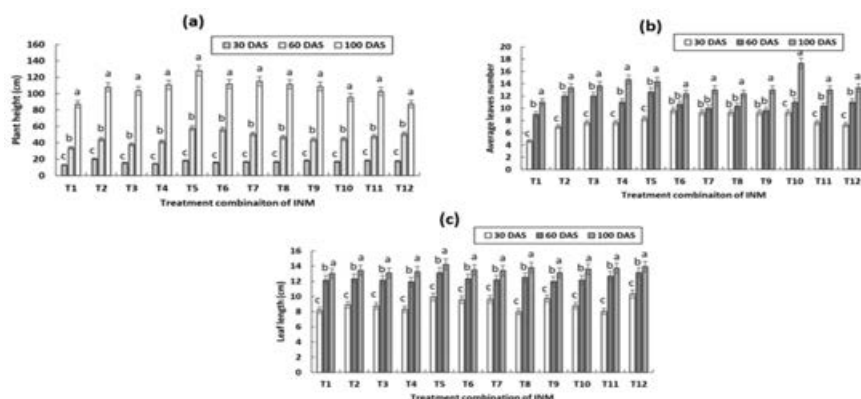


Figure 3. Effects of different treatment combinations of vegetative properties of okra a) plant height (cm) b) leaf number c) leaf length (cm). The lettering denotes the significance of the values at a 5% level of probability after Tukey's post hoc analysis among the treatments by DMRT test.

The vegetative parameters such as plant height, leaf length, and number of leaves were found significant at 30 DAS (Fig. 3). In those cases, the effect of chemical fertilizer on the plant height was found superior. The highest leaf length and number of leaves were observed in T₅ (vermicompost + 75% RFD) and T₆ (vermicompost + 100% RFD) treatment, respectively. In 60 DAS, the effect on plant height was found significant at a 1% probability level, on the other hand, the data for the leaf length and number of leaves were found significant at a 5% level of probability. In all the cases, vermicompost with 75% RFD was found superior. Our study results should correspond with the findings of Barani and Anburani (2004). The effect on leaf length was found statistically non-significant at 100 DAS but the data for the plant height and number of leaves were found significant at 5% and 1% probability level respectively. In these cases, the effect of vermicompost with 75% RFD on the plant height and leaf length was found superior whereas, compost, vermicompost, and trichocompost with 75% RFD produced the maximum number of leaves (Fig. 3). The use efficiency of inorganic fertilizers was also increased due to the controlled application of organic manures in the millet field of central Senegal (Tounkara *et al.*, 2020).

Plant height was documented at varying days after seeding (DAS) such as 30 DAS, 60 DAS, and 100 DAS. At 30 DAS, the height of the plant stood between 12.93 cm and 20.33 cm (Fig. 3a). The maximum height of the plant (20.33 cm) was determined from the T₂ treatment, and the minimal height of the plant was observed from T₁ (control). The effect of chemical fertilizer on the plant height was found superior at 30 DAS. At 60 DAS, plant height varied from 33.67 cm to 58.07 cm.

The number of leaves/plants at different stages of growth showed significant variation with the different doses of manures and fertilizer used in the soil. At 30 DAS, the number of leaves plant⁻¹ was found between 4.67 and 9.67. T₆ treatment showed the highest (9.67) number of leaves plant⁻¹ at 30 DAS and the lowest (4.67) was in T₁ (control). At 60 DAS, it ranged from 9.00 to 12.67. The highest number of leaves (12.67) was recorded from T₅ and the lowest (9.00) was observed in T₁ (control). At 100 DAS, the maximum number (17.33) of leaves was recorded for the treatment of T₁₀, and the lowest 11.00 was found from T₁ (control) treatment (Fig. 3b).

The leaf length was significantly varied in different INM treatments. The maximum leaf length of 30 DAS (10.32 cm), 60 DAS (13.17 cm), and 100 DAS (14.22 cm) were recorded

in the treatments T₁₂, T₁₂, and T₅ respectively (Fig 3c). The minimum leaf length 30 DAS (8.000 cm), 60 DAS (11.92 cm), and 100 DAS (13.00 cm) were noted in the treatments T₈, T₄, and T₁ respectively (Fig. 3c)

Table 4. The reproductive parameters of the studied okra plant

Treatments	Number of flower plants ⁻¹	Number of fruit plants ⁻¹	Individual fruit length (cm)	Individual fruit wt. (gm)	100 seeds wt. (gm)	Yield plant ⁻¹ (gm)	Yield (t ha ⁻¹)
T ₁	10.67	9.67	11.78	11.55	5.83	112.03	4.67
T ₂	29.00	27.33	11.85	13.68	5.73	373.63	15.57
T ₃	27.67	27.33	11.54	13.15	5.38	359.73	14.99
T ₄	26.00	25.67	11.70	13.19	5.69	339.83	14.16
T ₅	20.00	19.33	13.30	15.30	5.88	297.68	12.40
T ₆	25.67	24.00	11.59	13.08	5.32	313.84	13.08
T ₇	21.67	21.00	11.06	13.78	5.14	290.78	12.12
T ₈	19.00	19.00	11.13	12.72	5.81	228.96	10.54
T ₉	30.00	29.33	12.15	14.78	5.89	388.38	16.18
T ₁₀	24.00	23.67	10.56	11.46	5.31	271.69	11.32
T ₁₁	26.67	26.33	10.45	13.24	4.75	348.82	14.53
T ₁₂	20.33	19.67	11.63	12.11	5.64	239.34	9.97
LS	**	**	*	*	*	**	**
LSD at 5%	2.0057	3.2268	2.2055	2.4802	0.7120	69.727	69.727
CV	5.09	8.63	11.32	11.17	7.72	14.07	14.07

DAS: Days After Sowing, LSD: Least Significant Difference, LS: Level of Significance, CV: Coefficient of Variation, NS: Non-Significant, * indicates 5% Level of Significance, ** indicates 1% Level of Significance.

Vermicompost with 75% RFD was found superior in the case of individual fruit length and individual fruit weight (Sinha, 2009). But compost, vermicompost, and trichocompost with 75% RFD showed significantly better results in terms of the number of flowers, number of fruits, 100 seed weight, and yield. The data for the number of flowers, fruit, and yield was found statistically significant, whereas, the data for the individual fruit length, individual fruit weight, and 100 seeds weight was found non-significant (Table 4). The increase in yield of Okra was due to the combined application of organic manure and inorganic fertilizer is in line with the findings of Sanwal *et al.*, (2007) in turmeric, Patil *et al.*, (2004) in tomato, and Premsekhar and Rajashree, (2009) in Okra.

Assessment of yield and yield parameters

The number of fruits plant⁻¹ also varied with different treatments. Fruits number varied from 9.67 to 29.33 with the highest number of fruits plant⁻¹ (29.33) in T₉ and the lowest number of fruits plant⁻¹ (9.67) in treatment T₁. The effect of several fruits was found significant at the probability level of 1% (Table 4). Different treatments showed significant variation in the fruit length. The fruit length ranged from 10.45 cm to 13.30 cm. The highest fruit length (13.30 cm) was found in treatment T₅ (vermicompost + chemical fertilizer) and the lowest fruit length (10.45) was observed in treatment T₁ (control). The effect of fruit length was found significant at the 5% probability level (Table 3). The weight of the fruit varied significantly with different types of treatment techniques. The individual fruit weight was between 11.55 g and 14.78 g. The highest individual fruit weight (14.78 g) was found in treatment T₉ and the lowest value (11.55 g) was observed in treatment T₁. The effect of fruit length was found significant at the 5% probability level (Table 4).

The yield performance varied significantly in the dissimilar types of treatment. The cumulative yield ranged from 4.67 t/ha to 16.18 t/ha whereas the maximum yield (16.18 t/ha) was found in T₉ and the minimum yield (4.67 t/ha) was observed in T₁ (control). The effect of yield performance of okra was significant at the probability level of 1% (Table 4). Our study result was also supported by the previous observations (Sachan *et al.*, 2017).

Economic analysis

Variable costs for production, Benefit Cost Ratio (BCR) of okra cultivation in the combined effect of different treatments. The cost currency is converted from BDT (Bangladeshi Taka) to USD (\$) (1 BDT = 0.0125 \$). The price of okra is \$0.5/Kg. BCR is the ratio of variable costs and gross return.

Table 5. Costs in Treatment combination (USD), depending on the treatment applied

Cost items	Costs in Treatment combination (USD)											
	T ₁	T ₂	T ₃	T ₄	T ₅	T ₆	T ₇	T ₈	T ₉	T ₁₀	T ₁₁	T ₁₂
Labors	270	295	275	269	270	277	275	273	275	273	278	209
Fuel	435	455	425	420	422	428	425	427	425	425	428	422
Manure	-	-	150	150	160	155	165	161	144	144	208	96
Urea	-	56.5	36.5	46.5	36.5	46.5	35.5	49	36.5	49.5	-	34
TSP	-	46	28	36	28	35	28	43	28	42	-	28
MP	-	60	34.5	44	34.5	44.5	34.5	47	34.5	46.5	-	31
Gypsum	-	23	08	12	08	13.5	09	9.92	12	16	-	11
Irrigation	260	285	242	238	242	245	242	239.3	242	243	277	240
Pesticide	225	195	165	166	165	156	163	160.7	167	175	173	195
TVC	119	1415.	136	1381.	136	1400.	137	1410.	136	141	136	126
GR	0	5	4	5	6	5	7	1	4	4	4	7
NR	233	7785	749	7080	620	6540	606	5270	809	566	726	498
BCR	5	5	5	5	0	0	0	0	0	0	5	5
	114	6369.	613	5698.	483	5139.	468	3859.	672	424	590	371
	5	5	1	5	4	5	3	9	6	6	1	8
BCR	1.96	5.50	5.49	5.12	4.54	4.67	4.40	3.74	5.93	4.00	5.32	3.93

Notes: TVC: Total Variable Costs, GR: Gross Return, NR: Net Return, BCR: Benefit Cost Ratio, BCR = GR/TVC

CONCLUSIONS

This study explains that inorganic and organic fertilizer combinations are higher in almost all vegetative and reproductive parameters of okra. The combination of 75% recommended inorganic fertilizers and 25% organic manures derived from compost, vermicompost, and trichocompost exhibited the best beneficial performance in terms of soil physiochemical, economical, and production perspectives during okra cultivation. Blended applications of organic and inorganic nutrient sources are more productive and support soil fertility. Further multi-site and multi-trial research should be carried out to suggest the most appropriate combinations of inorganic and organic fertilizers for maximum benefit. Therefore, integrated nutrient management can be employed as an economically viable technique for the governance of sustainable farming.

REFERENCES

1. BARC (2012). Fertilizer Recommendation Guide. Pub. No. 41. Bangladesh Agricultural Research Council, Farmgate, Dhaka. Pp. 65.
2. Behera U.K., Sharma A.R. and Pandey H.N. (2007). Sustaining productivity of wheat-soybean cropping system through integrated nutrient management practices on the Vertisols of central India. Plant Soil. 297:185-199.
3. Chowdhury R. (2004). Effects of chemical fertilizers on the surrounding environment and the alternative to chemical fertilizers. IES-ENVIS Newsletter. 7: 4-5.

4. El-Kader, Abd A.A. Shaaban S.M. and Abd El-Fattah M.S. (2010). Effect of irrigation levels and organic compost on okra plants (*Abelmoschus esculentus* L.) grown in sandy calcareous soil. *Agric. Biol. J. N. Am.* 1 (3): 225–231.
5. Food and Agriculture Organization (FAO) (2016). FAOSTAT: 20-16. FAO, Rome.
6. Haque M.M., Ilias G.N.M. and Molla A.H. (2012). Impact of Trichoderma-enriched biofertilizer on the growth and yield of mustard (*Brassica rapa* L.) and tomato (*Solanum lycopersicon* Mill.). *Agriculturists.* 10(2): 109–119.
7. Kumar R. (2019). The Impact of chemical fertilizers on our environment and ecosystem. In Book: Research Trends in Environmental Sciences. Edition 2nd & Chapter 5: 69-86. Chandini, Randeep kumar, Ravendra kumar and Om Prakash.
8. Kumar V., Saikia J. and Nath D.J. (2017). Effect of integrated nutrient management on growth, yield, and quality of okra (*Abelmoschus esculentus* L.) Moench) cv. Arka Anamika. *International J. of Chemical Studies.* 5(5): 2001–2003.
9. Naidu A.K., Kuswah S.S. and Dwivedi Y.C. (2002). Influence of organic manures, chemical, and biofertilizers on growth, yield, and economics of brinjal. *South Indian Hort.* 50: 4–6.
10. Nanjappa H.V., Ramachandrappa B.K. and Mallikaryana B.O. (2001). Effect of integrated nutrient management on yield and nutrient balance in maize. *Indian J. Agron.*, 46 (4), 698–701.
11. Nath D.J., Ozah B., Baruah R., Barooah R.C. and Borah D.K. (2011). Effect of integrated nutrient management on soil enzymes, microbial biomass carbon, and bacterial populations under rice (*Oryza sativa*)–wheat (*Triticum aestivum*) sequence. *Ind. J. Agric Sci.* 81: 1143–1148.
12. Olaniyi J.O., Akanbi W.B., Olaniran O.A. and Ilupeju O.T. (2010). The effect of organo-mineral and inorganic fertilizers on the growth, fruit yield, quality, and chemical composition of okra. *J. Animal and Plant Sci.* 9 (1): 11351140.
13. Olsen S.R., Cole C.V., Watanabe F.S. and Dean L.A. (1954). Estimation of Available Phosphorus in Soil by Extraction with Sodium Bicarbonate. Circular 939, United States Department of Agriculture, Washington DC.
14. Page A.L., Miller R.H. and Keeney D.R. (1982). Methods of soil analysis, Part-2. Ameri. Soc. Agron. Madison, WI, USA.
15. Parihar C.M., Jat S.L., Singh A.K., Kumar B., Singh Y., Pradhan S. and Pooniya V. (2015). Conservation agriculture in irrigated intensive maize-based systems of North Western India: effects on crop yields, water productivity and economic profitability. *Field Crops Res.* 104–116.
16. Patil M.B., Mohammed R.G. and Ghadge P.M. (2004). Effect of organic and inorganic fertilizers on growth, yield, and quality of tomato. *J. Maharashtra Agric. Univ.* 29: 124–127.
17. Raj A.K. and Kumari V.L.G. (2001). Effect of organic manures and Azospirillum inoculation on yield and quality of okra (*Abelmoschus esculentus* L. Moench). *Veg. Sci.* 28(2): 179-181.
18. Sayed A., Sarker A., Kim J.E., Rahman M. and Mahmud M.G.A. (2020). Environmental sustainability and water productivity on conservation tillage of irrigated maize in red-brown terrace soil of Bangladesh. *Journal of the Saudi Society of Agricultural Sciences.* 19: 276–284.
19. Sachan S., Singh D., Kasera S., Mishra S.K., Tripathi Y., Mishra V. and Singh R.K. (2017). Integrated nutrient management (INM) in Okra (*Abelmoschus esculentus* (L.) Moench) for better growth and higher yield. *J. Pharm. Phytochem.* 6 (5): 1854–1856.
20. Sanwal S., Lakshminarayana K., Yadav R., Rai N., Yadav D. and Mousumi B. (2007). Effect of organic manures on soil fertility, growth, physiology, yield, and quality of turmeric. *Ind. J. Hort.* 64(4): 444 –449.
21. SAS (1993). Institute Inc. SAS Technical Report R-109, Conjoint Analysis Examples; SAS Institute Inc: Cary, NC, USA. Singh T.R., Singh S., Singh S.K., Singh M.P. and Srivastava B.K. (2004). Effect of integrated nutrient management on crop nutrient uptake and yield under okra-pea-tomato cropping system in molisol. *Indian J. of Hort.* 61 (4): 312–314.
22. Sohel M.H. and Ghosh M.K. (2021). Effect of Compost, Vermicompost, Trichocompost, and NPKS Fertilizers on the Growth, Yield, and Yield Components of Capsicum (*Capsicum annum* L.). *An Academic Journal of EXIM Bank Agricultural University Bangladesh.*, 3: 29-35.
23. Swanton C.J. and Murphy S.D. (1996). Weed science beyond the weeds: The role of integrated weed management (IWM) in agro-ecosystem health. *Weed Sci.* 44: 437–45.
24. Tounkara A., Clermont-Dauphin C., Affholder F., Ndiaye S., Masse D. and Cournac, L. (2020). Inorganic fertilizer uses the efficiency of millet crops increased with organic fertilizer application in rainfed agriculture on smallholdings in central Senegal. *Agric. Ecosys. Environ.* 294, <https://doi.org/10.1016/j.agee.2020.106878>.
25. Wikipedia Chapai Nawabganj District. (2020). In *Wikipedia*. https://en.wikipedia.org/wiki/Chapai_Nawabganj_District.
26. Wagh S.S., Laharia G.S., Iratkar A.G. and Gajare A.S. (2014). Effect of INM on nutrient uptake, yield, and quality of okra [*Abelmoschus esculents* (L.) Moench]. *An Asian J. Soil Sci.* 9 (1): 21–24.

Improvement of the urban microclimate with aromatic and medicinal plants

K. I. Maknea¹, N. Thymakis², J. N. Tzortzi³ and A. C. Asănică⁴

¹Administrative Region of Central, Thessaloniki, Greece

²Center of Agricultural Training, Balkan Botanic Garden of Kroussia, Hellenic Agricultural Organization Dimitra, Themi, Thessaloniki, Greece

³Politecnico Milano, Milano, Italy

⁴University of Agronomic Sciences and Veterinary Medicine of Bucharest, Bucharest, Romania

*Corresponding author kristinamaknea@gmail.com

ABSTRACT

The paper examines the bioclimatic contribution of urban gardening through the use of aromatic and medicinal plants, proposing an innovative approach to urban design. The research aims to evaluate the role of these plants in improving the urban microclimate, focusing on their ability to reduce temperature, increase relative humidity, and contribute to the creation of a synergistic ecosystem that attracts beneficial insects. The methodology involved experimental research at eight sites in Thessaloniki, where temperature, humidity, and solar radiation were recorded. At the sites with aromatic and medicinal plants, a temperature reduction of 10-15% was observed compared to the non-planted areas. Significant improvement was recorded in areas simulating balconies, where potted plants led to a temperature reduction of 5-10%. Furthermore, areas with dense vegetation and water features showed greater humidity, while air circulation was limited, creating adverse thermal comfort conditions. In locations with small arrangements of aromatic plants, a reduction in discomfort perception of up to 26% was observed. The study highlights the innovative potential of integrating aromatic and medicinal plants into urban design for improving the microclimate. Replacing grass in public spaces and using these plants on vertical and horizontal surfaces can improve thermal comfort, aesthetic value, and attract beneficial insects, such as bees.

Keywords: aromatic medicinal plants, urban microclimate, urban and bioclimatic design, landscape architecture, urban horticulture

INTRODUCTION

Rapid urbanization and climate change pose significant challenges to modern cities, particularly affecting the quality of the microclimate, thermal comfort, and the well-being of residents. The urban heat island effect and the increase in extreme weather events make it urgent to find sustainable solutions that improve the quality of life for urban populations. In this context, urban gardening, as a form of urban green space, offers an innovative strategy for improving the microclimate and enhancing city sustainability (Ali-Toudert and Thorsson, 2021; Shafique *et al.*, 2020). Urban gardening combines aesthetic,

environmental, and social benefits through bioclimatic approaches, reducing extreme temperatures, increasing humidity, and enhancing biodiversity (Petrovska, 2012; Hardy and Totelin, 2016).

This paper focuses on aromatic and medicinal plants and explores the impact of urban gardening or urban green spaces on the microclimate and quality of life in modern cities. Their incorporation into urban design offers solutions for improving public spaces and enhancing the energy efficiency of cities (Langemeyer *et al.*, 2021). Specifically, it examines the application of bioclimatic principles in landscape architecture, which leverage local climatic conditions to improve thermal comfort and reduce the impact of the urban heat island effect (Li *et al.*, 2020; Azunre *et al.*, 2019).

Furthermore, the study analyzes the significance of urban gardening for society, the economy, and public health. Citizen participation in gardening projects strengthens the connection with nature, promotes social interaction, and contributes to mental and physical well-being (Piedrahita *et al.*, 2020; Schreinemachers *et al.*, 2020). The development of therapeutic gardens and the integration of plants into public spaces have already proven effective in enhancing the quality of life for residents, while the creation of green oases in cities highlights the importance of sustainability and resilience (Chew-and Maller, 2023; Tsagalidou, 2013).

This research focuses on harnessing the potential of aromatic and medicinal plants to address urban development challenges, proposing strategies for their integration into urban design and upgrading public spaces. It aims to highlight the multiple benefits of these plants for the urban environment and the health of citizens, offering new perspectives for creating sustainable and thriving cities (Specht *et al.*, 2021; Raji *et al.*, 2021).

MATERIALS AND METHODS

Study Area

The experiments were conducted in eastern Thessaloniki to minimize external influences such as tall buildings, dense vegetation, or water bodies. The study area included the Research Institute of the Ministry of Agriculture of Northern Greece, the International Hellenic University, and the Urban Horticultural Garden of the School of Agriculture at Aristotle University of Thessaloniki. These locations provided a variety of urban and peri-urban conditions, ensuring comprehensive analysis. Eight experimental sites (Table 1) were selected, representing different surface types, including vegetated and non-vegetated areas, to evaluate the bioclimatic role of aromatic and medicinal plants.

Table 1. Experimental Sites

A	B	C	D	E	F	G	H
Concrete surface with shaded pots of aromatic plants	Soil area with aromatic and medicinal plants	Landscaped 'Mediterranean corner' with plants and natural elements	Bare soil with grass cover	Recreational area with plants, pond, and gravel	Urban garden with mixed vegetation	Concrete surface, no vegetation	Bare soil, no vegetation

Experimental Procedure

The study aimed to evaluate the bioclimatic role of aromatic and medicinal plants in urban environments, particularly their impact on thermal comfort. Eight experimental sites (A–H), representing varying vegetation types and controls, were mapped and characterized. Instruments, including weather stations, soil moisture sensors, and solar radiation

sensors, measured environmental data such as temperature and humidity at ground level to simulate human heat perception. Measurements were conducted on sunny days between 12:30 and 14:00 over a ten-day period (July 4 to August 7, 2023), comparing shaded and non-vegetated areas. Findings were validated using data from the ELGO weather station and Thessaloniki Airport, ensuring robust results representative of broader climatic patterns (Georgi and Zafiriadis, 2006). This framework highlights the potential of these plants to enhance thermal comfort and regulate urban microclimates.

To quantify the microclimatic impacts of vegetation, key parameters such as air temperature and humidity variations, solar radiation reduction, thermal comfort, and standard deviation were analyzed. Temperature and humidity variations were assessed by comparing measurements under shaded and sunlit conditions, highlighting the cooling effect and humidity enhancement provided by vegetation. Solar radiation reduction was evaluated as the percentage of radiation filtered by plant foliage, reflecting vegetation's shading efficiency (del Campo-Hitschfeld et al., 2023). Thermal comfort was measured using the Discomfort Index (DI), which incorporates air temperature and relative humidity to determine heat stress levels. The study compared DI values between vegetated and non-vegetated areas, calculating the reduction in discomfort in shaded environments (Xu et al., 2017; Nurmaya et al., 2022; Georgi and Dimitriou, 2010). The variability of temperature and humidity across different sites was analyzed using standard deviation to investigate the stability of microclimatic conditions. This process will be used to explore the dynamics of vegetated areas compared to non-vegetated surfaces, aiming to assess their respective contributions to microclimatic regulation. Previous studies suggest that vegetated areas are likely to demonstrate environmental variability, while impervious surfaces may exhibit consistent heat accumulation (Bady, 2014; Martinez and Bartholomew, 2017). These methods provide a framework for understanding the bioclimatic role of vegetation in urban environments.

The collected data were tabulated and analyzed using Microsoft Excel. Graphical representations were generated to illustrate comparisons between vegetated and non-vegetated points, enabling conclusions about vegetation's bioclimatic efficiency.

RESULTS AND DISCUSSIONS

The evaluation of environmental conditions (temperature, relative humidity, and brightness) at the eight experimental sites selected to study the impact of the environment on the cultivation of aromatic and medicinal plants reveals apparent differences related to site configuration and climatic conditions on the measurement days. The results are illustrated in graphs. (Figure 1).

At site A, covered with a black mesh for shading and used for potted plant cultivation, temperatures are relatively moderate, with maximum values ranging between 38-40°C on July 24 and 30. The shading effectively reduces temperatures, while humidity remains between 40% and 50%. Brightness levels are moderate due to the shading, with the highest intensity recorded on July 21.

At site B, which features unprotected soil, temperatures reach up to 40°C on July 19 and 23 due to direct solar radiation. Humidity levels are higher compared to other sites, peaking at 60% on July 20 and 24. Brightness is high as the site is fully exposed to the sun, with the most incredible intensity observed on July 21 and 25.

Site C, designed as a Mediterranean garden with various materials, has slightly lower temperatures, primarily due to shading and natural vegetation. Maximum temperatures range from 36-38°C on July 21 and 30, with humidity levels around 50%-55% on July 18 and 22. Brightness is high but mitigated by trees and natural elements in the area.

At site D, which consists of grass-covered ground, temperatures reached 40°C on July 25 and 28 due to intense solar radiation. Humidity remained between 45% and 50%, and brightness was also high as the site was exposed to sunlight without additional protection. At site E, temperatures are lower, with maximum values around 35°C on July 22 and 25. Due to the presence of water and vegetation, humidity is particularly high, reaching 60% on July 22 and 27. Brightness is high, with peak values recorded on July 21 and 25. At site F, located in the university's vegetable garden, temperatures are among the highest, reaching 42°C on July 20 and 23. Due to urban conditions, humidity remains low, approximately 30%-35%. Brightness is intense and stable, with peak values on July 21 and 27. At site G, a concrete area with no vegetation, temperatures peak at 45°C on July 21 and 30 due to the urban heat island effect. Humidity is the lowest among all sites, around 20% to 25%. Brightness is extremely high, as the area is fully exposed to sunlight. Finally, at site H, which consists of bare soil, temperatures are also very high, reaching 44°C on July 20 and 30. Humidity remains low, around 20% to 25%, while brightness is very high, especially on July 21 and 28.

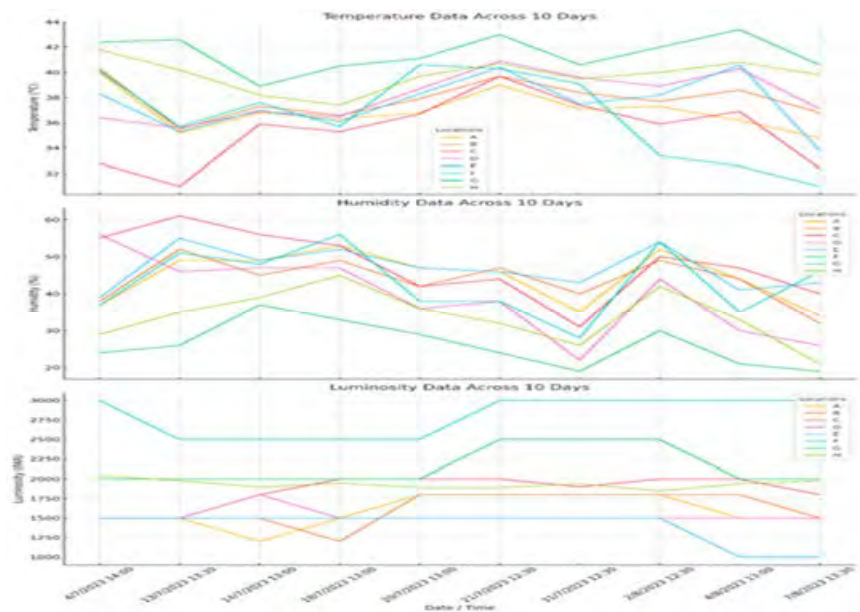


Figure 1. Comparative analysis of temperature, humidity, and brightness in experimental sites

Site D, characterised by a grass-covered surface, served as a reference point for comparing the temperature effects of other surfaces (Figure 2). Vegetated sites (C, E) exhibited consistently favourable variations, ranging from 8.9% to 16.4% relative to site D, confirming the effectiveness of dense vegetation in reducing temperatures. The highest positive variation was recorded at site C on August 2, 2023 (+14.5%), indicating the maximum cooling effect during days with high temperatures. Sites with limited vegetation or paved surfaces (A, B) showed more minor variations, generally below 5%, and occasionally recorded slightly negative values, suggesting a limited cooling effect. Non-vegetated sites (G and H) demonstrated significant negative variations compared to site D, with values reaching as low as -19.7% (site G, July 13, 2023), highlighting the exacerbation of the urban heat island effect on impervious surfaces.

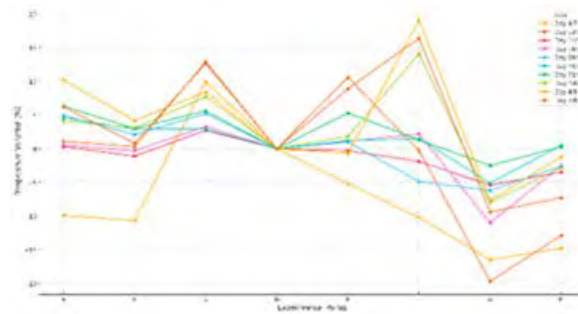


Figure 2. Temperature variation (%) relative to experimental point D across days

Site G, a reference point, is characterized by an impervious surface without vegetation and exhibits the most pronounced urban heat island effect (Figure 3). Vegetated sites (C, E, F) showed significant temperature reductions compared to site G. On August 4, 2023, site F recorded a positive variation of 24.9%, representing the highest cooling effect. Mixed-use sites (A, B) displayed moderate variations between 5% and 10%, with a weaker cooling effect than densely vegetated sites. Impervious surfaces (H) exhibited negative or nearly negligible variations relative to site G, confirming their lack of contribution to temperature reduction. The smallest variation was recorded on August 7, 2023 (-5.0%).

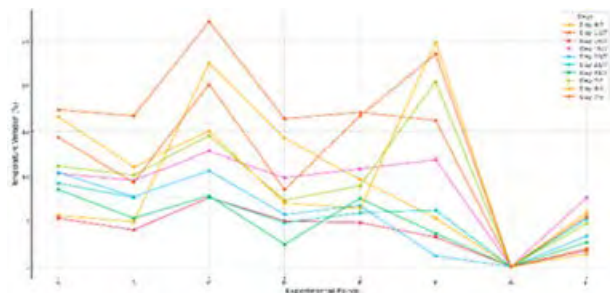


Figure 3. Temperature variation (%) relative to experimental point G across days

Site H, another impervious surface used as a reference point, corroborated the findings observed at site G. Vegetated sites (C, E) showed significant favourable variations, with the highest recorded on July 31, 2023, at site C (+10.3%), emphasizing the effectiveness of dense vegetation in lowering temperatures (Figure 4). Mixed-use sites (A, B) exhibited moderately favourable variations, ranging between 3% and 6%. Site G consistently showed negative variations, averaging -15.1%, indicating that its impervious surface significantly contributes to increased local temperatures.

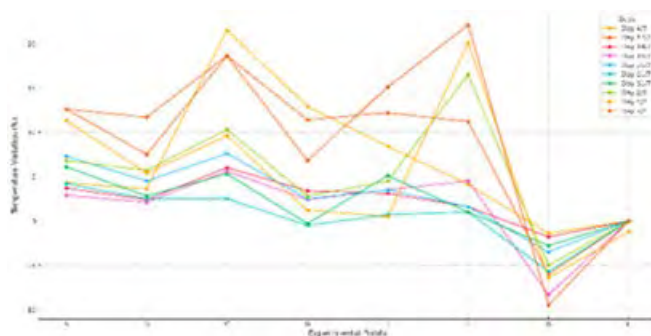


Figure 4. Temperature variation (%) relative to experimental point H across days

Days with higher temperatures (August 2 and 7) highlighted more pronounced differences between vegetated sites and impervious surfaces. For instance, on August 2, 2023, site C demonstrated maximum favourable variations of +14.5% (compared to site G) and +10.3% (compared to site H), underscoring the effectiveness of vegetation in mitigating heat stress conditions.

Figure 5 presents the parameters' average temperature, humidity, and standard deviations for the eight experimental sites studied. Data analysis reveals significant variations in environmental conditions directly associated with site configuration, the presence or absence of vegetation, and surface construction materials.

The calculations showed that the lowest standard deviation (STDEV) values were recorded at site G, covered with concrete (1.4), and site H, covered with soil (1.27). This suggests that surface materials at these test sites are influenced primarily by air conditions rather than other climatic factors. Analyzing the standard deviation (STDEV) and the mean (AVERAGE) provides valuable insights into the impact of urban surfaces on microclimates.

The average temperatures (AVERAGE) indicate that concrete surfaces exhibit the highest values (~41.51°C), demonstrating the pronounced impact of these materials on the urban heat island effect. Temperature standard deviation (STDEV), ranging from 1.27 to 3.47, reflects the variability in temperature across different areas. Higher values (e.g., 3.47) in cultivated areas suggest greater instability, potentially due to varying vegetation density or shading effects.

The average humidity values (AVERAGE), with the highest at 44.60% in shaded areas with potted plants, highlight the cooling effect of vegetation. Conversely, concrete areas exhibit the lowest humidity (~26.20%), illustrating the dehydrating conditions these surfaces create. Humidity standard deviation (STDEV), ranging from 5.53 to 10.75, is highest in cultivated areas (10.75), reflecting the influence of variable factors such as crop type or water management practices.

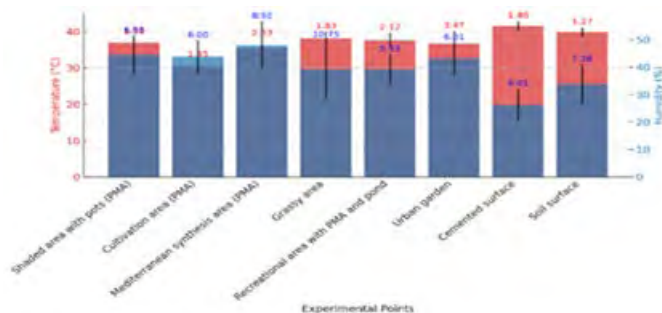


Figure 5. Average temperature and humidity with standard deviation

The graph in Figure 6 illustrates the variability of active solar radiation (%) for each measurement point (A, B, C, D, E, F, G) over 10 days. The analysis focuses on various surfaces, with measurements taken at eight distinct locations (A, B, C, D, E, F, G, H), representing different surface types that may influence the distribution and absorption of solar radiation.

Surfaces A (plants), B (crops), D (grass), and E (park with vegetation and shrubs) display relatively stable solar radiation values, around 24-26%, indicating good capacity for absorption and dispersion of solar energy. In contrast, surfaces G (concrete) and F (urban gardens) exhibit significant fluctuations and lower values, reaching as low as -62.25%. This suggests that these surfaces are more vulnerable to changes in weather conditions and the angle of solar radiation incidence.

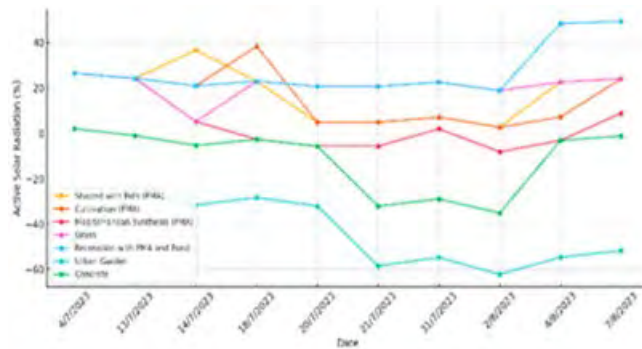


Figure 6. Active solar radiation (%) across different surfaces

Figure 7 illustrates the reduction in the Discomfort Index in shaded areas (DIsh) compared to sunny conditions (DI_{sun}) at site D. The most significant reductions are observed at sites C and E, where values reach up to 9% and 5%, respectively, highlighting the substantial cooling effect of shade. In contrast, sites A and B show less pronounced reductions, with some dates recording negative values. These negative values may be attributed to other environmental factors, such as fluctuations in temperature or humidity, that influence the Discomfort Index.

Additionally, the effectiveness of shade varies over the observation period, suggesting that the impact of shading is strongly dependent on weather conditions and changes in environmental parameters. This variability underscores the complex interplay between vegetation shading and local climatic factors in alleviating discomfort.



Figure 7. Reduction in Discomfort Index (DIsh) (%) across experiment points

CONCLUSIONS

This study highlights the innovative use of aromatic and medicinal plants to improve the urban microclimate, providing significant benefits for environmental quality and urban sustainability.

Temperature and humidity measurements indicate that areas with aromatic medicinal plants, such as site C-100 m² landscaped "Mediterranean corner" with succulents, aromatic medicinal plants, stones, slate, tree trunks, *Cupressus sempervirens*, soil, and black geotextile fabric, exhibited the most favourable conditions. In contrast, the concrete site (G-200 m² concrete surface with no vegetation (courtyard of the International Hellenic University)) recorded the highest temperatures and the lowest humidity levels. Significant differences were observed in vegetated areas, which enhanced natural cooling and humidity in contrast to sites covered with inorganic materials.

Aromatic and medicinal plants reduce ground-level temperatures and attract beneficial insects, enhancing urban biodiversity.

The findings suggest that incorporating such plants into public spaces and urban surfaces, such as pots or vertical concrete structures, can significantly improve sustainability and environmental aesthetics while reducing maintenance workload and irrigation requirements.

This study underlines the value of aromatic and medicinal plants as tools for urban design to enhance the urban microclimate. It recommends their broader application to develop sustainable and healthy cities. In the future, similar solutions should be implemented in larger urban spaces to enhance environmental quality and create more resilient and functional microclimates.

REFERENCES

1. Ali-Toudert F. and Thorsson S. (2021). Bioclimatic principles for urban landscape design. *Urban Climate*, 34, 100-115.
2. Azunre J. A., Mensah P., Johnson D. R., Amoako K., and Tetteh, S.(2019). Urban planning and sustainable cities: A bioclimatic approach. *Urban Sustainability Review*, 3(4), 47-59.
3. Bady M. (2014). Analysis of outdoor human thermal comfort in three major cities in Egypt. *Open Access Library Journal*, 1, e457.
4. Chew C. and Maller C. (2023). Therapeutic horticulture in urban environments: Benefits for health and wellbeing. *Journal of Environmental Psychology*, 71, 123-135.
5. del Campo-Hitschfeld, M. L., Arenas, N., Rivera, M.–and Ballesteros-Pérez, P. (2023). Application of spectrometry for determining solar radiation of deciduous trees' shade. *Buildings*, 13(5), 1130.
6. Georgi J. N. and Dimitriou D. (2010). The contribution of urban green spaces to the improvement of environment in cities: Case study of Chania, Greece. *Building and Environment*, 45(6), 1401–1414.
7. Georgi N. J. and Zafiriadi, K. (2006). The impact of park trees on microclimate in urban areas. *Urban Forestry and Urban Greening*, 5(2), 93–104.
8. Hardy M. P. and Totelin L. (2016). Herbal medicine in ancient Greece and Rome. *Pharmaceutical Histories*, 23, 212-225.
9. Langemeyer J., Baró F., Haase D., Gómez-Baggethun E. and Kronenberg J. (2021). Urban green infrastructure for climate adaptation. *Environmental Science & Policy*, 107, 12-22.
10. Li D., Sun, T., Zhao H., Cui, J. and Dong L. (2020). Designing green spaces to mitigate urban heat island effects. *Journal of Environmental Management*, 268, 110708.
11. Martinez M. N. and Bartholomew M. J. (2017). Interpreting and calculating different types of means and standard deviations. *Pharmaceutics*, 9(2), 14.
12. Nurmaya E., Abidin A., Hasanah N. and Asmara A. (2022). Heat Stress Analysis Using Discomfort Index Method: Impact on Macro-Environmental in Yogyakarta. *Journal of Ecological Engineering*, 23(1), 286-295.
13. Piedrahita A., Blanco J., Ramirez L., Torres, M. and Gonzalez E. (2020). Social impacts of urban gardening: Health and community engagement. *Urban Studies*, 57(6), 1125-1137.
14. Petrovska B. B. (2012). Traditional medicinal plants as natural remedies for improving human health. *Phytotherapy Research*, 26(1), 1-16.
15. Raji B., Smith T., Patel K., Zhou Y. and Nguyen L. (2021). Co-cultivation techniques for resilient urban landscapes. *Environmental Management*, 62(5), 767-778.
16. Schreinemachers P., Wu M., Uddin N., Ahmad S. and Hanson P. (2020). Nutrition and urban agriculture: Promoting healthy behaviors in low-income cities. *Agricultural Systems*, 178, 102736.
17. Shafique M., Kim R., Hwang J., Lee D. and Park S. (2020). The role of urban greenery in improving thermal comfort and air quality. *Landscape and Urban Planning*, 198, 103-118.
18. Specht K., Siebert R., Thomaier S., Freisinger U. and Sawicka M. (2021). Integrating ecology into urban landscaping: Challenges and opportunities. *Landscape Ecology*, 36, 1943-1955.
19. Tsagalidou E. (2013). Vertical farming: An innovative solution for urban gardening. *Urban Agriculture Magazine*, 32(4), 12-18.
20. Xu Z., Wang, Y., Chen, Q., Zhang H. and Li X. (2017). Urban heat island and discomfort index: A case study. *Energy and Buildings*, 156, 195–207.



Potato growing

Tuber yield components of potato (*Solanum tuberosum* L.) as influenced by earthing up and inflorescence removal

D. Mosneh Wudu¹, M. Workineh Kassa² and B. Negash Demissie²

**¹Mekdela Amba University, College of Agriculture and Natural Resource,
Department of Horticulture, Tulu Awliya, Ethiopia**

**²Debre Markos University, College of Agriculture and Natural Resource,
Department of Horticulture, Debre Markos, Ethiopia**

Corresponding author email: dresmosneh@gmail.com

ABSTRACT

Inappropriate agronomic practices, such as earthing up and inflorescence removal, are responsible for the great discrepancy between the potential and achievable yields of potatoes in Ethiopia. To assess the effects of the combination on the tuber yield and yield components of potato, a factorial combination of four earthing up rounds (without, one, two, and three rounds) and three inflorescence removal levels (without removal, bud removal, and flower removal) was used in a randomized complete block design with three replications. The number of marketable, unmarketable and total tubers per hill; and the marketable, unmarketable, and total tuber yields significantly differed in terms of the combined effects of factors. The maximum marketable tuber number (10.07 count/hill), the maximum total tuber number (13.17 count/hill), the marketable tuber yield (31.49 t/ha), and the total tuber yield (33.08 t/ha) were associated with bud removal combined with three rounds of earthing up. Thus, this combination of treatments can be advised for potato production.

Keywords: Assimilates, Reproductive part, Stolons, Tuber initiation, Tuberization

INTRODUCTION

Potato (*Solanum tuberosum* L.) from the Solanaceae family is the world's most important food security crop, owing to its ability to produce large amounts of crops in a short period and high nutritional products per unit input (Limenih and Tefera, 2014). In Ethiopia, it is the most important crop in terms of both economic importance and food production (Degebasa, 2019). Ethiopia has ideal climatic and edaphic conditions for high-quality potato production; it may have the highest potential for potato production in all of Africa. However, potato production in Ethiopia currently occupies only 2% of its potential land (Gebremedhin *et al.*, 2008).

In 2020/2021, compared with the average yield of 15 t/ha in Africa and 21 t/ha globally, the average yield was 13.28 t/ha, with a total production of 11.15 million tons in 85,988 hectares of land (FAOSTAT, 2022). Several production challenges have been identified as the causes of the low national yield. The major challenges are the limited availability and high price of seed tubers (Amede *et al.*, 2006), the occurrence of diseases and insect pests (Guluma, 2020), and non-optimal agronomic practices, including earthing up (Sakadzo *et al.*, 2019) and inflorescence management (Tekalign and Hammes, 2005; Gebregwergis *et al.*, 2021). Earthing up is a row cultivation model that is essential for increased tuber production and root system expansion, weed removal, and soil–nutrient mixing (especially with manures) (Khayatnezhad and Gholamin, 2011). Adequate earthing up applied at the right time and in the right way

favours high tuberization and consequently an increase in total yields (Gutema, 2016). In addition, the earthing up practice keeps the tuber from greening, provides support for the plants, captures runoff, aerates the rooting zone, and improves resistance to growing roots, thereby creating conditions for better root growth and utilization of the newly added soil minerals for plant growth, yield and quality improvement (Ren *et al.*, 2017; Ebrahimi *et al.*, 2021; Van Dijk *et al.*, 2021; Arora *et al.*, 2023). Furthermore, earthing up the soil helps maintain moderate soil moisture and temperature and could be a strategy to increase potato yields. Higher moisture and a lower temperature in the soil lead to early tuberization and greater yield (Rykaczewska, 2015). Earthing up also increases the soil depth, allowing plants to extend their roots widely and deeply to absorb more nutrients and water, as earthing up conserves moisture and reduces the leaching of nutrients (Sakadzo *et al.*, 2019). Specifically, when tubers are exposed outside of the soil, they become more vulnerable to pest attacks and greening, both of which lower tuber quality (Svubure *et al.*, 2015).

Moreover, the development of potato inflorescences (flowers and fruits) has a detrimental effect on tuber development because growing tubers actively compete with inflorescences for assimilation. The removal of the inflorescence improves the distribution of assimilates into underground structures and increases tuber yield (Tekalign and Hammes, 2005). The inflorescences are strong sinks for assimilates (mineral nutrients, sugars, and amino acids), and there could be a corresponding decrease in the amounts available for tuber growth. Potato plants distribute assimilates to developing fruits, tubers, and other vegetative structures based on the strength of the sinks. There must be competition between sink organs when assimilation is limited (Hassen *et al.*, 2013). This requires the removal of inflorescences to eliminate competition among sink organs. Thus, identifying the optimum earthing up frequency and the right stage of inflorescence removal (flowers, buds and fruits) must be considered to ease the existing problems of increasing potato tuber yield, especially under cool conditions, because potato responses are strongly influenced by environmental variability. Therefore, the present study evaluated the combined influences of earthing up and inflorescence removal on the tuber yield and yield components of potato crops.

MATERIALS AND METHODS

Description of the study area

The experiment was conducted in the Debre Markos, East Gojjam Zone of the Amhara Regional State in 2022 under irrigation. The experimental area is located at 10° 19' 43" latitude North and 37°44' 43" longitude East, with an altitude of 2,446 m.a.s.l. The 2021 average annual rainfall at the experimental site was 1,628 mm. The mean minimum and mean maximum temperatures of the experimental area were 11°C and 25°C, respectively. The soil of the area was characteristically Nitosol, and its pH was 5.6.

Experimental materials, treatments, design and procedures

The improved potato variety '*Gudenie*' was used for the experiment. It is a potential potato cultivar, with early maturation late blight tolerance, and a high yield, and it has been the most commonly used variety by farmers in the study area. A factorial combination of four earthing up rounds (without, one, two, and three rounds) and three inflorescence removal stages (without removal, bud removal, and flower removal) was used in a randomized complete block design with three replications. The first earthing up treatment was applied on the 15th day after complete emergence, the second treatment was applied on the 30th day, and the third treatment was applied on the 45th day. The soil was earthed up around the plant up 15–20 cm high (Nyawade *et al.*, 2018), except for the control. According to Sharma (2014), earthing up should start after full plant emergence when the shoot reaches 15 cm in height but before the start of tuber formation (Rodríguez *et al.*, 2020).

and it should be completed before the plant begins flowering. Bud and new leaf removal was performed early in bud formation, whereas the flower and new leaf removal was performed at the beginning of blooming (Faramarzi *et al.*, 2011). The inflorescence removal was performed daily until the plants ceased growing buds and flowers.

Data collection and analysis

To evaluate the combined influences of earthing up and inflorescence removal, data on the tuber yield and yield components of potatoes were collected. The marketable tuber number per hill was determined based on its size category, which was greater than or equal to 25 g and free from disease and rot. The unmarketable tuber number/hill (count) was determined by counting the tuber size category (< 25 g), including disease and insect attacks. The total tuber number was determined as the sum of the marketable tuber number and the unmarketable tuber number per hill. The marketable tuber yield (t/ha) was determined by measuring tubers that were free from damage, weighed greater than or equal to 25 g and converted into tons per hectare. The unmarketable tuber yield (t/ha) was determined by weighing diseased, green, and small (< 25 g) tubers and converting them into tons per hectare (Lung'aho *et al.*, 2007).

SAS version 9.2 statistical software (SAS, 2009) was used to perform the statistical analysis (ANOVA). The Fisher-protected least significant difference (LSD) test at a 5% level of significance was used to separate the means of significant treatment influences (Gomez and Gomez, 1984).

RESULTS AND DISCUSSION

Number of marketable tubers/hill

The combined effects of the earthing up and inflorescence removal practices significantly differed among the treatments ($p < 0.05$). The maximum number of marketable tubers/hill (10.07) was recorded from the bud removal combined with the three earthing up treatments, whereas the minimum number of marketable tubers (2.77) was recorded from the control (Table 1). The number of tubers increased when the earthing up frequency increased along with bud removal. This could be a result of the dry matter being distributed to the tubers and the soil being prepared for healthy stolon development and early tuberization, from which healthy and excellent superior tubers are formed. This result is consistent with that of Gebregwergis *et al.* (2021), who revealed that removing flower buds avoids competition for assimilation; as a result, most of the tubers reached marketable tuber size. Chitsinde (2018) reported that earthing up three times ensured a well-drained cool environment and produced the highest marketable tuber number. Removing buds with younger leaves increased the number of marketable tubers by approximately 50% (Desta and Tsegaw, 2008). Tekalign and Hammes (2005) also reported that potatoes whose flower buds were removed presented the greatest number of marketable tubers.

Number of unmarketable tubers/hills

The number of unmarketable tubers/hills was significantly ($p < 0.05$) influenced by the interaction of earthing up and inflorescence removal. Accordingly, after three rounds of earthing up and flower removal, the lowest number of unmarketable tubers/hill (1.07) was observed. Conversely, the control group had the greatest number of unmarketable tubers per hill (6.03) (Table 1). A lack of earthing up exposes tubers to damage, resulting in undersized and deformed tubers. To promote assimilation into tubers, create ideal soil conditions for tuber initiation and development, and prevent tuber damage, it was necessary to remove the flowers and earthing up the soil three times. This finding is consistent with that of Gebregwergis *et al.* (2021), who reported that a greater number of tubers were affected by disease, and sprouting was observed in control plants. Potato

plants that are not hilled have a poor protective and stabilizing soil layer, and tubers are more vulnerable to various types of damage (Montaldo, 1984; Svubure *et al.*, 2015; Gutema, 2016; Van Dijk *et al.*, 2021; Arora *et al.*, 2023). In contrast, a nonsignificant effect of flower bud removal on unmarketable tuber numbers was reported by (Tekalign and Hammes, 2005; Desta and Tsegaw, 2008). This difference is probably related to the higher precipitation and cooler temperatures in the current study area.

Number of total tubers/hill

Earthing up frequency, flower bud removal, and their interaction had highly significant effects on the number of tubers per hill ($p<0.01$). The maximum tuber number (13.17) was obtained from the combination of bud removal with three earthing up treatments followed by the combination of bud removal with one earthing up treatment (12.13), whereas the minimum (8.8) was obtained from the control (Table 1). It is assumed that the early growth of buds, flowers and fruits consumes the food materials necessary for tuber formation. A significant increase in the percentage ranging from 6.48% to 49.66% was observed in the response to bud removal and earthing up. This could be because of the development of more stolons and early tuberization, from which tubers are formed. The greater number of tubers in all the treatments than in the control group might be related to the increased initiation of stolons, which often results in increased tuber formation, development and tuber number (Van Dijk *et al.*, 2021). Similarly, more earthing up frequencies allow for the formation of more tubers on the buried stems (Khayatnezhad and Gholamin, 2011; Chitsinde, 2018). The number of tubers per plant significantly increased when the inflorescences were removed (Nazari, 2010; Asl, 2016).

Table 1. The number of marketable and unmarketable tubers of potato is influenced by the combined effects of earthing up and inflorescence removal practices.

Treatment		Parameter		
Inflorescence removal	Earthing up	Marketable tuber numbers/hill	Unmarketable tuber numbers/ hill	Total tuber numbers/ hill
Without removal	No earthing up	2.77 ^g	6.03 ^a	8.80 ^f
	One time	5.83 ^{ef}	5.00 ^b	10.83 ^{becd}
	Two times	6.97 ^{ceb}	3.90 ^{ced}	10.87 ^{becd}
	Three times	7.67 ^{cbd}	3.83 ^{ced}	11.50 ^b
Bud removal	No earthing up	6.63 ^{ced}	4.83 ^{cb}	11.46 ^b
	One time	8.33 ^b	3.80 ^{ed}	12.13 ^{ba}
	Two times	7.73 ^{cbd}	3.20 ^e	10.93 ^{bcd}
	Three times	10.07 ^a	3.10 ^e	13.17 ^a
Flower removal	No earthing up	4.73 ^f	4.63 ^{cbd}	9.37 ^{fe}
	One time	6.17 ^{efd}	3.47 ^e	9.63 ^{fecd}
	Two times	7.90 ^{cb}	3.13 ^e	11.03 ^{bc}
	Three times	8.40 ^b	1.07 ^f	9.47 ^{fed}
Least significant difference (0.05)		*	*	*
Coefficient of variation (%)		13.47	15.71	8.61

Values marked by a different letter(s) are significantly different at $p<0.05$, according to the LSD test.

Marketable tuber yield

There was significant ($p<0.05$) variation in the effect of the combination of earthing up and flower bud removal on the marketable tuber yield. Table 2 shows that the combination of bud removal and three rounds of earthing up produced the highest marketable yield (31.49 t/ha) of all the treatment combinations. Conversely, the control plots produced the lowest marketable yield (10.7 t/ha) (Table 2). A significant increase in

the percentage ranging from 106% to 187% was observed in the response to bud removal combined with earthing up. The increased marketable yield under conditions of bud removal and three rounds of earthing up could be due to the elimination of competitor parts and favourable growing soil conditions contributing to the synthesis of more assimilates and translocate to the tubers, so most of the initiated tubers reach a marketable size and are protected from damage and greening. Consistent with the present results, Gebregwergis *et al.* (2021) reported that the highest marketable tuber yield (30.25 t/ha) was obtained by removing flowers at the bud stage and earthing up after full emergence. The removal of inflorescences resulted in an increase in the yield of marketable tubers to as high as 4.3 t/ha (Faramarzi *et al.*, 2011). Compared with the inflorescence removal treatments, the control treatment (no removal) reduced the marketable tuber yield by approximately 22% (Tekalign and Hammes, 2005). Similarly, according to Gutema (2016), the yield increased by 27.5% under three rounds of earthing up compared with that of the control. Potato hilling prevents tuber greening and maintains adequate coverage for developing tubers, helping to guarantee that tubers are more uniformly sized, well-shaped, and less likely to sustain damage, all of which are components of marketable tuber yield (Bohl and Love, 2005; Xing *et al.*, 2011; Sakadzo *et al.*, 2019). Breaking the compaction of soil layers results in increased plant exploitation of water and improved soil physical properties (reducing bulk density and increasing aggregate stability and hydraulic conductivity), which are responsible for all vegetative growth and production properties (Ati *et al.*, 2015).

Unmarketable tuber yield

The unmarketable tuber yield was significantly ($p < 0.05$) affected by the combination of earthing up and flower bud removal. Table 2 presented the highest unmarketable tuber yield (3.99 t/ha) in the control. The flower buds and tubers may compete fiercely for assimilation, which reduces assimilate partitioning to tubers, encouraging stolon elongation instead of bulking, and most of the tubers are undersized and exposed to mechanical, insect, disease, and sunlight damage. This finding aligns with the findings of Gebregwergis *et al.* (2021), who reported that combining nonearthing up and without bud removal produced a greater unmarketable tuber yield than did bud removal and earthing up. Getachew *et al.* (2012) reported that inadequate earthing up may expose tubers to sunlight, extreme heat, disease, and insect damage, all of which could have an impact on tuber quality and yield. Gutema (2016) also reported a considerably greater unmarketable yield in the control treatment than in the other treatments. In contrast to the present results, Tekalign and Hammes (2005) and Desta and Tsegaw (2008) reported that flower bud removal did not significantly influence unmarketable tuber yield.

Total tuber yield

There was a significant ($p < 0.05$) variation in the total yield of potato tubers among the combination treatments. The combined effect of bud removal and three rounds of earthing up produced the highest total tuber yield (33.08 t/ha), whereas the control had the lowest total tuber yield (14.96 t/ha) (Table 2). The total tuber yield increase ranging from 68% to 121% was likely facilitated by the greater volume of loose soil available and the absence of competitors. This allows the synthesis and partitioning of additional dry matter into the tubers. Consequently, it helped to produce more tubers that were better in terms of quantity and size, which ultimately increased the yield. The present result is in line with those of Gebregwergis *et al.* (2021), who stated that the total tuber yield is obtained by removing buds and earthing up. Faramarzi *et al.* (2011) reported that, as a result of removing inflorescences, the total tuber yield increased by as much as 3.62 t/ha. Nazari (2010) reported that removing the inflorescence increased the potato yield by up to 2.68

t/ha (8.9%). The removal of flowers and fruit increased the total tuber yield by 2.2% and 17%, respectively, suggesting that reproductive growth inhibited the growth and development of tubers (Tsegaw and Zelleke, 2002). The removal of the reproductive parts of potato reduced the total tuber yield by 13% -19%, compared with that of plants in which the reproductive parts were not removed (Tekalign and Hammes, 2005; Asl, 2016). Gutema (2016) reported that, compared with the control, three rounds of earthing up significantly increased the total tuber yield by 24.7%. In addition, Stalham *et al.* (2007) reported that compacted soil delayed emergence, reduced rooting density and deep rooting, slowed the rate of leaf appearance and ground cover extension, and shortened the canopy cover duration of crops. Rodríguez *et al.* (2020) reported that two rounds of earthing up increased the total yield by 86.02%. Improving the physical properties and moisture content of the soil results in larger roots, more space for the roots to spread out, better nutrient absorption, better vegetative growth characteristics, enhanced photosynthesis, and greater productivity (Moreno *et al.*, 2008).

Table 2. The marketable, unmarketable, and total yields of potato tubers is influenced by the combined effects of earthing up and inflorescence removal practices.

Treatment		Parameter		
Inflorescence removal	Earthing up	Marketable tuber yield (t/ha)	Unmarketable tuber yield (t/ha)	Total tuber yield (t/ ha)
Without removal	No earthing up	10.97 ^g	3.99 ^a	14.96 ^f
	One time	21.58 ^d e	3.26 ^{bc}	24.84 ^d
	Two times	23.46 ^{bcd}	2.48 ^{def}	25.94 ^{bcd}
	Three times	24.83 ^{bcd}	2.21 ^{ef}	27.04 ^{bcd}
Bud removal	No earthing up	16.05 ^f	3.47 ^b	19.52 ^e
	One time	22.59 ^{cd}	2.55 ^{de}	25.14 ^{cd}
	Two times	26.28 ^b	2.07 ^{efg}	28.35 ^{bc}
	Three times	31.49 ^a	1.59 ^g	33.08 ^a
Flower removal	No earthing up	18.17 ^{ef}	2.87 ^{cd}	21.04 ^e
	One time	23.59 ^{bcd}	2.53 ^{def}	26.02 ^{bcd}
	Two times	25.41 ^{bc}	2.42 ^{def}	27.83 ^{bcd}
	Three times	26.71 ^b	2.03 ^{fg}	28.74 ^b
Least significant difference (0.05)		*	*	*
Coefficient of variation (%)		9.48	11.47	8.12

Values marked by a different letter(s) are significantly different at $p < 0.05$, according to the LSD test.

CONCLUSION

The results of the present study revealed that the combined effect of the earthing up and inflorescence removal practices significantly influenced the potato tuber yield components, contributing to an increase in the total marketable tuber yield. Based on the findings of the present study, the combination of the three rounds of earthing up and removing inflorescences can be recommended for the production of potato.

REFERENCES

1. Amede T., German L., Rao S., Opondo C., and Stroud A. (2006). Integrated natural resource management in practice: enabling communities to improve mountain livelihoods and landscapes: Proceedings of the African highlands initiative conference, 12-15 October, 2004, Nairobi, Kenya.

2. Arora S., Bhatt, R., Sharma V., and Hadda M. S. (2023). Indigenous practices of soil and water conservation for sustainable hill agriculture and improving livelihood security. *Environmental Management*, 72(2), 321-332. <https://doi.org/https://doi.org/10.1007/s00267-022-01602-1>
3. Asl A. N. (2016). The effect of density and inflorescence removing on yield and yield components of potato (*Solanum tuberosum* L.). *International Journal of Advanced Biotechnology and Research*, 7(3), 335-344.
4. Ati A. S., Ahmed S., and Same S. (2015). Effect of tillage system on some machinery and soil physical properties, growth and yield of potato *Solanum tuberosum* L. *IOSR J. Agric. Vet. Sci*, 8(4), 63-65. <https://doi.org/10.9790/2380-08436365>
5. Bohl W. H., and Lov S. L. (2005). Effect of planting depth and hilling practices on total, US No. 1, and field greening tuber yields. *American Journal of Potato Research*, 82, 441-450. <https://doi.org/10.1007/BF02872222>
6. Chitsinde A. (2018). *Effects of Frequency of Earthing up on the Yield of Irish Potato (Solanum tuberosum) BUSEJ*.
7. Degebase, A. C. (2019). Review of potato research and development in Ethiopia: achievements and future prospects. *Journal of Biology, Agriculture and Healthcare*, 9(19), 27-36.
8. Desta B., and Tsegaw T. (2008). The Effect of removal of buds and younger leaves on growth, tuber yield and quality of potato (*Solanum tuberosum* L.) grown under hot tropical lowland. *East African Journal of Sciences*, 2(2). <https://doi.org/https://doi.org/10.4314/eajsci.v2i2.40371>
9. Ebrahimi M., Souri M. K., Mousavi A., and Sahebani N. (2021). Biochar and vermicompost improve growth and physiological traits of eggplant (*Solanum melongena* L.) under deficit irrigation. *Chemical and Biological Technologies in Agriculture*, 8, 1-14.
10. FAOSTAT. (2022). *Food and Agriculture Organization of the United Nations. Crops and livestock products*. FAOSTAT. Retrieved 12 March from <http://www.fao.org/faostat/en/#data>
11. Faramarzi, A., Shahrokhi, S., Mehrpouya, H., and Panah, D. H. (2011). Evaluation of inflorescence removing and salinity anti-stress matter application effects on potato, *Solanum tuberosum* (cv. Agria) yield. <https://doi.org/173173565>
12. Gebregwergis F. H., Gebremicheal, M., Gebremedhin H., and Asefa A. (2021). The effects of flower removal and earthing up on tuber yield and quality of potato (*Solanum tuberosum* L.). *Journal of Agricultural Sciences, Belgrade*, 66(2), 121-137. <https://doi.org/https://doi.org/10.2298/JAS2102121F>
13. Gebremedhin W., Endale G., and Berga L. (2008). Overview of trends in root and tuber crops research in Ethiopia. *Root and Tuber Crops: the Untapped Resources. Ethiopian Institute of Agricultural Research*, 1-5.
14. Getachew T., Belew D., and Tulu S. (2012). Combined Effect of Plant Spacing and Time of Earthing up on Tuber Quality Parameters of Potato (*Solanum tuberosum* L.) at Degem District, North Showa Zone of Oromia Regional State. *Asian journal of crop science*, 5(1), 24-32. <https://doi.org/10.3923/ajcs.2013.24.32>
15. Gomez K. A., and Gomez A. A. (1984). *Statistical procedures for agricultural research*. John Wiley & sons.
16. Guluma D.-A. (2020). Factors affecting potato (*Solanum tuberosum* L.) tuber seed quality in mid and highlands: A Review. *International Journal of Agriculture & Agribusiness*, 7(1), 24-40.
17. Gutema C. (2016). Effect of earthing up frequencies and tuber seed form on yield and profitability of potato (*Solanum tuberosum*) production in Bale highlands. *Agricultural Research & Technology: Open Access Journal*, 2(4), 96-101. <https://doi.org/10.19080/ARTOAJ.2016.01.555592>
18. Hassen Y., Ali, 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), 7-13. <https://doi.org/https://doi.org/10.1155/2024/3918033>
19. Khayatnezhad M., and Gholamin R. (2011). Effects of planting depth and soiling time of the plant foot on the potato yield. *African Journal of Agricultural Research*, 6(16), 3804-3808.
20. Limenih B. N., and Tefera T. A. (2014). Knowledge gaps in potato technology adoption: The case of central highlands of Ethiopia. *Journal of Agricultural Extension and Rural Development*, 6(8), 339-346.
21. Lung'aho C., Lemaga B., Nyongesa, M., Gildermacher P., Kinyale, P., Demo, P., and Kabira, J. (2007). Commercial seed potato production in eastern and central Africa. *Kenya Agricultural Institute*, 140.
22. Montaldo A. (1984). Cultivation and improvement of the potato. Moreno, R. G., Álvarez, M. D., Alonso, A. T.,
23. Barrington S., and Requejo A. S. (2008). Tillage and soil type effect
24. on soil surface roughness at semiarid climatic conditions. *Soil and Tillage Research*, 98(1), 35-44. <https://doi.org/https://doi.org/10.1016/j.still.2007.10.006>
25. Nazari L. (2010). *The effect of the removal of inflorescences on four varieties of potato yields in Ardabil MA thesis in Agriculture. Islamic Azad University of Mianeh*].
26. Nyawade S. O., Karanja N. N., Gachene C. K., Schulte-Geldermann E., and Parker M. (2018). Effect of potato hilling on soil temperature, soil moisture distribution and sediment yield on a sloping terrain. *Soil and Tillage Research*, 184, 24-36. <https://doi.org/https://doi.org/10.1016/j.still.2018.06.008>

27. Ren X., Chen X., Cai T., Wei T., Wu Y., Ali S., Zhang P., and Jia Z. (2017). Effects of ridge-furrow system combined with different degradable mulching materials on soil water conservation and crop production in semi-humid areas of China. *Frontiers in Plant Science*, 8, 1877. <https://doi.org/https://doi.org/10.3389/fpls.2017.01877>
28. Rodríguez S. G., Pinedo Taco R. E., and Sulca Salazar F. (2020). Hilling effect on yield and profitability of native potato cultivars. *Ciencia y Tecnología Agropecuaria*, 21(3).
29. Rykaczewska K. (2015). The effect of high temperature occurring in subsequent stages of plant development on potato yield and tuber physiological defects. *American Journal of Potato Research*, 92, 339-349. <https://doi.org/DOI 10.1007/s12230-015-9436-x>
30. Sakadzo N., Tafirenyika F., and Makaza K. (2019). Effects of time of earthing up on yield and yield parameters of Irish potato (*Solanum tuberosum* L.) in Zaka District, Zimbabwe. *Agricultural Science*, 1(1), p39-p39. <https://doi.org/https://doi.org/10.30560/as.v1n1p39>
31. SAS. (2009). SAS/STAT @9.2 User's Guide, Second Edition. Cary, NC: SAS Institute Inc. .
32. Sharma A. K. (2014). Practices for quality seed potato production. Summer School,
33. Stalham M., Allen E., Rosenfeld A., and Herry F. (2007). Effects of soil compaction in potato (*Solanum tuberosum*) crops. *The Journal of Agricultural Science*, 145(4), 295-312. <https://doi.org/https://doi.org/10.1017/S0021859607006867>
34. Svubure O., Struik P., Haverkort A., and Steyn J. M. (2015). Yield gap analysis and resource footprints of Irish potato production systems in Zimbabwe. *Field Crops Research*, 178, 77-90. <https://doi.org/https://doi.org/10.1016/j.fcr.2015.04.002>
35. Tekalign T., and Hammes P. (2005). Growth and productivity of potato as influenced by cultivar and reproductive growth: II. Growth analysis, tuber yield and quality. *Scientia horticulturae*, 105(1), 29-44.
36. Tsegaw T., and Zelleke A. (2002). Removal of reproductive growth increases yield and quality of potato (*Solanum tuberosum* L.).(125). *Tropical agriculture*.
37. Van Dijk L. C., Lommen,W. J., de Vries M. E., Kacheyo O. C., and Struik P. C. (2021). Hilling of transplanted seedlings from novel hybrid true potato seeds does not enhance tuber yield but can affect tuber size distribution. *Potato Research*, 64(3), 353-374. <https://doi.org/https://doi.org/10.1007/s11540-020-09481-x>
38. Xing Z., Chow L., W. Rees H., Meng F., Monteith J., and Stevens L. (2011). A comparison of effects of one-pass and conventional potato hilling on water runoff and soil erosion under simulated rainfall. *Canadian journal of soil science*, 91(2), 279-290. <https://doi.org/https://doi.org/10.4141/cjss10099>



Fruit growing

Behaviour of some Bulgarian plum cultivars in the pedo-climatic conditions of the Pitesti – Romania area

M. Butac^{1*}, M. Nesheva², M. Chivu¹, A. Stan¹, E. Mareş¹ and S. Nicolae¹

¹Research Institute for Fruit Growing Pitesti, Romania

²Fruit Growing Institute – Plovdiv, Bulgaria

Corresponding author email: madalinabutac@yahoo.com

ABSTRACT

In Romania and Bulgaria, plum is one of the most widespread species, both countries carry out a large breeding program. ‘Stanley’ is the most widespread cultivar in the plum orchards of both countries. Although it is a very productive cultivar, further requested by farmers, it presents some defects such as the low fruit quality and the sensitivity to Plum pox virus. That is why in both countries an improvement of the assortment was imposed by creating new cultivars in their breeding program, but also by introducing valuable cultivars obtained in other breeding centers from Europe. For this reason, researchers from both countries have changed biological material. In 2015, three Bulgarian cultivars were planted in a field trial by the Research Institute for Fruit Growing Pitesti, Romania. This paper aimed to evaluate the behaviour of these cultivars in climatic conditions from the Pitesti-Maracineni area. All Bulgarian cultivars were noted by good production, large fruits with high soluble solids content, as well as by tolerance to Plum pox virus. These cultivars can be recommended for extension in the commercial orchards from Romania, taking into account the requirements of the private fruit growers. Also, these cultivars can be used as genitors in breeding programs.

Keywords: *Prunus domestica* L., yield, fruit quality, blooming, ripening, Plum pox virus.

INTRODUCTION

In Romania and Bulgaria, plum is one of the most widespread species, both countries carry out a large breeding program.

In Romania, the plum is the dominant species, the area cultivated with plum being 66,710 ha which ensures a production of 665,730 t, Romania holds the 2nd place in the world after China and the first one in Europe (Butac, 2020; Data FAO, 2024).

In Bulgaria, the area cultivated with plums is smaller than in Romania (9,400 ha), and the production is only 56,650 t. However, the plum culture is very important ranking third place after the walnut and sweet cherry regarding the surface and the first place in terms of production (Nesheva and Butac, 2021; Data FAO, 2024).

‘Stanley’ is the most widespread cultivar in the plum orchards of both countries (Butac, 2020; Nesheva *et al.*, 2021; Butac *et al.*, 2023). Although it is a very productive cultivar, further requested by farmers, it presents some defects such as the low fruit quality and the sensitivity to *Plum pox virus*. That is why in both countries an improvement of the assortment was imposed by creating new cultivars in their breeding program, but also by introducing valuable cultivars obtained in other breeding centers from Europe (Bozhkova, 2013; Bozhkova *et al.*, 2018; Nesheva *et al.*, 2021; Butac *et al.*, 2023). The most important plum breeding objectives in both countries are resistance to Plum pox virus, fruit quality

and high-yielding capacity. The new cultivar must respond to the requirements of growers and consumers who are constantly changing (Bozhkova, 2013; Nesheva *et al.*, 2021; Nesheva and Butac, 2021; Butac *et al.*, 2023). Also, in order to obtain high and constant productions the cultivars must be well adapted to the pedo-climatic conditions specific to each culture area. Due to the correlation between cultivars and environmental conditions, it is necessary to properly zone the cultivars introduced or released (Nesheva *et al.*, 2021; Nesheva and Butac, 2021). To respond to these objectives, researchers from both countries have changed biological material. Thus, in 2015, three Bulgarian cultivars were introduced in a field trial by the Research Institute for Fruit Growing Pitesti, Romania. This paper aimed to evaluate the behaviour of these cultivars in climatic conditions from the Pitesti-Maracineni area.

MATERIALS AND METHODS

Plant material. Three new Bulgarian plum cvs. grafted on ‘Mirobalan C5’ rootstock were evaluated in comparison with the most widespread plum cvs. in Romanian orchards, ‘Stanley’ (Table 1).

Table 1. List of plum cultivars studied

No.	Name of cultivar	Parentage	Year of registration and origin
1	Ulpia	President O.P	2011, FGI Plovdiv, Bulgaria
2	Sineva	Stanley O.P	2011, FGI Plovdiv, Bulgaria
3	Plovdivska Rencloda	Stanley O.P	2011, FGI Plovdiv, Bulgaria
4	Stanley	d’Agen x Grand Duke	1926, USA

Source: Zhivondov, 2010; Zhivondov and Bozhkova, 2010

Field trial and climatic conditions. The field trial was established in 2015, 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). The trees were planted at a distance of 4 x 3 m, in 3 replications with 3 trees per variant. The trees were trained as flat open center, without irrigation. Soil is medium-textured, heavy-clay, with low humus content. The 2020-2024 climatic conditions were analyzed comparative with the last 55 years period (1969-2023). During the study period, the average annual temperature was 2.2°C higher than the multiannual average, and the precipitation was 183.3 mm lower than the multiannual average (Table 2).

Table 2. The main meteorological parameters (2020-2024)

No.	Meteorological parameters	Years					
		2020	2021	2022	2023	2024 (until October)	1969- 2023
1	Average temperature (°C)	11.8	10.7	11.6	12.3	15.0	10.1
2	Maximum temperature (°C)	35.3	36.8	38.3	36.0	30.3	38.8
3	Minimum temperature (°C)	-10.2	-14.1	-12.0	-11.4	3.12	-24.4
4	Annual rainfalls (mm)	679.1	636.2	526.3	562.8	44.8	673.2

Measurements. In 2020 - 2024 period, the following parameters were appreciated and measured: flowering and ripening time by noting the calendar date, yield per tree (YT, kg) by weighting the fruits per tree (3 trees/each replication), fruit weight with an electronic balance (25 fruits/each cvs./each replication), fruit firmness with a penetrometer Qualitest HPE equipped with a plunger of diameter 0.10 cm² (25 fruits/each cvs./each replication); soluble solids content of the fruits with a digital refractometer (25

fruits/each cvs./each replication) and Plum pox virus susceptibility according to a rating scale from 1 to 9 (1=resistant, 2=very low susceptible, 3=low susceptible, 5=intermediate, 7=susceptible, 8=very susceptible, 9=extremely susceptible).

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 DISCUSSION

Flowering time

Usually in Romania plum blooms in mid-April depending on weather conditions. Late flowering is very important for plum cultivars because late spring frosts may occur in early April (Gravite and Kaufmane, 2017). Average flowering time of the Bulgarian plum cultivars was at the beginning of the second decade of April in the same period with ‘Stanley’ cv. (Table 3). The earliest flowering was in 2023 and 2024 (first decade of April) due to the high temperature from February and March. Our results regarding the flowering time confirm the results reported by Butac and Chitu (2007), Chitu *et al.* (2013), Florea *et al.* (2019). Respectively, due to the increased average air temperature there in the last periods was registered an advance of the beginning of the flowering stage with 12 days.

Ripening time

All three Bulgarian cultivars are earlier than ‘Stanley’ cv. ‘Ulpia’ and ‘Sineva’ cvs. ripe earlier than ‘Plovdivska Renclođa’ cv. The earliest year in terms of fruit ripening was 2024, and the latest year was 2023 (Table 3). In the climatic conditions of Romania, the plum ripening stage extends over three months (July, August and September). The cultivars with Bulgarian origin enter the cultivars group with medium - maturation (August 15-20).

Table 3. Phenological characteristics of plum cultivars studied (day.month)

Year	Ulpia	Sineva	Plovdivska Renclođa	Stanley
Flowering time				
2020	12.04	12.04	13.04	12.04
2021	20.04	20.04	21.04	19.04
2022	11.04	11.04	12.04	11.04
2023	06.04	07.04	07.04	06.04
2024	02.04	03.04	03.04	02.04
Average	10.04	11.04	11.04	10.04
Ripening time				
2020	16.08	16.08	21.08	26.08
2021	18.08	18.08	22.08	27.08
2022	12.08	12.08	18.08	20.08
2023	20.08	20.08	22.08	28.08
2024	08.08	08.08	12.08	18.08
Average	15.08	15.08	19.08	24.08

Yielding capacity

Statistical analysis of fruit yield (kg/tree), using Duncan’s multiple range test ($P \leq 0.05$), showed significant differences between the cultivars. The highest fruit yield was recorded ‘Plovdivska Renclođa’ cv. with 18.91 kg/tree, and the lowest fruit production was recorded at ‘Ulpia’ cv. (15.15 kg/tree on average). It can be seen that none of the Bulgarian cultivars has overcome the ‘Stanley’ cv. known as one of the most productive plum cultivars (Tabel 4).

Between the years of study, there are very significant differences of production, with yield per tree increasing from one year to another as expected in a young plum orchard. Our results are similar to results reported by Zhivondov and Bozhkova in 2010.

Table 4. The yielding capacity of plum cultivars studied

No	Cultivar	2020	2021	2022	2023	2024	Average
1	Ulpia	9.07±0.40 ^b	11.07±0.66 ^b	15.83±0.31 ^c	20.93±0.45 ^d	18.83±0.45 ^c	15.15±4.67 ^b
2	Sineva	11.30±1.10 ^a	13.47±0.91 ^{ab}	16.47±0.68 ^c	23.37±0.66 ^c	20.07±0.65 ^c	16.93±4.57 ^b
3	Plovdivska Recnclođa	12.47±0.70 ^a	14.60±1.05 ^a	18.43±0.45 ^b	26.37±0.42 ^b	22.70±0.82 ^b	18.91±5.32 ^{ab}
4	Stanley (Control)	12.17±1.76 ^a	16.20±2.33 ^a	20.47±1.86 ^a	29.40±0.87 ^a	27.57±1.22 ^a	21.16±6.93 ^a
	Average	11.25±1.68	13.83±2.29	17.80±2.09	25.07±3.36	22.29±3.57	18.04±5.78

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

Fruits characteristics

The results regarding fruit characteristics were similar to those obtained by Zhivondov, 2010; Zhivondov and Bozhkova, 2010.

Fruit weight. An important factor in the marketing of plum cultivars designated for fresh consumption is fruit size (Milošević *et al.*, 2013, 2018). The largest fruits were recorded ‘Plovdivska Rencloda’ cv. (50.88 g) and the lowest fruits had ‘Ulpia’ cv. (42.93 g), between cultivars being significant differences (Table 5). ‘Sineva’ and ‘Plovdivska Rencloda’ cvs., have larger fruits than ‘Stanley’ control. Making a correlation between yield and fruit weight it is observed that there is a positive correlation. ‘Sineva’ and ‘Plovdivska Rencloda’ cvs. had large fruits and good yield and ‘Ulpia’ cv. had small fruits and small production.

Table 5. The fruits weight of plum cultivars studied

No.	Cultivar	2020	2021	2022	2023	2024	Average
1	Ulpia	42.33±1.66 ^a	45.37±1.76 ^b	45.00±1.45 ^{ab}	46.60±2.09 ^b	35.33±2.73 ^c	42.93±4.51 ^c
2	Sineva	44.50±0.96 ^a	46.07±0.81 ^b	49.40±3.81 ^a	50.43±1.04 ^b	42.93±6.04 ^b	46.67±3.53 ^b
3	Plovdivska Recnclođa	45.83±1.34 ^a	48.33±0.66 ^a	49.30±0.70 ^a	60.77±5.93 ^a	50.17±3.79 ^a	50.88±5.99 ^a
4	Stanley (Control)	43.20±2.76 ^a	43.97±0.94 ^b	43.70±3.10 ^c	45.57±0.85 ^b	44.30±0.80 ^b	44.15±1.86 ^{bc}
	Average	43.97±2.07	45.93±1.91	46.85±3.45	50.84±6.85	43.18±6.02	46.16±5.15

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

Flesh firmness. Firmness is an important factor in stone fruits often related to taste and shelf life, and firmness assessment is widely used both in the marketing chain to judge overall fruit quality and by researchers in cultivar testing and programs including fruit quality (Sekse and Wermund, 2010). Generally, flesh firmness decreases during maturation and ripening (Crisosto, 1994). All Bulgarian cultivars had firm fruits but less firm than ‘Stanley’ cv. Of the Bulgarian cultivars, the ‘Sineva’ cv. was noted with a firmness of 52.54 HPE units (Table 6).

Table 6. The fruits firmness of plum cultivars studied

No	Cultivar	2020	2021	2022	2023	2024	Average
1	Ulpia	41.43±1.07 ^b	42.33±2.83 ^b	47.43±6.97 ^b	56.23±3.16 ^a	45.33±14.12 ^a	46.55±8.26 ^b
2	Sineva	44.63±1.92 ^b	46.53±0.57 ^b	65.30±13.52 ^a	60.77±8.36 ^a	45.47±18.45 ^a	52.54±12.91 ^b
3	Plovdivska Recnclođa	43.40±1.37 ^b	47.50±0.66 ^b	49.87±084 ^b	46.30±2.82 ^b	47.10±3.41 ^a	46.83±2.81 ^b
4	Stanley (Control)	64.21±8.24 ^a	62.28±8.84 ^a	72.73±1.85 ^a	61.83±2.14 ^a	52.50±1.83 ^a	62.71±8.19 ^b
	Average	48.42±10.28	49.66±8.82	58.83±12.82	56.28±7.61	47.60±10.50	52.16±10.82

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

Fruit soluble solids content (SSC). Statistical analysis of data on fruit soluble solids content (%) showed insignificant differences between the cultivars. The Bulgarian plum cultivars had higher fruit soluble solids content than 'Stanley' cv. (Table 7).

Table 7. The fruits soluble solids content of plum cultivars studied

No.	Cultivar	2020	2021	2022	2023	2024	Average
1	Ulpia	16.27±0.25a	19.50±0.36a	17.23±1.89a	17.13±1.46a	17.60±2.26ab	17.55±1.67a
2	Sineva	15.73±0.38a	18.10±0.20b	15.00±1.67a	16.70±1.71a	19.70±2.71ab	17.05±2.22a
3	Plovdivska Recncloda	16.10±0.30a	18.20±0.20b	16.40±0.26a	17.90±1.04a	20.73±1.04a	17.87±1.80a
4	Stanley (Control)	14.23±0.35b	14.20±0.62c	16.20±1.90a	16.23±0.74a	16.40±0.46b	15.45±1.34b
	Average	15.58±0.88	17.50±2.10	16.21±1.59	16.99±1.27	18.61±2.38	16.98±1.98

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

Correlations between the main traits

There are significant correlations between: fruit weight and yield (r=0.328*); fruit soluble solids content and firmness (r=-0.543**) (Table 8).

Table 8. The Pearson Correlations between characteristic studied

Indicator	Yield	Weight	SU	Firmness
Yield	1	0.328 *	0.125	0.247
Weight	0.328 *	1	0.125	0.016
SU	0.125	0.125	1	-0.543 **
Firmness	0.247	0.016	-0.543 **	1

* Correlation is significant at the 0.05 level (2-tailed);

** Correlation is significant at the 0.01 level (2-tailed);

Behaviour at Plum Pox Virus (PPV)

Plum Pox Virus, also called Sharka, is considered one of the most serious virus diseases of stone fruit in Europe. In Romania, Sharka causes great yield losses, especially to sensitive plum cultivars (Zagrai et. al., 2001).

Bulgarian cultivars are very low susceptible to PPV, showing symptoms only on leaves (note 2).

'Stanley' cv. is medium sensitive to PPV, showing symptoms on leaves (note 5) and few symptoms on fruits (note 3).

Table 9. Behaviour of Bulgarian plum cultivars on the Plum Pox Virus

No.	Cultivar	Plum Pox Virus	
		On leaves	On fruit
1	Ulpia	2	1
2	Sineva	2	1
3	Plovdivska Recncloda	2	1
4	Stanley (Control)	5	3

1=resistant, 2=very low susceptible, 3=low susceptible, 5=intermediate, 7=susceptible, 8=very susceptible, 9=extremely susceptible.

CONCLUSIONS

All Bulgarian cultivars were noted by good production, large fruits, high soluble solids content, as well as by tolerance to Plum pox virus.

These cultivars can be recommended for extension in the commercial orchards from Romania, taking into account the requirements of the private fruit growers.

Also, these cultivars can be used as genitors in breeding programs.

REFERENCES

1. Butac M. and Chițu E. (2007). Impact of climatic changes on the phenological dynamics of some plum cultivars in the fruit growing Arges area. Scientific papers of RIFG Pitești, vol. XXIII: 139-147.
2. Butac M. (2020). Plum breeding. In: *Prunus*. Intech Open, London, United Kingdom (Open Access books).
3. Butac M., Mareș E., Stan A., 2023. Plum cultivars grown in Romania – a comparison between traditional cultivars widely grown and promising new ones. V Balkan Symposium on Fruit Growing, Zagreb, Croatia (in the process of publishing).
4. Bozhkova V. (2013). Plum genetic resources and breeding. Acta Hort., 734: 31-45.
5. Bozhkova V. and Savov P. (2018). Evaluation of introduced plum cultivars under agroclimatic conditions of Plovdiv region, Bulgaria. Scientific papers, series B., Horticulturae, vol. LXII, 77-82.
6. Chițu, E., Giosanu, D., Mateescu, E. (2013). Seasonal and Annual Extreme Temperature Variability and Trends of the latest three decades in Romania. Advances in Environmental Sciences - International Journal of the Bioflux Society, Online ISSN 2065-7647. Volume 5, Issue 2: 70-88; <http://www.aes.bioflux.com.ro>
7. Crisosto C. (1994). Stone fruit maturity indices: a descriptive review. Postharvest News and Information, vol. 5, no. 6: 65-68.
9. Florea, A., Chițu, E., Păltineanu, C. (2019). Dynamics of phenological stages due to climate change in plum trees in southern Romania. Acta Hortic. 1289, 205-212. <https://doi.org/10.17660/ActaHortic.2020.1289.29>.
10. Gravite I., Kaufmane E. (2017). Evaluation of German plum selections in Latvia. Proceedings of the Latvian Academy of Sciences. Section B, vol. 71, no. 3 (708): 166-172.26.
11. Milošević T., Milošević N., Glišić I. (2013). Agronomic properties and nutritional status of plum trees (*Prunus domestica* L.) influenced by different cultivars. Journal of Soil Science and Plant Nutrition, 13 (3), 706-714.
12. Milosevič T. and Milosevič N. (2018). Plum (*Prunus* spp.) breeding. Chapter 5. In: Advances in Plant Breeding Strategies: Fruits. Springer International Publishing AG, part of Springer Nature 2018: 165-215.
13. Nesheva M., Bozhkova V., Butac M., 2021. Performance of Romanian plum cultivars in the agro-environmental conditions of Plovdiv, Bulgaria. Romanian Journal of Horticulture, vol. II: 57-62.
14. Nesheva M. and Butac M., 2021. Fruit quality of new introduced plum cultivars under the conditions of Bulgaria. Gruit Growing Research, vl. XXXVII: 45-49.
15. Sekse L. and Wermund U. (2010). Fruit flesh firmness in two plum cultivars: comparison of two penetrometers. Acta Hortic. 874: 119-124.
16. Zagrai I., Ardelean M., Maxim A., Zagrai L. (2001). Researchers regarding the influence of Plum pox virus on production at different plum cultivars, clone and hybrids. Jubilee session of Horticulture Faculty from Iasi. Series Hort. 44: 150-151.
17. Zhivondov A., 2010. Plovdivska Renkloda – anew plum cultivar. Acta Hort. 874: 305-311.
18. Zhivondov A. and Bozhkova V., 2010. New Bulgarian plum cultivars. Acta Hort. 874: 345-351.
19. Food and Agricultural Organization (2024). www.fao.org/statistics/yearbook.

Results of some apple hybrid populations to the attack of scab and powdery mildew under natural conditions

G. M. Guzu^{1,2}, S.D. Roşu-Mareş¹, C. Moldovan¹, I. Zagrai¹, L.A. Zagrai¹
and M.I. Cordea^{2*}

¹Fruit Research and Development Station Bistriţa, Romania

²Faculty of Horticulture and Business in Rural Development, University of Agricultural Sciences and Veterinary Medicine Cluj-Napoca, Romania

*Corresponding author email: mcordea@usamvcluj.ro

ABSTRACT

Apple scab, caused by the fungus *Venturia inaequalis*, and powdery mildew, caused by the fungus *Podosphaera leucotricha*, are two of the most significant diseases affecting apple production worldwide. These diseases can lead to substantial crop losses and often require frequent fungicide applications, with negative environmental impacts and the development of fungicide resistance. Apple breeding programs frequently aim to develop cultivars exhibiting resistance to scab and powdery mildew. This is also the case of a breeding program initiated at FRDS Bistriţa to obtain hybrids resistant/tolerant to scab and powdery mildew, accompanied by superior fruit qualities and a later harvest period. Thus, the pollinations were performed on apple trees in 2023. The experiment was organized into two hybridization schemes involving six apple cultivars. Scheme 1 consisted of five maternal genitors ('Auriu de Bistriţa', 'Jonathan', 'Idared', 'William's Pride', and 'Bistriţean') and one paternal genitor ('Generos'). Scheme 2 consisted of one maternal genitor ('Generos') and five paternal genitors ('Auriu de Bistriţa', 'Jonathan', 'Idared', 'William's Pride', and 'Bistriţean'). The aim of this study was to evaluate the hybrids of these crosses in terms of early response to natural infection with apple scab and powdery mildew. Results showed no symptoms of scab infection on the leaves of the hybrids, while 83.8% of the total showed no symptoms of powdery mildew infection. The hybrid combination with the lowest percentage of infected hybrids was 'William's Pride x Generos' (10.4%), closely followed by the 'Auriu de Bistriţa x Generos' hybrid combination (12.5%). The highest percentage of infected hybrids was observed in the 'Jonathan x Generos' hybrid combination (20.0%).

Keywords: controlled pollination, monogenic resistance, *Podosphaera leucotricha*, polygenic resistance, *Venturia inaequalis*

INTRODUCTION

Apple scab, caused by the fungal pathogen *Venturia inaequalis*, remains one of the most economically significant diseases affecting apple (*Malus domestica*) production worldwide. The disease can cause considerable crop losses due to the infection of leaves, fruit, and shoots, making resistance to *V. inaequalis* a major focus in modern apple breeding programs (Parisi *et al.*, 1993). Traditional management of apple scabs often relies on frequent applications of fungicides; however, the increasing concerns regarding environmental impact and the development of fungicide resistance highlight the need for durable genetic resistance as a more sustainable solution (MacHardy, 1996).

The two primary forms of genetic resistance to apple scab are vertical (monogenic) and horizontal (polygenic) resistance (Švara *et al.*, 2024). Vertical resistance, typically conferred by single resistance (R) genes, such as *Vf* derived from *Malus floribunda* 821, provides strong and specific resistance to certain races of *V. inaequalis*. However, this form of resistance is often short-lived due to the ability of the pathogen to rapidly evolve virulent strains that overcome single-gene resistance, as has been observed with the breakdown of *Vf* resistance in several apple-growing regions (Bus *et al.*, 2011). As a result, the durability of vertical resistance is limited, making it less effective in the long term (Calenge & Durel, 2006). In contrast, horizontal (polygenic) resistance is generally regarded as more durable and robust. This form of resistance is controlled by multiple genes, each contributing to a partly but cumulative resistance effect. Polygenic resistance does not completely prevent infection but significantly reduces the severity of the disease, slowing down the pathogen's ability to adapt and evolve virulence (Parisi *et al.*, 1993). Because polygenic resistance is less specific to individual pathogen races, it provides a broader and more stable defense over time, making it an attractive target for sustainable apple breeding programs (Gessler, Pertot & Perazzolli, 2014).

Another widespread disease that affects the apple crop is powdery mildew, one of the most dangerous harmful fungal diseases. Production is reduced, by at least half in the orchards where no treatments are applied, compared to those where only sulphur compounds are applied (Holb and Kunz 2016). Some studies attest to the dramatic effect that powdery mildew has not only on the production of the current year but also on the following year, in the case of sensitive cultivars (Yoder, 2000).

Apple's resistance to powdery mildew (*Podosphaera leucotricha*) is primarily associated with specific genes identified in wild *Malus* species. Key genes include Pl-1 from *Malus robusta*, Pl-2 from *Malus zumi*, and Pl-w from *Malus* × *micromalus* or ornamental crabapples like "White Angel" (Alston, 1976). These genes have been incorporated into breeding programs for resistant cultivars, often supported by marker-assisted selection methods to improve resistance efficiency and durability in orchards.

The number of genetically resistant cultivars to powdery mildew is much lower than those with scab resistance but there are some examples: 'Ariwa' - *Pl 1* gene, 'Bramleys Seedling' - *Pld* gene, 'Erbachhofer' - *Plm* gene, etc. (Höfer *et al.*, 2021).

The current breeding efforts focus on crossing apple varieties with desirable technological traits, such as improved fruit quality and storage capabilities, with those possessing polygenic resistance to scabs or specific diseases. By focusing on the integration of multiple resistance genes, we aim to develop cultivars that offer long-term resistance to *V. inaequalis* and *Podosphaera leucotrichia* without sacrificing fruit quality, ultimately contributing to more sustainable apple production.

MATERIALS AND METHODS

Controlled pollinations were carried out in 2023 at the Fruit Research and Development Station Bistrița (FRDS Bistrița), Romania, and the biological material consisted of six apple cultivars (Table 1) and their F1 hybrids. In our experiments, we utilized the Romanian apple cultivar 'Generos', which was registered in 1983 by the Fruit Research Station Voinesti, in Romania. 'Generos' is known for its resistance to *Venturia inaequalis* and has been used in breeding programs due to its favorable agronomic characteristics, including good fruit quality and enhanced disease resistance. The polygenic resistance to apple scab in 'Generos' makes it a valuable resource in the development of new apple cultivars with durable resistance (Militaru *et al.*, 2018). This cultivar's resistance originates from the use of *Malus kaido* in its pedigree, which has contributed to its robust defense against multiple

races of *Venturia inaequalis*, further supporting the importance of polygenic resistance in modern breeding programs.

Table 1. The selection criteria for the apple cultivars used as genitors in the hybrid combinations

Cultivars	Selection criteria of the genitors	The behavior to scab infections (references)	The behavior to powdery mildew infections/references
Auriu de Bistrița	Superior fruit characteristics, ripening time, local cultivar	Tolerant (Guzu <i>et al.</i> , 2021; Ștefan <i>et al.</i> , 2018)	Low susceptible (Roșu-Mareș <i>et al.</i> , 2022; Ștefan <i>et al.</i> , 2018))
Jonathan	Harvest period	Susceptible (Afunian <i>et al.</i> , 2004)	High susceptible (Roșu-Mareș <i>et al.</i> , 2022)
Idared	Very good storage capacity in the warehouse	Susceptible (Roșu Mares <i>et al.</i> , 2022)	High susceptible (Roșu-Mareș <i>et al.</i> , 2022)
Bistrițean	The presence of the <i>Vf</i> gene, resistance to specific diseases, fruit appearance, local cultivar	Resistant (Militaru <i>et al.</i> , 2020; Ștefan <i>et al.</i> , 2018))	Low susceptible (Roșu-Mareș <i>et al.</i> , 2022; Ștefan <i>et al.</i> , 2018))
Williams Pride	Harvest period, resistance to specific diseases, taste qualities of the fruit, presence of the <i>Vf</i> gene	Resistant (Kaymak <i>et al.</i> , 2013)	Resistant (Swezey, 2000)
Generos	Polygenic resistance, indigenous cultivar, valued for its taste qualities	Resistant (Militaru <i>et al.</i> , 2018)	Medium sensitive (Roșu-Mareș <i>et al.</i> , 2022)

For artificial pollination in apple species, classical hybridization steps were followed (Cordea, 2014). The controlled hybridizations in this experiment are organized into two cyclical schemes as detailed in Table 2.

Table 2. The cyclical schemes of hybrid combinations implemented in the experiments conducted in 2023

Scheme 1	Scheme 2
23.01 - ♀Auriu de Bistrița x ♂Generos	23.06 – ♀Generos x ♂Auriu de Bistrița
23.02 – ♀Jonathan x ♂Generos	23.07- ♀Generos x ♂Jonathan
23.03 – ♀Idared x ♂Generos	23.08 – ♀Generos x ♂Idared
23.04 – ♀Williams Pride x ♂Generos	23.09 – ♀Generos x ♂Williams Pride
23.05 – ♀Bistrițean x ♂Generos	23.10 – ♀Generos x ♂Bistrițean

After refrigeration, in February 2024, the seeds were sown in a mixture of soil and sand in a 2:2 ratio and the germinated seedlings were then raised in a greenhouse. During the seedling stage, hybrids were selected based on their development. After assessing the number of hybrids suitable for planting from both hybridization schemes, the selected plants were transferred in the field (in April, 2024) by establishing

an experimental plot surrounded by large apple orchards infected by *Venturia inaequalis* and/or *Podosphaera leucotricha*. Thus, an appropriate natural infection pressure was secured. Then the behavior of the hybrids to the two specific apple diseases (scab and powdery mildew) was assessed. In order to classify the hybrids according to their response to the two diseases, the frequency of attack (F%) for the target pathogen was determined, according to phytosanitary methodologies.

To uniquely identify each hybrid, a six- or seven-digit code was assigned. The first two digits indicate the year of artificial pollination, the next two represent the hybrid combination number, and the final two or three digits denote the individual hybrid's sequential number.

The statistical determination of the results was conducted using the XLSTAT - Addinsoft software (Addinsoft, 2022), which runs on the Microsoft Office Excel platform. ANOVA analysis was used to assess variance, followed by a Duncan's test with a significance level of $p < 0.0001$.

RESULTS AND DISCUSSIONS

From the cyclic hybridization schemes 1 ('Generos' cv. used as the paternal parent) and 2 'Generos' cv. used as the maternal parent), a total of 1,203 seedlings were obtained as follow in table 3:

Table 3. The total number of hybrids obtained from each hybrid combination

Hybrid combination	No. of hybrids
Auriu de Bistrița x Generos	72
Jonathan x Generos	43
Idared x Generos	412
Williams Pride x Generos	161
Bistrițean x Generos	35
Generos x Auriu de Bistrița	231
Generos x Jonathan	50
Generos x Idared	112
Generos x Williams Pride	38
Generos x Bistrițean	42

From the two schemes, only the plants suitable for planting were selected, so from the over 1,200 hybrids obtained, only 31.84% (383 hybrids) fulfilled the requirements for transplantation.

Following the analysis of scheme 1 (Figure 1), it can be observed that the hybrids from four hybrid combinations were suitable for planting. The best percentages of hybrids suitable for planting in pots were obtained in two combinations: ultimately 'Auriu de Bistrița' x 'Generos' with 38.5% and 'Jonathan' x 'Generos' with 37.2%. Statistical analysis of the results shows that there are significant differences in the number of hybrids suitable for pot planting depending on the hybrid combination. Thus, three significant classes were identified. The number of hybrids suitable for planting was significantly higher in the combinations 'Auriu de Bistrița' x 'Generos' and 'Jonathan x Generos', compared to the other combinations.

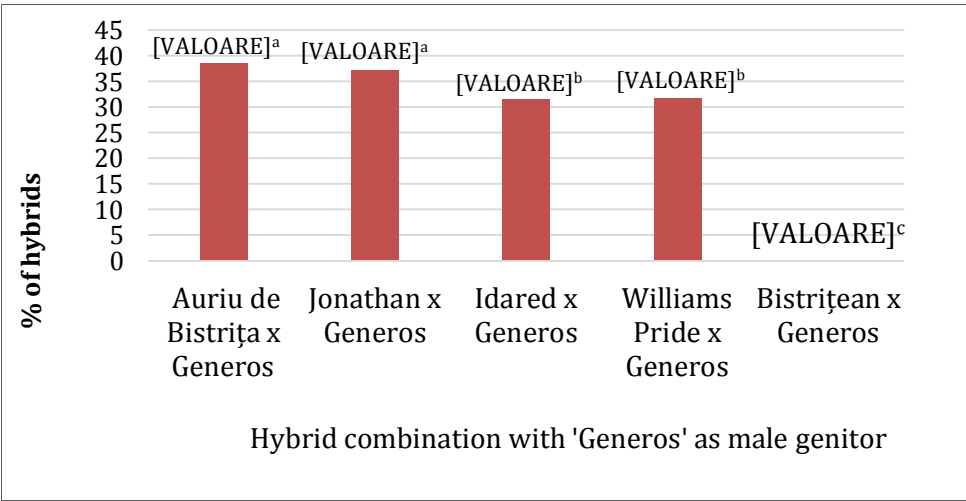


Figure 1. The percentage of hybrids suitable for planting in individual pots (scheme 1)

From cyclic scheme number 2, out of the five hybrid combinations, only three produced hybrids suitable for planting in individual pots (Figure 2). Leading the ranking is the combination 'Generos' x 'Idared' (53.6%), closely followed by the combination 'Generos' x 'Jonathan' (46.0%).

According to statistical results, the hybrid combinations from scheme 2 were classified into four significance classes.

The hybrids resulting from the combinations 'Generos' x 'Bistrițean' and 'Bistrițean' x 'Generos' did not survive.

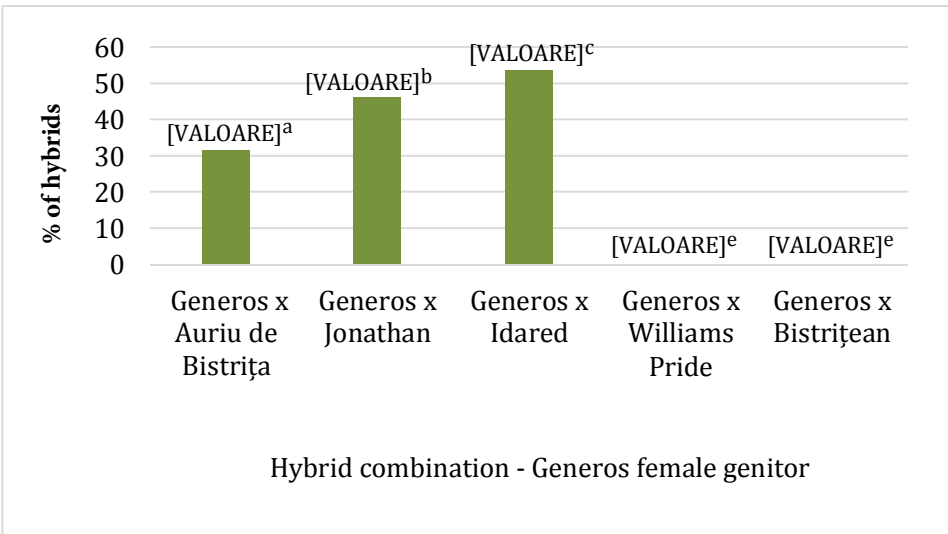


Figure 2. The percentage of hybrids suitable for planting in individual pots (scheme 2)

Following the second objective of this study, the early behavior of hybrids in terms of scab and powdery mildew infections, the results were encouraging. Regarding scabs, no typical symptoms were observed on the hybrids obtained in the year of monitoring (2024).

In contrast, the behavior of hybrids to powdery mildew infections, highlights the fact that some of them showed typical symptoms of powdery mildew (Figure 3).



Figure 3. Powdery mildew symptoms observed in the developed apple hybrids

Interpretation of the obtained results highlights that the infection rate of hybrids with powdery mildew, in the case of scheme 1 ('Generos' cultivar as paternal parent), ranged between approximately 10% and 20% (Figure 4). The hybrid combination with the lowest percentage of infected hybrids was 'William’s Pride' x 'Generos' (10.4%), followed by the hybrid combination 'Auriu de Bistrița' x 'Generos' (12.5%). The highest number of infected hybrids was found in the hybrid combination 'Jonathan' x 'Generos' (20.0%), followed by 'Idared' x 'Generos' (16.5%). The statistical interpretation of the obtained results revealed that the four hybrid combinations are classified into three significance classes. Thus, there are significant differences between the 'Jonathan' x 'Generos' respectively 'Idared' x 'Generos' combinations and the other hybrid combinations in terms of the infection rate by powdery mildew of the hybrids. The combinations 'Auriu de Bistrița' x 'Generos' and 'William’s Pride' x 'Generos' are classified in the same significance class, therefore there are no statistically significant differences.

In the case of the second scheme ('Generos' cultivar as a maternal parent), the percentages ranged between 13.6% ('Generos' x 'Jonathan' hybrid combination) and 19.1% ('Generos' x 'Idared') (Figure 5).

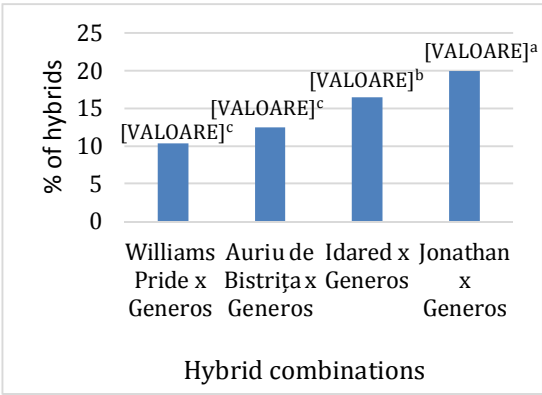


Figure 4. Infection rate of hybrids with powdery mildew (scheme 1)

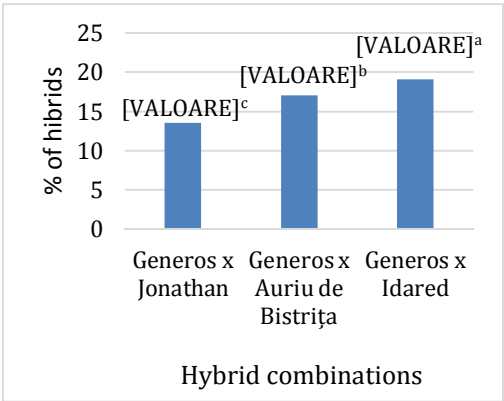


Figure 5. Infection rate of hybrids with powdery mildew (scheme 2)

The results were categorized into three levels of significance, with each hybrid combination belonging to a distinct level. The Duncan test revealed statistically significant differences among all hybrid combinations.

Overall, out of the total of 383 hybrids obtained and monitored, 83.8% did not exhibit powdery mildew symptoms, while the remaining 16.2% were infected with powdery mildew in various percentages.

CONCLUSIONS

Following the selection process and disease monitoring the hybrids did not phenotypically display any symptoms characteristic of apple scab. Regarding the response to powdery mildew, over 80% of the hybrids exhibited typical symptoms of this disease during the experimental year (2024).

ACKNOWLEDGEMENTS

We are grateful to Fruit Research & Development Station Bistrita for the generous help and assistance, as well as to The University of Agricultural Science and Veterinary Medicine of Cluj-Napoca.

REFERENCES

1. Bachelier G. (1968). Contribution à l'étude de la minéralisation du carbone des sols. Sa mesure en laboratoire et sur le terrain, sa nature, son dynamisme et son intérêt. OROSTOM: p33- 52.
2. Afunian M.R., Goodwin P.H. and Hunter D.M. (2004). Linkage of Vfa4 in *Malus x domestica* and *Malus floribunda* with Vf resistance to the apple scab pathogen *Venturia inaequalis*. Plant Pathology, 53(4): 461–467.
3. Alston, F. H. (1976). Inheritance of powdery mildew resistance in apples (*Malus domestica* Borkh). Euphytica, 25, 383–390.
4. Bus V.G.M., Laurens F.N.D., Van de Weg W.E., Rusholme Pilcher R.L and Calenge F. (2011). The Vh8 and Vh2 gene regions conferring scab resistance to apple are located on chromosomes 2 and 11, respectively. Genome, 54(6): 448-459.
5. Calenge F. and Durel C.E. (2006). Both Effector-Triggered Immunity and Quantitative Disease Resistance to apple scab are present in natural populations of *Malus*. New Phytologist, 170(4): 882-893.
6. Cîrstea G., Călinescu M., Ducu C., Moga S., Mihăescu C., Sumedrea D., Ungureanu C., Butac M., Vălu M. V., 2019. Bioformulări de produse fitosanitare împotriva fitopatogenilor *Podosphaera leucotricha* și *Venturia inaequalis* (Bioformulations of plant protection products to control *Podosphaera leucotricha* Fruit Growing Research, Vol. XXXV, 2019.
7. Gessler C., Pertot I. and Perazzolli M. (2014). *Venturia inaequalis*: the casual agent of apple scab. Phytopathology, 104(6): 1380-1391.
8. Guzu G., Zagrai I., Zagrai L., Cordea M. and Moldovan C., (2021). Molecular screening of some apple progenies for detection Vf gene using Marker Assisted Selection. Bulletin of University of Agricultural Sciences and Veterinary Medicine Cluj-Napoca. Horticulture, 78(2): 36-40.
9. Höfer M, Flachowsky H, Schröpfer S, Peil A. (2021). Evaluation of Scab and Mildew Resistance in the Gene Bank Collection of Apples in Dresden-Pillnitz. Plants. 16, 10(6):1227.
10. Holb I. J., and Kunz S. (2016). Integrated control of apple scab and powdery mildew in an organic apple orchard by combining potassium carbonates with wet table sulfur, pruning, and cultivar susceptibility. Plant Disease, 100(9): 1894-1905.
11. Kaymak S., Kaçal E. and Öztürk Y. (2013). Screening breeding apple progenies with vf apple scab (*Venturia inaequalis* (Cke.) Wint.) disease resistance gene specific molecular markers, Integrated protection of fruit crops IOBC-WPRS Bulletin Vol. 91, 2013: 361-365.
12. MacHardy W.E. (1996). Apple scab: Biology, Epidemiology, and Management. St. Paul, MN: APS Press.
13. Militaru M., Călinescu M., Butac M., Chivu M. and Sestraș A. (2018). Response to apple scab and powdery mildew attack of direct and reciprocal F1 apple hybrids. Fruit Growing Research, 34: 38-44.
14. Militaru M., Sturzeanu M., and Iancu A. (2020). Molecular screening of some Romanian apple cultivars for scab resistance genes, Fruit Growing Research, Vol. XXXVI: 5-11.
15. Parisi L., Lespinasse Y., Guillaumes J. and Kruger J. (1993). A new race of *Venturia inaequalis* virulent to apples with Vf gene. Phytopathology, 83(5): 533-537.

16. Roşu Mares S., Guzu G., Zagrai I., and Puia C. (2022). An apparent breakdown of *Vf* resistance occurring in an apple orchard in Bistrita area, Bulletin of University of Agricultural Sciences and Veterinary Medicine Cluj-Napoca. Horticulture 79(1): 47-53.
17. Roşu-Mareş S.D., Sofron A.M., Guzu G.M., Jakab Z., and Carmen P. (2022). The response of some Romanian and foreign apple cultivars to the natural infections caused by *Podosphaera leucotricha*, in Bistriţa area: 151-156.
18. Švara A., De Storme N., Carpentier S., Keulemans W. and De Coninck B. (2024). Phenotyping, genetics, and “-omics” approaches to unravel and introgress enhanced resistance against apple scab (*Venturia inaequalis*) in apple cultivars (*Malus domestica*). Horticulture Research, 11(2) uhaee 002; 1-19.
19. Swezey S.L. (Ed.). (2000). Organic apple production manual (Vol. 3403). UCANR Publications.
20. Ştefan N., Glăman Gh., Branişte N., Stănică F., Duţu I. and Coman I. (2018). Romanian Pomology (in Romanian, publisher Ceres, Bucureşti
21. Yoder S.K., (2000). Effect of powdery mildew on apple yield and economic benefits of its management in Virginia. Plant disease. 84: 1171-1176.

The efficacy of some ecological insecticides against the defoliating larvae of *Lepidoptera* spp. on European plum under controlled conditions

C. Moldovan^{1*}, S.D. Roşu-Mareş¹, G.M. Guzu¹, I. Zagrai¹,
L.A. Zagrai¹, Z. Jakab-Ilyefalvi¹ and A.M. Chiorean¹

¹Fruit Research and Development Station Bistriţa, Romania

*Corresponding author email: claudiu.moldovan@scdp-bistrita.ro

ABSTRACT

The European plum (*Prunus domestica* L.) is the most important fruit species in Romania, and the second most cultivated in temperate regions, after apple. The plum is challenged by a number of pests and defoliating larvae from the order *Lepidoptera*, that can cause significant losses. Therefore, at Fruit Research and Development Station Bistrita (FRDS Bistrita), the efficacy of four ecological products: Oleorgan (0.3%), Laser 240 SC (0.06%), BactoSpeine DF (0.1%) and Konflic (0.3%), was tested under laboratory conditions in two consecutively years (2021 and 2022). The experiment was conducted on a sample of 50 larvae, the treatments were applied by spraying, and observations were made over a four-days period after the application of the treatments. The results obtained highlighted the potential of some ecological products in controlling defoliating larvae. Thus, Laser 240 SC achieved significant results, causing a mortality rate of up to 80%. Also, BactoSpeine DF demonstrated significant potential in controlling these pests, with a mortality rate of up to 50%. In contrast, Oleorgan and Konflic were less efficient in controlling defoliating larvae. Data collected over the two years laboratory experiments revealed the real potential of some ecological products under controlled conditions.

Keywords: eco-friendly, mortality rate, pest, phytosanitary treatments, *Prunus domestica*

INTRODUCTION

The interest in sustainable and environmentally friendly agricultural systems has grown significantly in the last 20 years according to The World of Organic Agriculture Statistics and Emerging Trends (2023). In the European Union, the support of farmers through non-reimbursable funds for the development of organic crops has encouraged sustainable agricultural methods. However, in fruit growing, major challenges include managing diseases and pests, which can cause significant economic losses. Although organic farming research is still in its early stages, the sector has the potential for rapid evolution due to the growing interest in sustainable practices and their positive impact on the environment. The success of organic farming depends on the intensification of research, the development of organic technologies and financial support for farmers who adopt these methods.

Prunus domestica L., commonly known as the European plum, is a highly popular and valued fruit-bearing species worldwide being consumed fresh or processed in a diversity of products (Gil *et al.*, 2002; Botu *et al.*, 2008; Ortega-Vidal *et al.*, 2022; Sottile *et al.*, 2022).

Plum continues to be the predominant fruit species in Romania (FAOSTAT, 2024), and it is desirable for at least a portion of its production to be ecological. However, managing the primary diseases and pests in orchards represents a significant challenge due to the limited information regarding the actual effectiveness of ecological phytosanitary products. Despite Romania's important position in the global plum production market, a large portion of pest and disease control remains largely reliant on chemical treatments that are now being withdrawn from the European market due to their toxicity.

Due to global warming, some *Lepidoptera* species in Romania have longer flight periods and more generations per year compared to the last century (Roşu-Mareş *et al.*, 2020). This further complicates matters for farmers, who must keep their crops healthy with less means to control the pest populations (Sjöberg *et al.*, 2015). Defoliating moths such as *Adoxophyes reticulana* (summer fruit tortrix), *Hedya nubiferana* (marbled orchard tortrix), *Archips podana* (fruit tree tortrix), and *Spilonota ocellana* (eye-spotted bud moth) are significant pests in European orchards, including those in Romania (Roşu-Mareş, 2021). These larvae feed on plum leaves, fruits and buds. They typically exhibit one to two generations annually and have the ability to overwinter in various stages, requiring integrated control strategies that combine eco-friendly approaches with pest biology knowledge (Charmillot, 2000).

The use of pheromone traps for monitoring *Lepidopterae* populations using species-specific pheromones have been successfully used in Romania (Jakab *et al.*, 2009, (Kutinkova *et al.*, 2009, Roşu-Mareş *et al.*, 2021) to monitor adult populations, allowing growers to time treatments more accurately and reduce unnecessary applications of insecticides (Gündüz & Karagüzel, 2020).

Biological control plays a central role in eco-friendly pest management. Additionally, maintaining habitats for predators like lady beetles (*Coccinellidae*) and birds contributes to natural pest control. Biological insecticides such as the small parasitoid wasp *Trichogramma* spp. (Keszthelyi and Péntes, 2014) or *Bacillus thuringiensis* (Cross *et al.*, 1999) are effective against lepidopteran larvae while being safe for non-target species, including pollinators.

By incorporating biological control agents, microbial and botanical insecticides and environmentally safe cultural practices, growers can reduce the reliance on synthetic chemicals, promoting both sustainable agriculture and long-term orchard health.

In this context, research conducted at FRDS Bistriţa has focused on controlling larvae from the *Lepidoptera* order, one of the most widespread pests affecting plum trees, which can cause significant damage. The purpose of the study was to identify the best ecological treatment options for *Lepidopterae* order larvae, among those currently existing on the Romanian insecticide market.

MATERIALS AND METHODS

The studies were conducted at the Fruit Research and Development Station Bistrita (FRDS Bistrita) in laboratory conditions, during 2021 and 2022, where the efficacy of four ecological products with insecticidal effects was evaluated for controlling defoliating larvae of the *Lepidoptera* order on plum. Performing the tests under controlled conditions was chosen to limit the interference of external factors as well as for the accuracy of determining the mortality of the larvae.

The adults captured on pheromonal traps in the experimental orchard in 2021-2022 were from the tortricid species: *Adoxophyes reticulana* (summer fruit tortrix), *Hedya nubiferana* (marbled orchard tortrix), *Archips podana* (fruit tree tortrix) and *Spilonota ocellana* (eye-spotted bud moth). *Adoxophyes reticulana* (figure1), was the most prevalent species in pheromonal traps in both years but the biological similarities between the larvae of the

above mentioned species determined us to consider the results for the whole group of tortricid moths existent in our orchard.



Figure 1. *Adoxophyes reticulana* adult and defoliating larvae

The ecological products tested were: Oleorgan, Laser 240 SC, BactoSpeine DF, and Konflic (Table 1). The ecological insecticides tested were selected based on their manufacturer-claimed insecticidal properties. The specific concentrations used in the experiments were based on the manufacturer recommended guidelines.

Table 1. List of ecological products evaluated in experiments aimed at controlling *Lepidoptera* defoliating larvae

No.	Commercial name	Concentration (%)	Active substance
1.	Oleorgan	0.30%	40% saponified oil extract from the Neem tree
2.	Laser 240 SC	0.06%	240 g/l spinosad
3.	BactoSpeine DF	0.10%	<i>Bacillus thuringiensis</i> subsp. <i>kurstaki</i> , 54%
4.	Konflic	0.30%	Potassium salt from vegetable oil extract 50% and <i>Quassia amara</i> extract 50%

In the experiment, plum shoots from the Stanley variety were collected from an untreated orchard. In the laboratory, the shoots were placed in plastic containers after being shortened to optimize space, with each treatment variant allocated its own container. The tests were conducted on a sample of 50 tortricid moths larvae, which were kept in plastic containers according to the treatment variant (Figure 2).

The results obtained for the four ecological products were compared to an untreated control variant. The data were analyzed using XLSTAT software (version 2019.3.2) from Addinsoft, operating on the MS Office Excel Professional Plus 2019 platform. All data, collected from laboratory, were organized in a completely randomized block design. XLSTAT was then employed to perform an analysis of variance (ANOVA, Fisher, 1925), followed by Duncan's Multiple Range Test (Duncan, 1955) to determine significant differences between the treatment variants at a significance level of $p < 0.0001$.



Figure 2. Preparation and application of treatments for controlling defoliating larvae of the *Lepidoptera* order

RESULTS AND DISCUSSIONS

The two-year study (2021-2022) demonstrated promising results for certain ecological products in controlling defoliating larvae. The results obtained in 2021, compared to those in 2022 indicate similar mortality rate of four organic products in controlling tortricid moths larvae, minor variations were observed particularly for Laser 240 SC, which showed a 10% difference in effectiveness (Figure 3).

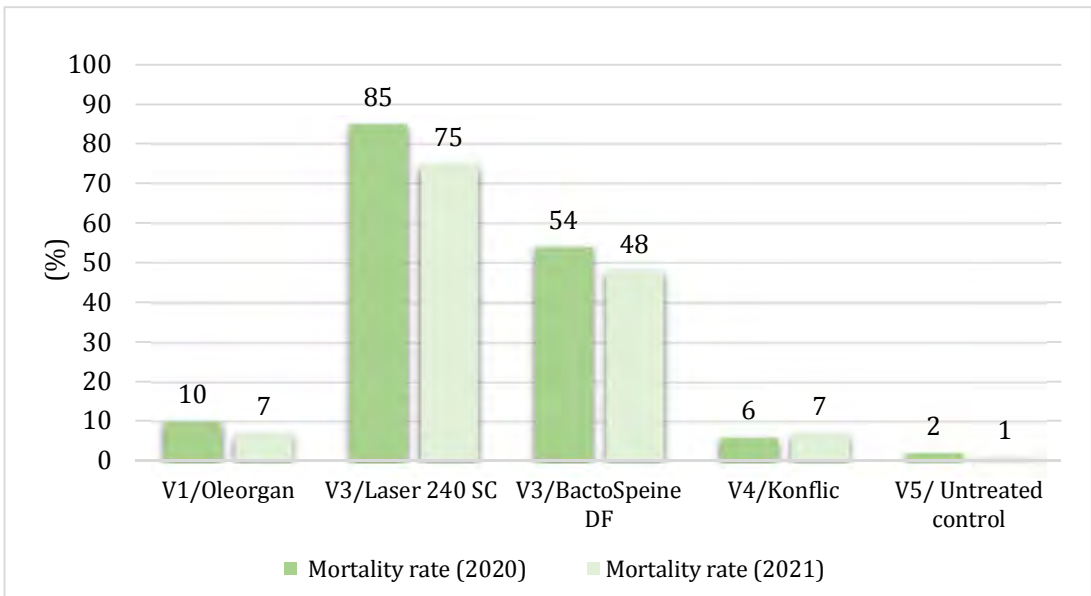


Figure 3. The efficacy of the products tested in the laboratory during the study

The results obtained in the laboratory over the two years (2020-2021), four days after applying the treatments, assessed that the organic product Laser 240 SC (0.06%, 240 g/l Spinosad) demonstrated the highest effectiveness, causing an average mortality rate of 80%. BactoSpeine DF (0.1%, *Bacillus thuringiensis* subsp. *kurstaki**, 54%), followed with a mortality rate of 51%. Oleorgan (0.3%) and Konflic (0.3%), exhibited significantly lower mortality rates, at 8.5% and 6.5%, respectively. The untreated control variant recorded a mortality rate of 1.5% (Table 2).

Table 2. The data obtained regarding the mortality rate of defoliating larvae during the study

No.	Product	Concentration (%)	Average mortality rate (2020-2021)
1	V1/Oleorgan	0.3%	8.5%
2	V2/ Laser 240 SC	0.3%	80%
3	V3/ BactoSpeine DF	0.06%	51%
4	V4/ Konflic	0.1%	6.5%
5	V5/ Untreated control	-	1.5%

***Note:** the best results obtained in the experience are marked in bold

Following the statistical analysis of multiple years data using the Duncan test to evaluate the effectiveness of various organic products in controlling defoliating larvae, significant differences were found among the tested products in the laboratory. The results were divided into four significance classes. The product with the best performance, Laser 240 SC, was found to be significantly better than the other tested products. Bactospeine DF was significantly more effective than the products Oleorgan and Konflic, which were in the same significance class. As expected, the untreated control variant was placed in the lowest significance category (Table 3).

Table 3. Statistical processing of multi-year data on the control of defoliating larvae

Produs	The effectiveness of ecological products tested in the laboratory
V3/Laser 40 SC	80.000 ± 2.200 ^a
V4/Bactospeine DF	51.000 ± 1.852 ^b
V1/Oleorgan	8.500 ± 0.600 ^c
V4/Konflic	6.500 ± 2.042 ^c
V5/ Untreated control	1.500 ± 0.700 ^d
Pr > F(Model)	<0,0001
Signification	Yes

Of the four organic products tested, Laser 240 SC (0.06%) achieved the best results. Similar to our findings, Laser 240 SC, based on spinosad, has been reported to have a strong insecticidal effect in controlling pests from the *Lepidoptera* order, such as *Spodoptera littoralis*, *Helicoverpa zea*, *Helicoverpa armigera*, *Tuta absoluta*, and *Helicoverpa amigera* (Brickle *et al.*, 2001; Aydin & Goulson, 2006; Abouelghar *et al.*, 2013; Singh *et al.*, 2022; Erasmus *et al.*, 2023; Jimu *et al.*, 2023). Spinosad has been demonstrated

as an effective pest control agent, particularly in managing *Lepidopteran* insect pests (Wanner *et al.*, 2000). Specialized studies have shown that the mortality of defoliating larvae is directly influenced by the concentration of spinosad applied (Jat and Ameta, 2013). Higher concentrations of Spinosad resulted in the highest mortality rates, that highest concentrations showed a 95% mortality 95% (Nawaz *et al.*, 2019). Galvan *et al.* (2005) reported that spinosad reduced the survival rate of first instar larvae, prolonged their development time to adulthood, decreased weight gain, and lowered the fertility of female *Harmonia axyridis*. Spinosad, a naturally derived insecticide produced by the fermentation of *Saccharopolyspora spinosa*, acts on the insect's nervous system, causing rapid mortality in targeted pests while having minimal impact on beneficial organisms (Cross *et al.*, 1999). Most studies on the toxicity of spinosad in *Lepidoptera* pests have primarily focused on larval stages, with limited research on its sublethal effects in adult target species (Sparks *et al.*, 1998; Pineda *et al.*, 2004). However, Pineda *et al.* (2007) observed reproductive effects in *Spodoptera littoralis* when adults were exposed to spinosad through both residual contact and ingestion. Spinosad acts as a neurotoxin on insects, disrupting their nervous activity and causing paralysis and death. Beneficial effects have also been reported in the control of pests from the *Coleoptera* order, to *Sitophilus oryzae* and *Rhyzopertha dominica* species (Andrić *et al.*, 2018; Perišić *et al.*, 2022; Abdelgaleil *et al.*, 2024; Bohórquez *et al.*, 2024).

Among the eco-friendly substances tested, spinosad has proven to be the most effective in controlling *Lepidoptera* order larvae in plum orchards during our recent trials.

The product Bactospeine DF (0.3%) demonstrated high potential in controlling defoliating larvae, causing over 50% mortality in four days after treatment. The products Oleorgan (0.3%) and Konflic (0.3%) showed unsatisfying results, causing a low mortality rate. Regarding these two products, based on *Azadirachta indica* (Neem tree) and *Quassia amara* oil, the results obtained recommend them at most as an auxiliary in a program to control the larvae of defoliating lepidoptera.

Identifying efficient ecological solutions for controlling the main pests and diseases of plum remains an important topic of discussion in the context of the European Green Deal, which aims to ensure that a significant part of agriculture is practiced in eco-friendly systems. By developing sustainable and eco-friendly strategies, we can protect plum orchards and ensure a resilient agricultural future.

CONCLUSIONS

The results obtained after two years of study (2021-2022) highlight, overall, the potential of certain organic products in controlling defoliating larvae from the *Lepidoptera* order, which can cause serious issues in plum orchards. The organic substance spinosad could represent an effective solution for controlling defoliating larvae in organic plum cultivation, as well as in Integrated Pest Management (IPM). The other biologic substances tested did not performed very well in our two-year study for tortricid moth control and probably require future improvment.

ACKNOWLEDGEMENTS

The study is part of the research program funded by Romanian Ministry of Research and Innovation, PCCDI-UEFISCDI, project number PN-III-P1-1.2-PCCDI-2017-0662, within PNCDI III. We are grateful to Fruit Research & Development Station Bistrita for the generous help and assistance.

REFERENCES

1. Abdelgaleil S.A., Gad H.A., Al-Anany M.S. and Atta AA. (2024). Monoterpenes improve the insecticidal efficacy of spinosad against *Sitophilus oryzae* (L.) on stored wheat. *International Journal of Pest Management*, 70(3), 508-518.
2. Addinsoft XLSTAT Statistical and Data Analysis Solution; Addinsoft: New York, NY, USA, 2022.
3. Andrić G., Kljajić P., Golić M.P., Trdan S., Bohinc, T., and Solarov M.B. (2019). Effectiveness of spinosad and spinetoram against three *Sitophilus* species: Influence of wheat endosperm vitreousness. *Journal of Stored Products Research*, 83, 209-217.
4. Aydin H. and Gürkan M.O. (2006). The efficacy of spinosad on different strains of *Spodoptera littoralis* (Boisduval) (Lepidoptera: Noctuidae). *Turkish Journal of Biology*, 30(1), 5-9.
5. Bohórquez, G., Jumbo, L. O. V., Albino-Bohórquez, A., Oliveira, E. E., & Bacca, T. (2024). Comparative efficacy of alternative insecticides for *Rhyzopertha dominica* management in Colombia: Beyond organophosphates and pyrethroids. *Journal of Stored Products Research*, 109, 102465.
6. Botu I., Botu M., Achim G. and Baciú A. (2008). Plum culture in Romania: Present situation and perspectives. In: IX International Symposium on Plum and Prune Genetics. *Breed. Pomol.* 874, 365-372.
7. Brickle D.S., Turnipseed S.G. and Sullivan M.J. (2001). Efficacy of insecticides of different chemistries against *Helicoverpa zea* (Lepidoptera: Noctuidae) in transgenic *Bacillus thuringiensis* and conventional cotton. *Journal of economic entomology*, 94(1), 86-92.
8. Charmillot P.J. and Gut L. J. (2000). Mating disruption to control codling moth and oriental fruit moth in fruit orchards. *IOBC/WPRS Bulletin*, 23, 65-79.
9. Cross J.V., Solomon M.G., Chandler D., Jarrett P., Richardson P.N. and Winstanley D. (1999). Biocontrol of pests of apples and pears in northern and central Europe: 1. Microbial agents and nematodes. *Biocontrol Science and Technology*, 9(2), 125-149.
10. Duncan D.B., Multiple range and multiple F tests. *Biometrics* 1955, 11, 1-42.
11. Erasmus R., van den Berg J., van Rensburg P.J. and du Plessis H. (2023). Residual activity of spinosad applied as a soil drench to tomato seedlings for control of *Tuta absoluta*. *Pest Management Science*, 79(5), 1860-1867.
12. Fisher R.A., *Statistical Methods for Research Workers*; Oliver & Boyd: Edinburgh, UK, 1925.
13. Food and Agriculture Organization of the United Nations (FAOSTAT), (2024).
14. Galvan T.L., Koch R.I. and Hutchison W.D. (2005). Effects of spinosad and indoxacarb on survival, development, and reproduction of the multicolored Asian lady beetle (Coleoptera: Coccinellidae). *Biol. Control* 34 (1): 108-114.
15. Gil M.I., Tomás-Barberán F.A., Hess-Pierce B. And Kader A.A. (2002). Antioxidant Capacities, Phenolic Compounds, Carotenoids, and Vitamin C Contents of Nectarine, Peach, and Plum Cultivars from California. *J. Agric. Food Chem*, 50: 4976-4982.
16. Gündüz E.A. and Karagüzel O. (2020). Population dynamics of tortricid moths in apple and plum orchards in Turkey. *Turkish Journal of Agriculture and Forestry*, 44(2), 154-162.
17. Jakab-I. Zs. Platon I. Ganska Lucia. (2009). Researches concerning the pheromonal control of summer fruit tortrix moth (*Adoxophyes orana*. Hb) using the biotechnique "Attract and kill", *Bulletin UASMV Cluj Napoca*, 66: 633-640.
18. Jat S.K., and Amet, O.P. (2013). Relative efficacy of biopesticides and newer insecticides against *Helicoverpa armigera* (Hub.) in tomato. *Bioscan Ranchi (Ranchi)* 8 (2), 579-582.
19. Jimu F., Mapuranga R., Mubvekeri W. and Kutywayo D. (2023). Efficacy of Spinosad 48SC on the Management of Cotton Bollworms *Diparopsis castanea* (Hubner) and *Helicoverpa amigera* (Hampson) in Zimbabwe. *Asian Journal of Research in Crop Science*, Volume 8, Issue 4, Pg 56-63.
20. Keszthelyi S. and Péntes B. (2014). Effectiveness of mating disruption against *Adoxophyes orana* in plum and apple orchards in Hungary. *Horticultural Science (Prague)*, 41(3), 110-115.
21. Kutinkova H., Dzhuvinov V., Platon I. and Roşu-Mareş S. (2009). Field monitoring of codling moth, *Cydia pomonella* L. (Lepidoptera: Tortricidae), by Pheromone Traps in Bulgaria and Romania; *Acta Horticulture*, 825: 371-376.
22. Nawaz A., Ali H., Sufyan M., Gogi M.D., Arif M.J., Ranjha M.H., ... and Ghramh H.A. (2019). Comparative bio-efficacy of nuclear polyhedrosis virus (NPV) and Spinosad against American bollworm, *Helicoverpa armigera* (Hubner). *Revista Brasileira de Entomologia*, 63(4), 277-282.
23. Ortega-Vidal J., Cobo A., Ortega-Morente E., Gálvez A., Martínez-Bailén M., Salido, S. and Altarejos J. (2022). Antimicrobial activity of phenolics isolated from the pruning wood residue of European plum (*Prunus domestica* L.). *Industrial Crops and Products*, 176: 114-296.
24. Perišić V., Perišić V., Rajčić V., Luković K. and Vukajlović F. (2022). Spinosad application in process of integrated pest management against *Rhyzopertha dominica* F. in stored small grains. In *Book of proceedings: 26. International Eco-Conference*, 21-23. Novi Sad pp. 125-132.

25. Pineda S., Budia F., Schneider M.I., Gobbi A., Viñuela E., Valle J. and Del Estal P. (2004). Effects of two biorational insecticides, spinosad and methoxyfenozide, on *Spodoptera littoralis* (Lepidoptera: Noctuidae) under laboratory conditions. *Journal of economic entomology*, 97(6), 1906-1911.
26. Pineda S., Schneider M.I., Smagghe G., Martínez A.M., Del Estal P., Viñuela, E., ... and Budia F. (2007). Lethal and sublethal effects of methoxyfenozide and spinosad on *Spodoptera littoralis* (Lepidoptera: Noctuidae). *Journal of economic entomology*, 100(3), 773-780.
27. Roşu-Mareş S.D. (2021). The outbreak of *Sponota ocellana* (Denis & Schiffermüller), the eye-spotted bud moth on apple trees in Bistriţa region", *Scientific Papers Series B. Horticulture Volume LXV* (2): 56-61.
28. Roşu-Mareş S.D., Pojar Feneşan M., Ciotlăuş I., Balea A. and Andreica A. (2021). Pheromone monitoring of harmful Lepidopterae present in Bistrita area in apple and plum orchards. *Romanian Journal of Horticulture*, II, 99-106.
29. Roşu-Mareş S., Şofron A. and Moldovan C. (2020). Preliminary results on the changes in the flight of *Cydia pomonella*(L.) in Noth- Eastern Transylvania, under the influence of climate change. *Scientific Papers Series B. Horticulture Volume LXIV* (1): 183-185.
30. Singh K., Raju S.V.S. and Sharma K.R. (2022). Field efficacy of novel insecticides emamectin benzoate and spinosad against fruit borer, *Helicoverpa armigera* (Hubner) on tomato. *Journal of Entomological Research*, 46(1), 106-110.
31. Sjöberg P., Ramert B., Thierfelder T. and Hillbur Y., (2015). Ban of a broad-spectrum insecticide in apple orchards: effects on tortricid populations, management strategies, and fruit damage. *Journal of Pest Science*. 88(4):767-775.
32. Sottile F., Caltagirone C., Giacalone G., Peano C., and Barone E. (2022). Unlocking plum genetic potential: Where are we at?. *Horticulturae*, 8(2): 122-128.
33. Sparks T.C., Thompson G.D., Kirst H.A., Hertlein M.B., Larson L.L., Worden T.V. and Thibault S.T. (1998). Biological activity of the spinosyns, new fermentation derived insect control agents, on tobacco budworm (Lepidoptera: Noctuidae) larvae. *Journal of Economic Entomology*, 91(6), 1277-1283.
34. *The World of Organic Agriculture Statistics and Emerging Trends*, (2023), Edited by Helga Willer, Bernhard Schlatter and Jan Trávníček.
35. Wanner K.W., Helson B. V. and Harris B.J. (2000). Laboratory and field evaluation of spinosad against the gypsy moth, *Lymantria dispar*. *Pest Management Science: formerly Pesticide Science*, 56(10), 855-860.

Spread and development of *Verticillium* wilt or cotton wilt in the western part of Azerbaijan

L. Huseynova

Institute of Plant Protection and Industrial Crops, Republic of Azerbaijan

*Corresponding author e-mail: fitopatoloq.Lale@mail.ru

ABSTRACT

Almost 136 thousand hectares of cotton crops in Azerbaijan, the unique climate allows growing almost all cultivars of cotton. More than half of the cotton crops are in the Ganja-Kazakh geographical zone of the country. The development of cotton growing in Azerbaijan as a highly profitable sector of the economy is inextricably linked not only with active selection work, the introduction of new cultivars and progressive technologies, but also with the creation of an effective set of protective measures. In recent years, with the increase in areas under cotton and the growth of its productivity, the likelihood of the spread of harmful mycoses has significantly increased. Among the widespread diseases in cotton crops, *Verticillium* wilt occupies a special place. *Verticillium* wilt is a very harmful disease. During its development in the plant, the osmotic pressure and cell turgor decrease, transpiration increases, the flow of water and minerals to the roots and aboveground organs of the plant slows down, the parenchymatous cells of the wood die off and the leaves fall prematurely, which leads to a sharp decrease in the yield and quality of cotton. In connection with the above, the objective of our research was to study and diagnose *verticillium* wilt of cotton in the conditions of the western part of Azerbaijan. In the 2022-2024 research years, an assessment was made of the prevalence and intensity of *verticillium* wilt of cotton in the region, and a scientifically based and improved control system was developed. For this purpose, the following preparations were tested in the fight against *verticillium* wilt of cotton: Kolosal Pro, EC, Fitolavin, VRK and control without chemical treatment. Analysis of the conducted field studies show that the fungicide Fitolavin, VRK on all cultivars of cotton showed high biological efficiency against the disease.

Keywords: cotton, *verticillium* wilt, prevalence, development, harmfulness

INTRODUCTION

Cotton is one of the most valuable agricultural plants of the spinning group. The main product for which it is cultivated is fiber. It is formed in the form of hair-like growths on the seeds. This distinguishes cotton from bast spinning plants, in which the fiber develops in the bast part of the stem (Mukhamedzhanov, 1960).

Despite the great successes achieved in the production and use of various types of artificial fiber, cotton fiber, due to its versatility, still retains its exceptional importance. It is mainly used for the production of various cotton fabrics. There is not a single branch of the national economy that would not use cotton materials and products to one degree or another (Kurbanov *et al.*, 2018). In addition to regular yarn and a wide cultivar of clothing and underwear fabrics, cotton fiber is used to produce sewing threads, ropes, cables, fishing tackle, drive belts, conveyor belts, special fabric for rubber hoses (sleeves), filters, electrical insulation winding, artificial silk (viscose), and cotton wool. The fiber of some cultivars of cotton is used to produce special cord fabrics used in the automobile industry for tire gaskets, for making parachutes, tarpaulin, etc. Cotton fiber can also be used to make celluloid, photographic films, varnishes, high grades of writing paper, and many

other different materials and products. It is also used as an admixture to wool and silk (Firsov 2006; Fedotov *et al.*, 2015).

Cotton seeds are of great value; after the fibers are removed, they are partly used for sowing, and mainly processed to obtain various products, in particular oil. It is used as food and for technical purposes. Refined cottonseed oil has very high nutritional qualities and is considered almost equivalent to Provencal oil, so it is widely used in the canning industry. It is also used to make margarine, artificial lard, laundry and toilet soap, technical oils and drying oil. Glycerin, stearin and a number of other products are obtained from it. The cottonseed cake and meal remaining after oil separation are good concentrated feed for some domestic animals (Baranov *et al.*, 1994; Stupin *et al.*, 2014). Gossypol is extracted from the seed kernel, from which polymers, varnishes, heat-resistant coatings, medicines, dyes and some other materials are produced. A waste product of the cotton oil industry, gossypol resin (tar), is very heat-resistant and can be used to produce heat-resistant varnishes, and in foundry - to make molds. A polymer used to make very durable products has been isolated from cotton tar, and as an admixture it can be used for hard road surfaces. Cotton is an excellent honey plant, so beekeeping can be successfully developed near its crops (with some restrictions on the use of pesticides to control pests and diseases). In total, more than 150 types of various materials and products are produced from cotton fiber, seeds and other parts of the plant (Pasypanov 2015; Savelyev 2019).

All types and forms of cotton make up one genus - gossypium (*Gossypium* L.), which is part of the mallow family (*Malvaceae* Juss.) (Figure 1). It originated in the tropical zone of the globe, where the air temperature in the coolest month of the year is not lower than 18 °C. Cotton is a perennial woody plant. In its homeland, it grows not only in culture, but also in the wild, representing bushes and trees of various sizes up to 6-7, less often up to 10-12 m in height. The longevity of different forms of cotton is not the same. Usually tall forms are more durable. Low-growing forms (small bushes), cultivated in the former USSR and other countries, are also perennial by nature. However, in the cotton-growing regions of the former USSR (including Azerbaijan), there are frosts in winter, so cotton can only grow as an annual crop. If it is placed in a greenhouse during the period of frost, it will live for several years (Kozlovskaya 2015). The first centers of cotton cultivation and the use of its fiber for spinning and weaving were India and, apparently, Southeast and Central Africa in the Old World; Peru, Mexico and the Antilles in the New World (Torikov *et al.*, 2020).

Verticillium wilt (*Verticillium dahliae* Kleb.) is registered everywhere in the cotton area of the former USSR. The disease is most severe in Uzbekistan, Kyrgyzstan, a number of regions of Tajikistan, Kazakhstan and Transcaucasia (Peresypkin 1989; Nerozin 2005; Beloshapkina 2015). The causative agent of tracheomycotic wilt of cotton is the fungus *Verticillium dahliae* Kleb. It has only an anamorphic stage in its life cycle. Structurally, it is represented by microsclerotia up to 0.1 mm in size and septate colorless mycelium with whorled conidiophores, on the sterigmata of which unicellular, colorless conidia are formed in the heads. With the help of microsclerotia, the fungus is able to survive in the soil for several years. In humid conditions, a white sporulation coating appears on the affected cotton. The causative agent of the disease is polyphage. Since, in addition to cotton, it affects many field crops, grasses and tree species.

The main symptom of cotton wilt is tracheomycotic wilt, caused by damage to the vascular system, due to mechanical blockage of vessels by a mass of pathogens or their destruction by toxins (Pospelov *et al.*, 1979; Durinina *et al.* 1984; Karbozova *et al.*, 2015).

The aim of our work was to study the prevalence, intensity of development and harmfulness of verticillium wilt (*Verticillium dahliae* Kleb.) in the conditions of the western part of Azerbaijan, as well as to develop a system of measures to protect cotton bushes from this disease.

MATERIALS AND RESEARCH METHODS

The investigations took place within Goranboy region describe the place, located in the west of Azerbaijan. For three consecutive years (2022-2024) the spread, development and harmfulness of verticillium wilt of cotton (*Verticillium dahliae* Kleb.). The soil of the experimental plot is chestnut. The area of the experimental plot is 20 hectares, the experiment was repeated 4 times. Different varieties of cotton were used in the studies ('*Ganja-160*', '*AzNIHI-195*', '*Agdash-3*' and '*Marash*'). The disease was monitored from the beginning to the end of the growing season. Cotton bushes affected by the disease were examined in the fields and samples were taken from their infected plant organs. They were studied in the laboratory. Microscopic analysis of the collected material was carried out in the Central Phytosanitary Laboratory of the Azerbaijan Institute of Food Safety. Meteorological conditions during the years of study were generally favorable for the growth and development of cotton. The prevalence and development of the disease were considered according to M.I. Dementyeva. (Dementyeva, 1985). The intensity of damage to cotton bushes by verticillium wilt (*Verticillium dahliae* Kleb.) was assessed on a scale: 0 points - no damage; 1 point - 1 to 10% of the leaf surface is damaged; 2 points - 11 to 25% of the leaf surface is damaged; 3 points - 25 to 50% of the leaf surface is damaged; 4 points – more than 50% of the leaf surface is damaged.

RESULTS AND DISCUSSION

As a result of the conducted route surveys, verticillium wilt of cotton (*Verticillium dahliae* Kleb.) was registered in all western regions of Azerbaijan (Table 1).

Table 1. Spread and intensity of development of verticillium wilt of cotton (*Verticillium dahliae* Kleb.) in the conditions of the western part of Azerbaijan (Goranboy district, 2022-2024)

Cultivars	2022 year		2023 year		2024 year	
	P (%)	R (%)	P (%)	R (%)	P (%)	R (%)
' <i>Ganja-160</i> '	23.4	15.8	23.8	16.0	24.1	16.3
' <i>AzNIHI-195</i> '	56.7	29.8	57.9	30.1	59.5	30.8
' <i>Agdash-3</i> '	61.1	30.3	62.7	30.7	63.8	31.5
' <i>Marash</i> '	22.0	15.0	22.5	15.2	22.6	15.2

Note: P – disease prevalence, %; R – disease development intensity, %



Figure. 1. Cotton field (*a*-close-up and *b*-view from a distance)

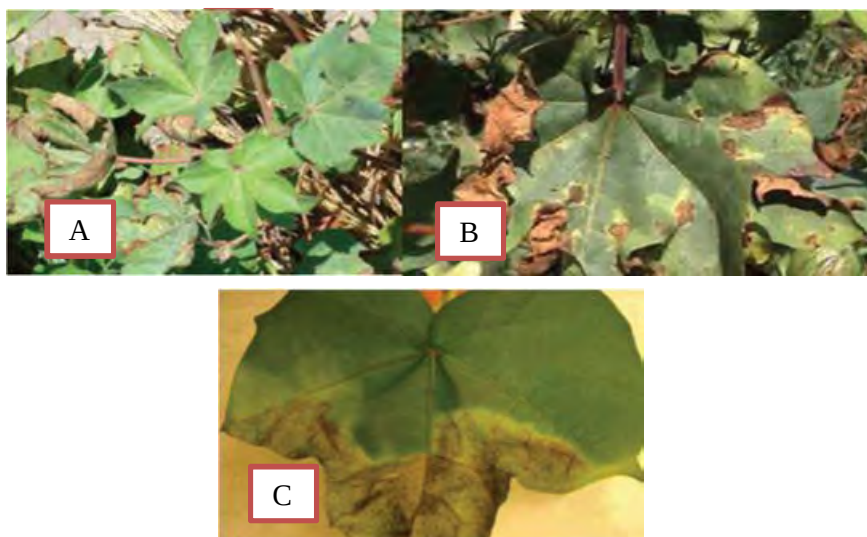


Figure. 2. *a* – affected leaves; *b* - the formation of necrotic areas on diseased leaves; *c* - drying cotton leaves affected by wilt

The first signs of wilt appear in the phase of 3-4 true leaves between the veins. Intensive manifestation of the disease is observed at the beginning of flowering. The affected leaf tissue first loses turgor, then becomes pale green or yellow, and then dries up (Figure 2). The disease, reaching the capsules, penetrates the seeds. The affected tissue of the conducting vessels of the leaves, petioles, stems and capsules becomes dark brown, which is a characteristic sign of the disease. In case of epiphytotic, the crop acquires the appearance of being burnt by fire. Populations of *Verticillium dahliae* Kleb. are heterogeneous in terms of virulence. The causative agent of the disease most severely affects cotton at an air temperature of 23-25°C and a soil moisture of 60%. The disease is most intense in irrigated agriculture and in areas with high saturation of field crop rotations with cotton and untimely replacement of susceptible varieties with resistant ones.

Verticillium wilt (*Verticillium dahliae* Kleb.) is an extremely harmful disease of cotton, since its infestation can exceed 60%. The disease is most harmful to medium-fiber varieties (*Gossypium hirsutum* L.). Fine-fiber cotton (*Gossypium barbadense* L.), although affected by wilt, exhibits a certain tolerance to *Verticillium dahliae* Kleb., so its yield losses from the disease are significantly less.

In Azerbaijan, located in the very center of the "Silk Road", they were also engaged in the production of snow-white fiber. During expeditions, archaeologists of the country discovered ancient irrigation systems, which indicated that cotton was grown in our country in ancient times. Cotton (*Gossypium* L.) is affected by many diseases, among which Verticillium wilt (*Verticillium dahliae* Kleb.) causes considerable harm, reducing the quantity and quality of the harvest or causing premature death of plants (Figure 4).

The conducted studies have established that in the western part of the country (in the Goranboy region), verticillium wilt of cotton is very widespread. Regarding the response of the cultivars to this disease, in the years 2022-2024, the greatest damage was observed in the cultivars 'AzNIHI-195' and 'Agdash-3' cv. and 'Agdash-3' where disease prevalence was between 61.1 % and 53.8 for 'Agdash-3', and 56.7 and 59.5 for 'AzNIHI-195' cv.

At the opposite pole, the results showed high tolerance to this disease for the cotton cultivars "Ganja-160" and 'Marash'. For the latter, the prevalence of the disease in the three years of studies was between 22.0% and 22,6 % for the Marash cv. and varied between 232.4 % and 21.1for the *Ganja-160*cv.



Figure 3. The cutting of an affected cotton stem



Figure 4. Death of a cotton bush under the influence of wilt

Therefore, the cultivation of resistant cultivars of cotton (*Gossypium* L.) to the cryptogram disease attack could be one of the agronomic control measures for the western part of Azerbaijan.

As already noted, we paid some attention to the development of a chemical method against verticillium wilt of cotton (*Verticillium dahliae* Kleb.). The following fungicides were tested for this purpose: Kolosal Pro, EC (300 g/l propiconazole + 200 g/l tebuconazole) and Fitolavin, VRK (BA-120000 EA/ml), applied in the periods mentioned in table 2.

Table 2. Effect of fungicides on the spread and development of verticillium wilt of cotton (*Verticillium dahliae* Kleb.) (2022-2024)

Fungicides	Period and number of treatments	Cultivars	2022 year			2023 year			2024 year		
			P (%)	R (%)	BE (%)	P (%)	R (%)	BE (%)	P (%)	R (%)	BE (%)
Kolosal Pro. EC (300 g/l propiconazole + 200 g/l tebuconazole)	In the phase after flowering (3)	'AzNIHI-195'	28.0	16.7	50.1	26.1	14.9	57.3	22.0	13.6	63.0
		'Agdash-3'	26.0	15.9	64.0	25.5	15.0	66.1	20.1	14.7	67.0
		'Marash'	15.5	7.9	74.0	15.0	6.9	76.0	15.0	6.8	77.0
		'Ganja-160'	14.4	6.6	77.0	14.5	6.7	76.0	14.2	6.1	79.4
Fitolavin. VRK (BA-120000 EA/ml)	Before flowering (3)	'AzNIHI-195'	20.0	13.2	61.0	19.2	12.0	66.0	18.5	10.0	73.0
		'Agdash-3'	19.9	9.8	78.0	19.4	9.0	80.0	19.0	8.8	80.3
		'Marash'	15.0	7.1	76.3	15.0	7.0	76.0	14.5	6.5	75.3
		'Ganja-160'	14.5	6.3	78.0	14.2	6.1	78.1	14.0	6.2	79.1
Control (without chemical treatment)	x	'AzNIHI-195'	66.9	33.5	0	71.0	34.9	0	75.5	36.6	0
		'Agdash-3'	77.7	44.0	0	77.9	44.3	0	80.5	44.6	0
		'Marash'	49.0	29.9	0	48.2	28.9	0	49.1	29.0	0
		'Ganja-160'	48.8	28.7	0		27.9	0		29.6	0

Note: **P** – disease prevalence, %; **R** – disease development intensity, %; **BE** - biological efficiency, %

CONCLUSIONS

The article presents the results of stationary and route studies on the study of *Verticillium* wilt of cotton, analyzes the parasitic features of the fungus *Verticillium dahliae* Kleb. The results and analyzes of field experiments conducted over 3 years are presented. The experiment included 4 cultivars of cotton ('*Ganja-160*', '*Agdash-3*', '*AzNIHI-195*' and '*Marash*'). In the field experiment of 2022-2024, cotton bushes were treated with fungicides - Kolosal Pro, KE (300 g / l propiconazole + 200 g / l tebuconazole) and Fitolavin, VRK (BA-120000 EA / ml). Analysis of the obtained data showed that in 2024, as well as in 2023, the best result against wilt was obtained in the case of Fitolavin.

REFERENCES

1. Mukhamedzhanov M.V. (1960). Cotton. "Academy of Sciences of Uzbekistan", 1960, 335 p.
2. Kurbanov A.E., and Avtonomov V.A. (2018). Creation of cotton varieties resistant to pathogens. Moscow: "Lambert", 74 p.
3. Fedotov V.A., Shchedrina D.I., and Kadyrov S.V. (2015). Plant growing. Moscow: "Lan", 106 p.
4. Baranov V.D., and Ustimenko G.V. (1994). The world of cultivated plants. Moscow: "Mysl", 158 p.
5. Firsov I.T. 2006. Plant growing technology. Moscow: "Kolos", 250 p.
6. Stupin A.S., and Naumkin V.N. (2014). Plant growing technology. M.: "Lan", 264 p.
7. Saveliev V. A., (2019). Plant growing. M.: "Lan", 194 p.
8. Pasypanov G. S. (2015). Plant growing: practical course. M.: "INFRA-M", 92 p.
9. Kozlovskaya I. P. (2015). Technological foundations of plant growing. Minsk: "IVC of the Ministry of Finance", 105 p.
10. Torikov V. E., Belous N. M., and Melnikova O. V., (2020). Plant growing. M.: "Lan", 53 p.
11. Nerozin S. A. (2005). Pest and disease control of cotton. Tashkent, 6 p.
12. Dementeva M. I. (1985). Phytopathology. M.: "Agropromizdat", 122 p.
13. Beloshapkina O. O. (2018). Phytopathology. M.: "INFRA-M", 118 p.
14. Peresypkin V. F. (1989). Agricultural phytopathology. M.: "Agropromizdat", 336 p.
15. Pospelov S. M., Arsenyeva M. V. (1979). Plant protection. M.: "Kolos", 122 p.
16. Dyakov Y. T., and Elansky S. N. (2021). General phytopathology. M.: "Yurait", 117 p.
17. Durinina E. P., and Velikanov L. L. 1984. Soil phytopathogenic fungi. M.: "Publishing House of Moscow University", 37 p.
18. Zheldakova R.A., and Myamin V.E. (2006). Phytopathogenic microorganisms. Minsk: "Publishing House of the Belarusian State University", 93 p.
19. Zezyulina G.A. (2017). Agricultural phytopathology. Minsk: "IVC of the Ministry of Finance", 333 p.
20. Karbozova R.D., and Tulengutova K.N. (2015). Phytopathology. Alma-Ata: "Dayip", 183 p.

Effectiveness of active ingredients in controlling fireblight (*Erwinia amylovora*) in modern apple orchards

F.C. Marin^{1*}, M. Călinescu¹, M. Sumedrea¹ and E. Chițu¹

¹Research Institute for Fruit Growing, Pitesti, Romania,
Orchard Technology and Plant Protection Laboratory

*Corresponding author: c_marin69@yahoo.com

ABSTRACT

This study investigated the effects of different registered active ingredients aiming to control fireblight *Erwinia amylovora* Burill Winslow, in modern apple bearing orchards. Five type active ingredients and combinations were assessed as regard their protective effect against fire blight on the apples. *Copper oxiclорure* provided an average efficacy E% 78.74-80.81 Better results were obtained with *copper hydroxide* E% 80.70-81.66. The mixture *copper oxiclорure+hydroxide* displayed a superior field efficacy ranging between 81.45 - 83.91. *Aluminum fosetyl*, applied during the flowering period and fruit set provided an average efficacy E% of 90.70 -81.26. The other mixture tested *fluopyram+aluminum fosetyl* offered an average efficacy E% in fireblight control, within 91.20 - 93.18 especially on growing shoots and fruits. However, in order to increase the bactericides efficacy in apple crop protection, it will be useful and opportune additional researches, on new active ingredients complementarity, water quality monitoring, spray equipment calibration, and improvement of applications quality.

Keywords: apple, orchards, fireblight, chemical control.

INTRODUCTION

Fireblight - *Erwinia amylovora* Burrill 1882 Winslow *et al.* 1920 (Phylum *Pseudomonadota*, Class *Gammaproteobacteria*, Order *Enterobacterales*, Family *Erwiniaceae*) is a contagious bacterial disease affecting More than 200 species from 40 genera, worldwide, in 46 countries and among these, 75 members of *Rosaceae* Family are susceptible, which is a major concern for the pome fruits producers. (Momol and Aldwinckle 2000; Babadoost, 2005; Beckerman, 2007; Sundin, 2014). This destructive crop bacterium initially originated in North America. Today, *E. amylovora* can currently be found in all the provinces of Canada, as well as in some parts of the USA. Nowadays it is believed that the pathogen was first introduced into Northern Europe in the 1950s through fruit containers, contaminated with bacterial ooze, imported from the USA. During the following decades, *E. amylovora* spread through much of Northern Europe. In Romania was reported first time in 1991 and since then spread constantly especially among sensitive and highly sensitive commercial varieties. (Amzăr and Ivașcu, 2003). *Bacteria overwinter in cankers. In the spring, bacteria multiply fast at the canker margins and in adjacent bark tissues, giving rise to inoculum* (Van der Zweet and Beer 1995, Hartman and Hershman, 2002). *Schroth, 2010; Cooley et al., 2015; Ivey & Ellis, 2016; Nischwitz and Hubbel, 2018; DuPont, 2023; Peter, 2023*). The bacteria infect first the trees flowers travels more quickly in one- and two-year-old wood, and within 2-3 years especially the young trees are killed. Honeybees and other insects are attracted to the colonies ooze and can spread bacteria to susceptible tissue, such as flower stigmata. Birds, rain and wind can also transmit the bacterium to susceptible tissue, the colonization of

which will be heavily decided by temperature (21-28-31 °C is most favourable) and moisture either from rain or heavy dew. A few minutes of heavy hail can spread the disease throughout an entire orchard, killing the trees. (Thomson, 1986; Steiner *et al.*, 2000; Babadoost, 2005; Teviotdale, 2011, Malnoy *et al.*, 2012; Cooley *et al.*, 2015; Johnson, 2000; Johnson, 2015; Koski and Jacobi, 2015; Smith, 2017; Nischwitz and Hubbel, 2018; DuPont, 2023; Martinez, and Pearce, 2023).

Climate changes, strong winds and drought periods, also can contribute to spread the disease in the apple orchards. (Roşu-Mareş *et al.*, 2024).

Most commercially successful apple cultivars (12.5% autochthonous and 44.0% foreign ones) grown in recent years which includes: 'Elstar' 'Fuji', 'Gala', 'Braeburn', 'Golden Delicious', 'Red Delicious', 'Granny Smith', 'Idared', 'Jonathan', 'Jonagold', 'Mutsu', 'Rome Beauty', (Braniste and Andries, 1990; Janick, 1996; Beckerman, 2007; Sobiczewski, 2014; Elis, 2016; DuPont, 2023), 'Topaz', 'Auriu de Bistrita' (Jakab-Ilyefalvi and Platon, 2012; Militaru *et al.*, 2012) and other valuable varieties, are more vulnerable to fireblight than many other cultivars. Due to the damages produced by this terrible disease, for its containment researches are carried out in many research units and entities from major apple producing countries (USA, 14; Canada, 1; Australia, 1 New Zealand 1; UK, 2; Germany, 2; Italy, 6; France, 2; Switzerland, 2, Poland, 1; Belarus, 2; Romania, 7).

Therefore, the purpose of this work was to assess the effectiveness of some registered active ingredients in control of fireblight under the harsh conditions of the latest years.

MATERIALS AND METHODS

The researches were conducted between 2021 - 2024, at the Research Institute for Fruit Growing Pitesti Romania. The experimental device was located on a plain terrain placed on the second terrace of the Argeş River, on a low to medium fertile illuvial clay unit (more than 30% of clay; humus less than 1.7%; nitrogen index 0.33-1.43; PAL 1.3-2.5 mg/100g, but well supplied with potassium, up to 40 mg/100 g). Soil reaction is slightly acid (pH=5.8-6.8). The orchard floor was covered with grass between the tree rows and cleared with total herbicides on stripes of 1.0-1.2 m wide, along the tree rows. The fertilization was well balanced in nutrients by applying N200-P240-K280-Ca20 (1.0: 1.2: 1.4: 0.2 ratio) recipe and 4-5 foliar fertilizations with Haifa products. During the dry periods the experimental plots received 6-7 l water/ m²/day.

Biological material consisted in 'Jonathan' and 'Idared' varieties grafted on low vigour vegetative rootstocks grown under super-intensive system, with over 2222 trees /ha, trained as 'slender spindle' and supplied with water and nutrients by fertirrigation. Each trial variant included 4 replicates of 5 apple trees.

During the study period 5 type active ingredients and combinations were assessed as regard their protective effect against fire blight on the apples following EPPO and national and international guidelines. The variants experimented in detail are presented in Table 1. The treatments were precise forecasted, based on the reserve of the pathogens which survived over winter, the apple trees phenology stages, and also related to the evolution of the climatic parameters, monitored and registered with automate iMetos weather station and were stored, processed and analyzed using its software Field Climate, with fireblight early warning module. Using this module, the risk of infections with fireblight was assessed using the Cougar scale (where '0'=no risk; '1'=low infection risk; '2'=medium infection risk and '3'=high infection risk). In the experimental plots the phytosanitary treatments with fungicides were applied with the atomizer STIHL, 400 series. The coverage quality was verified using Novartis sensitive paper and assessed using Snap Card mobile application, developed by the University of Western Australia and the Department

of Agriculture and Food. The rests of products, solution, and water from washing the spraying equipment were neutralized in installations type Phytobac, Heliosecc or RemDry. The damages caused in by fireblight on apple shoots were estimated periodically, both as attack frequency/incidence $F\% = [(n/N) \times 100]$. The attack intensity/severity (I) was evaluated with notes upon a 0-4 scale damages degree was calculated using the formula $DD\% = [(F\% \times I) \times 100]$. The tested products biological main indicator, biological efficacy, the was calculated upon Abbott's formula, $E\% = [(1-T/UT) \times 100]$. The experimental data were stored, processed and analysed, using MS Office Excel, 2010, statistic and graphic facilities and verified once more with SPSS.14 statistic software.

RESULTS AND DISCUSSIONS

Romania holding the 8th place on regard the apple production (593.700 t /year) and planted surfaces (53.820 ha) (FAOSTAT, 2021).

The diversity of host tissues susceptible to infection, combined with the limited number of management tools available to control the disease, has made it difficult to stop or slow the progress of fire blight epidemics (Norelli et al, 2007; Fellows 2024).

a) Results regarding the orchard microclimate infections and infection risks.

Assessment of the Table 2 revealed that during the study period (2021-2023) the monthly average temperature was favorable for the infection's occurrence from 15.1-16.4 C at the beginning of May and 22.4-23.3 C in August. The average number of wet hours ranged between 110-255 h in June, during the active growth of the shoots and 62.3-189.8 h in September. Assessment of the figures 1-3 highlight the infection risks with the *Erwinia amylovora* registered with the aid of the early warning module of iMetos software.

In this sense, can be observed that in 2021, first light infections (100 DIV) occurred in April-May, with peaks (DIV 300-400) min 21 May, and second and third decade of June. Then during the entire vegetation period, the infection risk was maximum until 21 September (DIV 400). Another period of maximum infection risk (DIV 400) was in the mid of September and in 2021 October, a medium one (DIV 200). Last infections (DIV 100) were in mid of November. In 2022, first light infections (100 DIV) occurred in 22 April, the medium ones (DIV 200) in 19-20 May, then the infection risk was medium or higher (DIV 200-400), from the end of May till 22 September, maximum (DIV 400) at the end of September and debut of October. Since 22 of October till 22 November, the infection risk was medium (DIV 200). In 2023 first light infections (100 DIV) occurred in April-May, with a small peak in 23 May, and a medium one (DIV 200) in first decade of June. The infection risk was maximum (DIV 300-400) from the last decade of June till the end of September. Another maximum risk of infections was registered between 20-23 September, and a medium one (DIV 100-200) between 20-25 September and 20 November.

We can conclude, that according the early warning module of iMetos software, in our area, the risk of infections with fireblight, stretched from late April to late November.

Under the most favorable conditions coupled with varietal sensitivity and the lack of treatments, the fireblight infections progresses fast on every apple organs especially in the growing shoots (see Fig. 4 and Fig. 5) which lead to the necessity of preventive, quality treatments with various registered active ingredients, multisite or with a specific action chain.

b) Results with the active ingredients tested against fireblight.

Assessment of Fig. 6 reveal that among the studied and accepted active ingredients tested many of them were polyvalent, at reasonable rate of application controlling well and very well fireblight and more than two major pathogens of the apple crop, which explain their use in practice for a long period. Some active substances, copper compounds, aluminum fosetyl and the ones acting on phosphonates metabolism, seem to trigger the defense mechanisms of the plants. A particular attention must be allocated during the growing season to the use of the

copper hydroxide not to overpass the accepted rate (6.0 kg metallic copper/ha/season). To increase the active efficacy and avoid any mistakes, it can be displayed better at the beginning and the end of the vegetation period (Fig. 6). Examination of the figure 7 it can be seen that the number of applications per season ranged between 2 and 3 and the interval between treatments ranged according to the active ingredients tested between 10 and 14 days, with the exception of the mixture copper hydroxide + copper oxychloride, on which the interval between treatments was in average 7 days. Assessment of figure 8 reveal the variation of the field efficacy of the active ingredients tested, against fireblight attack on apple shoots. In this sense, copper oxychloride provided an average efficacy E% ranging between 78.74-80.81 (stdev=0.8703; var=1.0917). Better results were obtained with copper hydroxide which provided an average efficacy E% between 80.70-81.66 (stdev=0.5404; var=0.6669). The mixture hydroxide + copper oxychloride displayed a superior field efficacy ranging between 81.45 - 83.91 (stdev=1.3539; var=1.6440). All these coppers based active ingredients showed a good contact (multisite) effect against the infections with *Erwinia amylovora*. Aluminium fosetyl, applied during the flowering period and fruit set provided an average efficacy E% ranging between 90.70-81.26 (stdev=0.2802; var=0.3080). Belonging to the ethyl-phosphonates group of fungicides, acts by translaminar effect, against fireblight progression in young shoots and fruitlets. The mixture fluopyram + aluminum fosetyl offered an average efficacy E% in fireblight control, within 91.20-93.18 (stdev=0.9954; var=1.0791), especially on growing shoots and fruits. The mixture insured the fireblight control during the maximum risk infection periods and provided the simultaneously control of some others key pathogens of the apples such as, apple scab, powdery mildew and dry cancer due the fluopyram component which acts on inhibition of sterol biosynthesis DMI of mentioned pathogens.

Our researches results confirm the newest ones that active ingredients effectiveness are very strong influenced by the yearly orchard conditions and to many changes. (See Table 2). However, to increase the bactericides efficacy in apple crop protection, it will be useful and opportune additional researches, on new active ingredients complementarity and products agreed in biological and integrated production systems, water quality monitoring, spray equipment calibration, and improvement of applications quality.

In USA, various copper liquid formulation *Bacillus subtilis* QST 713 strain (Blossom Protect), essential oils or peracetic acid-peroxide products (e.g. Thyme Guard, Cinnerate, Oxidate 5.0, Jet Ag) were applied in apple orchards in several trials at petal fall have also had control similar good effects in control fireblight (DuPont *et. al* 2023). In Germany, complexed sulfuric clays (Myco-Sin) showed good results to prevent infection after artificial hail simulation. (Bantleon, G. *et al.*, 2021).

Table 1. Work variants RIFG Pitesti Romania, Lat. N 44.513; Long. E 24.52; Alt 287m, 2021-2023.

Variant	Active ingredient	BBCH	Rate [kg/ha/appl.]	Applications [n]
V1	Untreated	-	-	-
V2	copper oxiclорure (50% metallic copper)	54;72; 75	2.0	3
V3	copper hydroxide (50% metallic copper)	54;72	3.0	2
V4	copper oxiclорure+hydroxide (280 g copper/kg)	56;72	2.0	2
V5	aluminum fosetyl (800 g/kg)	59;73	3.75	2
V6	fluopyram+aluminum fosetyl (50+666 g/kg)	72;75	3.0	2

Table 2. Orchard microclimate conditions during the study period
RIFG Pitesti Romania, Lat. N 44.513; Long. E 24.52; Alt 287m, 2021-2023

Month/ Year	Temperature									Degree Days			Chill Hours			Rain Fall [mm]			Wet Hours			Wet Days			Rainy Days			
	High			Low			Mean																					
	2021	2022	2023	2021	2022	2023	2021	2022	2023	2021	2022	2023	2021	2022	2023	2021	2022	2023	2021	2022	2023	2021	2022	2023	2021	2022	2023	
January	13.3	17.8	20.3	-14.1	-12.0	-4.2	0.5	0.8	4.0	0.8	4.4	8.7	438.5	396.3	540.5	73.6	6.4	101.7	0.0	0.0	3.8	25.0	13.0	28.0	21.0	6.0	15.0	
February	23.3	17.8	19.6	-10.3	-7.6	-11.4	3.0	3.1	2.6	13.9	7.8	10.7	365.3	385.5	348.0	12.4	11.0	7.9	2.0	0.3	1.3	18.0	12.0	13.0	7.0	9.0	7.0	
March	18.8	22.1	21.7	-6.2	-9.3	-5.7	4.1	3.6	7.1	16.7	29.1	36.7	456.8	363.8	467.3	66.8	19.4	18.8	0.0	2.5	9.5	17.0	14.0	20.0	15.0	10.0	8.0	
April	25.3	24.5	21.3	-3.3	-3.8	-1.0	8.6	10.1	9.7	47.4	75.2	52.8	432.3	333.5	384.5	38.4	88.0	80.4	8.3	38.5	45.5	22.0	20.0	28.0	12.0	13.0	20.0	
May	28.4	31.5	27.1	2.5	2.9	3.9	15.6	16.4	15.1	184.0	211.3	165.6	102.3	117.0	107.3	65.4	72.6	77.4	132.0	96.5	150.3	25.0	23.0	28.0	16.0	13.0	16.0	
June	34.0	36.8	32.6	7.0	11.0	11.0	19.7	19.3	21.1	19.4	278.2	329.3	281.8	24.8	0.0	2.3	104.0	25.6	78.7	225.0	154.3	154.5	29.0	25.0	26.0	18.0	100.0	18.0
July	36.8	38.3	35.2	12.5	8.7	8.9	23.5	22.8	23.2	408.6	383.7	402.8	0.0	3.3	2.5	33.5	25.3	77.7	66.0	92.8	110.5	18.0	17.0	27.0	9.0	12.0	12.0	
August	36.4	35.2	36.0	9.0	12.9	9.8	22.4	22.6	23.3	372.4	384.3	398.6	2.8	0.0	0.5	74.0	142.1	17.0	82.3	128.3	63.8	16.0	25.0	17.0	9.0	15.0	7.0	
September	30.4	29.4	32.1	2.7	2.3	8.2	15.6	15.6	19.4	178.4	184.5	280.8	129.0	111.8	15.5	14.3	49.6	26.1	62.3	189.8	111.3	27.0	29.0	18.0	6.0	11.0	9.0	
October	22.7	27.1	29.5	-2.0	1.2	-0.8	8.6	12.0	13.5	27.5	104.5	140.5	360.0	304.5	240.0	36.1	4.3	4.9	29.5	46.0	55.0	23.0	30.0	26.0	9.0	3.0	6.0	
November	26.3	26.7	20.3	-2.2	-3.7	-5.7	6.7	7.6	6.6	25.4	31.9	25.9	514.0	531.3	436.8	25.6	40.8	53.8	8.8	7.5	47.0	26.0	27.0	25.0	11.0	13.0	16.0	
December	13.1	16.9	19.6	-11.6	-7.1	-4.8	1.6	2.8	3.1	0.6	5.8	10.2	500.3	500.0	487.0	91.9	40.4	18.4	0.5	8.8	0.0	25.0	28.0	26.0	16.0	16.0	10.0	
AVG	25.7	27.0	26.3	-1.3	-0.4	0.6	10.8	11.5	12.3	129.5	146.0	151.3	277.2	253.9	252.7	53.0	43.8	46.9	51.4	63.8	62.7	22.6	21.9	23.5	12.4	18.4	12.0	
STDEV	8.04	7.50	6.50	8.40	8.22	7.29	8.19	8.12	7.77	151.24	149.20	152.88	206.97	196.97	215.45	30.30	40.23	34.74	69.01	66.84	56.69	4.34	6.52	5.13	4.72	25.95	4.82	
VAR	31.25	27.77	24.76	62.99	21.92	13.28	75.86	70.31	63.41	116.79	102.20	101.07	74.67	77.57	85.26	57.17	91.87	74.08	134.28	104.81	90.40	19.20	29.73	21.81	38.00	140.92	40.20	



Figure 1. *Erwinia amylovora* attack risk
RIFG Pitesti Romania, Lat. N 44.513; Long. E 24.52; Alt 287m, 2021.



Figure 2. *Erwinia amylovora* attack risk
RIFG Pitesti Romania, Lat. N 44.513; Long. E 24.52; Alt 287m, 2022



Figure 3. *Erwinia amylovora* attack risk
RIFG Pitesti Romania Lat. N 44.513; Long. E 24.52; Alt 287m, 2023.



Figure 4. Severe infections with fireblight on growing shoots and fruitlets (untreated).



Figure 5. Severe infections with fireblight on growing shoots and fruits (untreated).

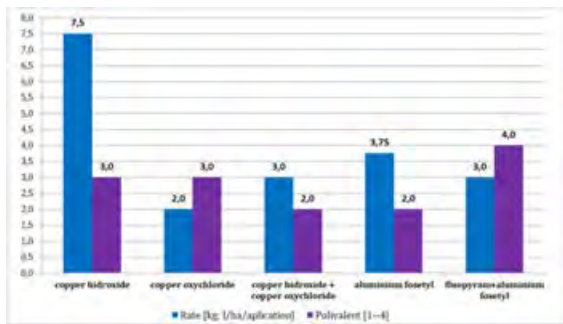


Figure 6. Active ingredients tested against fireblight type and the used rates limits.

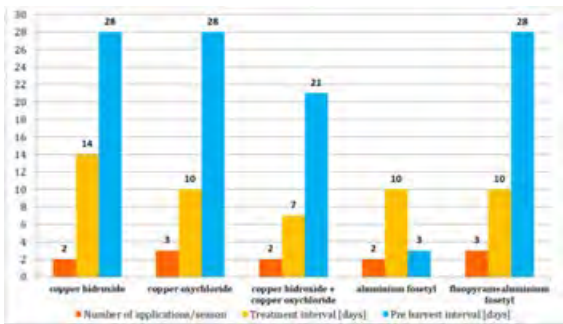


Figure 7. Active ingredients tested against multipurpose and some Good Agriculture Practice features.

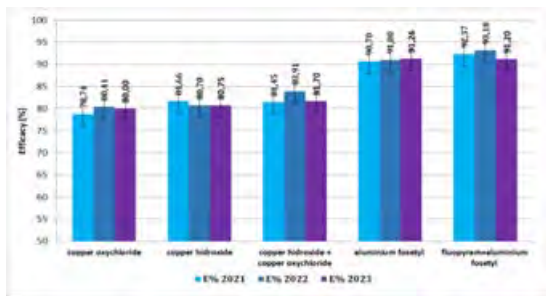


Figure 8. Efficacy of the tested active ingredients against fireblight growing shoots.



Figure 9. 'Idared' apples protected against fireblight attack.

Table 3. ANOVA analysis actives effectiveness correlated with the study years, ingredients dosage and number of their applications per season.

		Sum of Squares	df	Mean Square	F	Sig.
Year	Between Groups	.000	5	.000	.000	.000
	Within Groups	12.000	12	1.000		
	Total	12.000	17			
Rate	Between Groups	28.000	5	5.600	.	.
	Within Groups	.000	12	.000		
	Total	28.000	17			
Applications	Between Groups	14.500	5	2.900	.	.
	Within Groups	.000	12	.000		
	Total	14.500	17			
Efficacy	Between Groups	18722.000	5	3744.400	5616.600	.000
	Within Groups	8.000	12	.667		
	Total	18730.000	17			

CONCLUSIONS

During 2021-2023, the meteorological conditions were very favorable to infections produced by *Erwinia amylovora* on apples.

The apple varieties behave different on the on the fireblight infections according their genitors, the affected organ (flowers, bark, shoots, fruits) or the entire tree/rootstock combination, meteorological conditions and local microclimate, the growing system and applied technology, production level, etc.

To increase the bactericides efficacy in apple crop protection, it will be useful and opportune additional researches, on new active ingredients complementarity, water quality monitoring, spray equipment calibration, and improvement of applications quality. Technological interventions as fertigation and sanitation pruning and growing resistant or tolerant varieties, should be used as parts of an integrated fire blight management program, which includes using a risk assessment model, as well as cultural control when necessary.

ACKNOWLEDGEMENTS

The authors acknowledge the financial support received from the Romanian Ministry of Agriculture and Rural Development for the Project ADER 25.2.1, within the framework of which this research was conducted.

REFERENCES

1. Amzăr V., and Ivascu A (2003). Ghid de identificare al principalelor boli și dăunători la speciile pomicele. București. Editura MEDRO. 200 pp. ISBN 973-8487-02-1.
2. Babadoost M. (2005) Fireblight of apples. University of Illinois. College of of Agricultural, Consumer and Environmental Sciences. Department of Crop Sciences. RPD N. 801, 8 pp.
3. Bantleon G., Scheer, C., and Hartung K.. (2021). Extensive efficacy tests of non-antibiotic fire blight control agents. J. Plant. Pathol. 103: 155-165. <https://doi.org/10.1007/s42161-020-00694-0>.
4. Beckerman J. (2007). Fire Blight on Fruit Trees in the Home Orchard. Purdue University. Extension Factsheet BP-30-W. pdf. 3 pp. [online].
5. Cooley R. D., Autio R. W., Clements J., & Cowgill Jr W. P. (2015). An annual fire blight management program for apples: an update. Fruit Notes, 80, 18-26.
6. DuPont Tianna, and Youfu Zhao. (2023). Fireblight of Apples and Pears. Washington State University. Extension Factsheet pdf. 54 pp. <https://treefruit.wsu.edu/crop-protection/disease-management/fire-blight/>.
7. Elis M.A. (2016). Fireblight of apple and pears. Ohio State University. College of Food, Agricultural and Enviromental Sciences. PLPATH-FRU-22, 9 pp. <https://ohioline.osu.edu/factsheet/plpath-fru-22-0;>
8. Fellows Suzane. (2024). Controlling fireblight organically. GrowOrganic.com. pdf. 9 pp. [online].
9. Hartman J.,and Hershman D. (2002). Fire Blight. University of Kentucky. College of Agriculture and Life Sciences. Plant Pathology Department. Extension. PP Information Note 34 (PPA 034). [online].

10. Ivey M.Land . Ellis, M.A. (2016). Fire Blight of Apples and Pears. Ohio State University. Agricultural Research and Development Centre. Wooster, OH. <https://ohioline.osu.edu/factsheet/plpath-fru-22-0>.
11. Johnson K.B. (2015). Fireblight of Apples and Pears. Plant Health Instructor. Oregon State University. <https://www.apsnet.org/edcenter/disandpath/prokaryote/pdlessons/Pages/FireBlight.aspx>.
12. Koski R.D. and Jacobi W.R. (2015). Fireblight. Factsheet. 2907, Colorado State University. Extension Service, 6 pp. [online].
13. Malnoy M., Martens S., Norelli J. L., Barny Marie-Anne, Sundin G.W., Smits, Theo H.M. and Duffy B., (2012). Fire Blight: Applied Genomic Insights of the Pathogen and Host. Annual Review of Phytopathology. 50 (1). Annual Reviews: 475-494. doi:10.1146/annurev-phyto-081211-172931.
14. Martinez, A., Pearce M. (2023). Fireblight. Symptomes, Causes and Treatment. University of Georgia, Extension Circular 871, https://secure.caes.uga.edu/extension/publications/files/pdf/C%20871_5.PDF.
15. Militaru M., Braniște N., Butac M., Călinescu, M., Sestraš A., Fodor M., (2012). Evaluation of some apple varieties to fireblight (*Erwinia amylovora*) susceptibility in natural field conditions.. Fruit Growing Research. Vol. XXVIII 6 pp.
<https://publications.icdp.ro/publicatii/lucrari%202012/06.%20Militaru%20Madalina.pdf>
16. Momol, M.T. and Aldwinckle S. (2000) Genetic Diversity and Host Range of *Erwinia amylovora*. In J. L. Vanneste (ed.), Fire Blight Disease and its Causative Agent, *Erwinia amylovora*. CABI. New Zealand.
17. Nischwitz C, Hubbel M.A. (2018). Fire Blight of Pears and Apples. Utah State University Extension and Utah Plant Pest Diagnostic Laboratory. pdf. 3pp. https://digitalcommons.usu.edu/extension_curall/1843/
18. Norelli, J.L., Jones, A.L. and Aldwinckle, Herb S., (2007), Fire Blight Management in the Twenty-first Century: Using New Technologies that Enhance Host Resistance in Apple. Plant Disease. 87(7):756-765.
19. Peter, and K. A. (2023). Fire Blight in Home Apple and Pear Orchards. Pennsylvania State University. Extension Factsheet. pdf. 3pp. <https://extension.psu.edu/fire-blight-in-home-apple-and-pear-orchards>
20. Ritchie D.F.,and Sutton T.B. (2002). Fire Blight of Apple and Pear. North Carolina State University, College of Agriculture and Life Sciences, Plant Pathology Department, Extension, Fruit Disease Information Note 3 (FDIN 003). pdf. 2pp.
21. Schroth, M.N. (2010). Fire Blight of Apple and Pear, Plantdiseases.org. Archived (PDF);
22. Roșu-Mareș S.D., Zagrai L.A, Zagrai I., Jakab-Ilyefalvi Z., Chiorean A.M., and Vasile F. (2024). Climate Change and the Natural Infections of Apple Trees with *Erwinia amylovora* Burrill in Northern Transylvania, Scientific Papers. Series B, Horticulture. Vol. LXVIII, No. 1, 2024. Print ISSN 2285-5653, Online ISSN 2286-1580, ISSN-L 2285-5653.
23. Sobiczewski, P., Peil, A., Mikiciński, A., Richter, K., Lewandowski, M., Żurawicz, E., and Kellerhals, M. (2014). Susceptibility of apple genotypes from European genetic resources to fire blight (*Erwinia amylovora*). European Journal of Plant Pathology 141, 51-62
22. Smith Cheryl A. (2017). Fire Blight. New Hampshire University. Extension Pest Factsheet 32. pdf. 3 pp. https://extension.unh.edu/sites/default/files/migrated_unmanaged_files/Resource002817_Rep4172.pdf
23. Smith T.J. (2010). The development and use, of Cougar Blight 1990-2010. A situation-specific fireblight risk assessment model for apple and pear. Washington State University. <https://extension.wsu.edu/chelan-douglas/agriculture/treefruit/pestmanagement/cb2010fireblightriskmodel/>
24. Steiner P.W., Van der Zwet T., and Biggs A.R. (2000). Fireblight (*Erwinia amylovora*). West Virginia University. Plant Pathology Department. Keraneysville Tree Fruit Research and Education Center. WW. pdf. 7pp.
25. Sundin G.W. (2014), Compendium of Apple and Pear Diseases and Pests. APS Press. St. Paul, MN.
24. Teviotdale B.L., (2011). Pest Notes: Fireblight. Kearneysville Agricultural Research and Extension Center. in UC Davis, ANR, Publication 7414, pdf, 4 pp.
26. Thomson S.V., (1986), The role of the stigma in fire blight infections, Phytopathology 76(5): 476-482. doi:10.1094/Phyto-76-476;
27. van der Zwet, T., and Beer, S.V. (1995). Fire Blight - Its Nature, Prevention, and Control: A Practical Guide to Integrated Disease Management. U.S. Department of Agriculture, Agricultural Information Bulletin No. 631.
28. Vanneste J.L., (2000), Fire Blight: The Disease and Its Causitive Agent, *Erwinia amylovora*. CABI Publishing, London, UK: 370 pp.;
29. British Columbia Government. (2018). Fire Blight of Apple and Pear, <https://www2.gov.bc.ca/gov/content/industry/agriculture-seafood/animals-and-crops/plant-health/insects-and-plant-diseases/tree-fruits/fire-blight>
30. Cooperative Agricole Ferraresi Riunite, (2000). Il Colpo di Fuoco Batterico delle Pomacee (*Erwinia amylovora*), CAFER, Monography, p. 1-10;
31. Food and Agricultural Organization (2021). FAOSTAT. <https://www.fao.org/faostat/en/#data/QCL>.
32. Ontario Government, Canada, Fireblight in Pears and Apples, <https://www.ontario.ca/page/government-ontario>
33. University of California, Natural Resources Department, Models: Fire Blight of Pear and Apple--UC IPM, <https://ipm.ucanr.edu/DISEASE/DATABASE/fireblight.html>



Viticulture and Oenology

The influence of climatic factors on the quality of grapes in the vineyards Murfatlar and Iasi

V. Artem^{1*}, A. Ranca¹, C. Ciobanu¹, I. Dina¹, A. Nechita² and G. Zaldea²

¹Research and Development Station for Viticulture and Oenology
Murfatlar, Romania

²Research and Development Station for Viticulture and Oenology Iasi,
Romania

*Corresponding author e-mail: artemvictoria@yahoo.com

ABSTRACT

The objective of the present study was to evaluate the influence of key climatic factors (temperature and precipitation) during the growing season on the ripening and chemical composition of grapes for white and red wines in two vineyards renowned for the quality of their wines: Murfatlar and Iași, over two consecutive years (2023 and 2024). The climatic variability, characterized by high air temperatures and prolonged periods without precipitations contributed to a forced ripening of the grapes, with harvesting taking place in 2024 one week earlier than in 2023 in both vineyards. The high temperatures during ripening accelerated the accumulation rate of sugars, resulting in concentrations exceeding 200 g/L for white wine grape cultivar and over 220 g/L for red wine grape cultivars in the Murfatlar wine center, and concentrations ranging between 192.6-206.0 g/L for white grape varieties and 200.7-221.0 g/L for red grape cultivar in the Copou Iasi wine center. The results obtained, analyzed statistically, highlighted significant differences in the primary metabolites of grape berries (sugars and organic acids), influenced by vineyard and climatic conditions over the two studied years. Maintaining traditional viticultural regions remains a challenge for producers, requiring various strategies to compensate for these imbalances and to produce high-quality, balanced wines.

Keywords: extreme temperatures, drought, grape ripening, sugars

INTRODUCTION

The gradual climate change is affecting the evolution of natural factors in viticultural ecosystems; summers are becoming warmer and drier, with long autumns and uneven precipitation, alternating between periods of excessive drought and short bursts of heavy rainfall. High temperatures during the active growing season promote the development of shoots, but when they exceed 35°C, combined with low precipitation levels, they contribute to the onset of heat stress, which has a negative impact on vine phenology. Grape berries are vulnerable to heat stress, which have repercussions on the synthesis of primary metabolites such as sugars (Pillet *et al.*, 2012), organic acids (Sweetman *et al.*, 2014), and amino acids (Lecourieux *et al.*, 2017), as well as secondary metabolites (polyphenols, aromas) responsible for wine sensorial attributes. Water deficit can impact vegetative growth, flowering and berry development, although this depends on the phenological stage, as well as the severity and duration of the water deficit (Hardie *et al.*,

1976). One of the main objectives of wine grape producers throughout history has been to achieve full maturity, meaning the maximum concentration of soluble sugars at the highest berry weight. Recently, this objective has lost relevance, as an increasing number of consumers in the internal and international markets prefer lighter wines characterized by a moderate alcohol content (Ilina-Dumitru *et al.*, 2024). The grape ripening process is an extremely complex phenomenon that involves a combination of environmental conditions, genetic characteristics, hormonal activity, pigment biosynthesis, and the metabolism of sugars, acids, and aroma compounds. Key indicators of grape quality include total soluble substances, total acidity, and their ratio. In the dynamics of grape ripening, an increase in sugar content is observed due to the synthesis and accumulation of glucose and fructose, while titratable acidity tends to decrease (Fernandez *et al.*, 2015). The ratio of sugars to organic acids correlates with the quality of aromas and determines the optimal harvest time, as it is considered a quality index (Kafkas *et al.*, 2007). Figure 1 highlights the effect of temperature fluctuations on the synthesis of primary metabolites (sugars and organic acids) and secondary metabolites (aromas and phenolics) in grape berries, and consequently, on wines.



Figure 1. The effect of temperatures on the biochemistry of grape berries (Arias *et al.*, 2022)

Climatic variability resulting from rising air temperatures and prolonged periods of precipitation absence generated over time effects on grapevine development and grape composition, namely: (i) the onset of flowering and veraison phases occurs earlier; (ii) accelerated grape ripening in terms of sugar accumulation, which, in turn, leads to the higher alcohol content in the wine; (iii) faster depletion of organic acids in the must and increased pH values, which trigger high microbiological instability of the must during pre-fermentation; (iv) as a result of excessive temperatures, the aromatic profile may atypically shift towards over-ripening; (v) technological ripening (which occurs earlier) and phenolic ripening (which occurs later) are decoupled, with negative effects on the aromatic and phenolic profile, especially for red grape cultivars (vi) increased frequency of berry withering, extending to damage caused by sunburn (Pallioti *et al.*, 2014). Although the grapevines can adapt over time, the immediate consequences are inevitable, namely: a shortened phenological development period, plants that are less prepared for the cold season and more sensitive to extreme temperatures, forced ripening of grapes, and an advance of harvest dates with the berries being poorer in terms of their natural components (Lorenzo *et al.*, 2013). As a result of global warming, maintaining traditional viticultural regions remains a challenge for producers, requiring various strategies (optimizing a complex set of agrotechnical measures, vineyard and winemaking practices) to compensate for these imbalances and to produce wines that are both qualitatively and physico-chemically balanced and organoleptically pleasing.

This study aimed to highlight the changes and impact of key climatic factors (temperatures and precipitations) on the ripening and composition of grapes in two wine centers from

Murfatlar and Iasi vineyards. The research is useful for winemakers in order to assess the advantages and risks of these climatic changes, so they can adapt their viticultural practices and maintain the typicity of white and red wines from the two studied wine regions, given the fierce competition in the wine industry and the increasing consumer demand for quality wines that reflect the terroir.

MATERIALS AND METHODS

The study was conducted on white wine grape cultivars 'Chardonnay', 'Sauvignon blanc', and 'Columna' for both wine regions. Regarding red wine grape cultivars, 'Fetească neagră', 'Pinot noir' and 'Mamaia' were studied for the Murfatlar vineyard, and 'Fetească neagră', 'Cabernet sauvignon' and 'Arcaș' for the Iasi wine region. All the mentioned cultivars are authorized for producing quality wines with a Controlled Designation of Origin. Data were analyzed over two consecutive years, 2023 and 2024.

The vineyards in the Murfatlar and Iasi wine centers were established after 2009 on Berlandieri x Riparia rootstock, selection Oppenheim 4, clone 4 (SO4-4). The rows are N-S oriented, with a planting distance of 2.2 m between rows and 1.2 m within rows, resulting in a density of 3,787 plants/ha. The training system is a bilateral cordon with a trunk height of 0.8 m. Climatic parameters (maximum and minimum temperature and precipitation) were recorded with an iMetos 3.3 weather station (Murfatlar) and a AgroExpert weather station (Iasi). Grape must sugar (g/L) equated according to the total soluble solids content (refractometric method) and total acidity (g/L as tartaric acid) and pH were determined using the standard methods specified in the OIV Compendium of International Methods of Wine and Must Analysis. Data were expressed as the mean ± standard deviation. One-way analyses of variance of means (ANOVA) and the mean differences of the various samples were compared by a post-hoc test (Duncan multiple mean comparison test). A difference of $p < 0.05$ was considered as significant.

RESULTS AND DISCUSSION

Analyzing the evolution of the monthly average air temperatures over the two studied years presented in Table 1, a difference-in the values of this parameter compared to the multi-annual average can be observed. As seen, in 2024, the recorded values at Murfatlar are significantly higher in July, 26.1°C and August, 28.5°C compared to Iasi, which recorded values of 22.7°C in July and 25.0°C in August. Similar differences of at least 2°C are observed in 2023 for the summer months between Murfatlar and Iasi. Observations indicate that for both vineyards, the average air temperatures were higher compared to the multi-annual average by 2.8°C to 4.4°C at Murfatlar and by 0.94°C to 2.84°C at Iași.

Table 1. The evolution of average monthly air temperatures for the two studied wine growing areas

Vineyard	Murfatlar			Iasi		
	Average temperatures	2023	2024	Average temperatures	2023	2024
IV	10.5	10.8	16.2	10.8	8.1	14.2
V	16.2	17.4	16.6	16.5	16.2	16.7
VI	20.4	22.8	26.1	20.2	20.4	22.7
VII	22.6	26.8	28.5	22.0	23.0	25.0
VIII	22.6	26.9	26.1	21.4	24.6	24.4
IX	17.6	22.2	-	16.1	19.9	-
Average	18.3	21.2	22.7	17.8	18.7	20.6

Climatic variability, particularly of minimum and maximum air temperatures, as well as the lack of precipitation plays an important role in the biosynthesis of sugars and organic acids in grapes. The evolution of minimum and maximum temperatures recorded during the two studied viticultural years in the two vineyards, Murfatlar and Iasi, is illustrated in Figure 2.

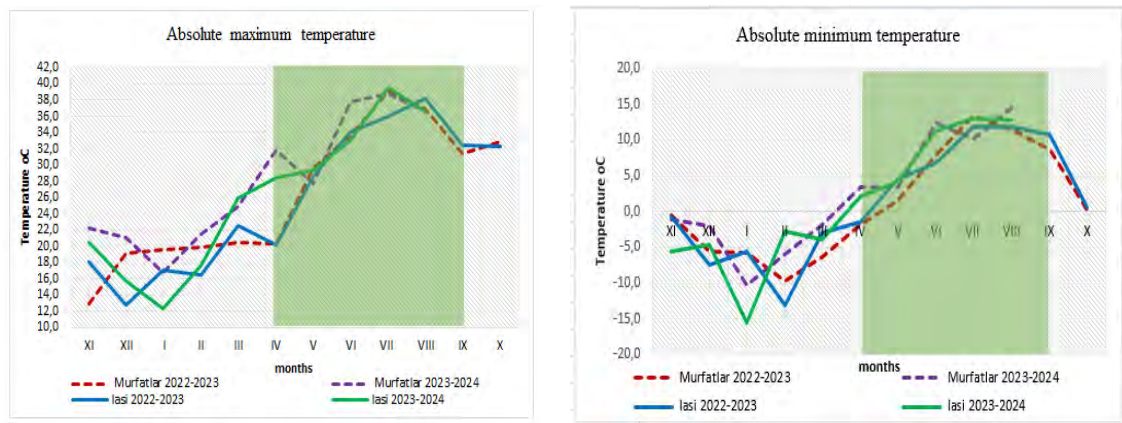


Figure 2. Evolution of maximum and minimum temperatures for the two vineyards

In the spring months, both vineyards show very large differences (on average 24.5°C) between minimum temperatures (-1.9°C to 3.4°C in April and 1.5°C to 4.6°C in May) and maximum temperatures (20.3°C to 31.9°C in April and 27.7°C to 29.7°C in May). Summer months (June, July, August) had minimum values ranging from 6.8°C to 13.4°C, with temperatures at least 2°C higher at Murfatlar (10.3°C to 14.5°C) compared to Iasi (6.8°C to 12.8°C).

Regarding the evolution of maximum temperatures during the summer months, temperatures exceeding 30.6°C can be observed, with recorded temperatures reaching up to 39.1°C in July 2023 at Murfatlar and in the same month in 2024 at Iasi.

In September 2023, minimum temperatures dropped to a range of 8.6°C to 10.7°C, while maximum temperatures fluctuated within a relatively narrow range of 31.4°C to 32.4°C.

Among the ecological factors, water plays a crucial role, being the most important component of the vegetative organs of the vine, supporting numerous metabolic functions, participating in biochemical reactions, and facilitating the transport of substances and synthesized products.

The precipitation regime, represented as the number of rainy days classified into three categories based on quantity (>0.1 mm; >5 mm; >10 mm), is shown in Table 2. It can be observed that for Murfatlar in 2023 the highest number of rainy days was recorded, especially those with >0.1 mm (40 days) compared to Iasi, where there were 36 days, whereas in 2024, the number of rainy days decreased, with 35 days in Murfatlar and 26 days in Iasi. Regarding the number of days with rainfall >10 mm, Murfatlar recorded a maximum of 3 days in April 2023 and in April and August 2024, while Iasi had 6 days in the same month of April 2023 (Table 2).

Table 2. The number of days with rain for the two wine growing areas

Year/ Vineyard	2023						2024					
	Murfatlar			Iasi			Murfatlar			Iasi		
	No.of days with rain			No.of days with rain			No.of days with rain			No.of days with rain		
Month	>0,1	>5	>10	>0,1	>5	>10	>0,1	>5	>10	>0,1	>5	>10
IV	13	7	3	11	1	6	10	5	3	6	1	1
V	8	1	0	3	1	1	8	1	1	4	1	2
VI	7	1	1	6	3	0	4	1	0	4	1	3
VII	8	0	2	8	0	3	6	1	2	6	2	2
VIII	3	0	0	1	0	1	8	5	3	6	2	1
IX	1	0	0	6	0	0	-	-	-	-	-	-
Total	40	9	6	35	5	11	36	13	9	26	7	9

Analyzing the precipitation quantity recorded during the growing season (Figure 3), it can be observed an uneven distribution, alternating in 2023 between periods of pluviometric excess - April at Murfatlar and April and July at Iasi, as well as periods of deficit - May, June, July, August, September at Murfatlar and May, June, August, September at Iasi. The total precipitation recorded was 170.9 mm at Murfatlar and nearly double, at 349.3 mm at Iasi, with values lower compared to the multi-annual average of 245.7 mm at Murfatlar and 384.3 mm at Iasi. In 2024 the total precipitation quantity recorded was 169.4 mm at Murfatlar and 250.0 mm at Iasi, with a decrease of 0.87% for Murfatlar and 28.42% for Iasi by comparison with 2023.

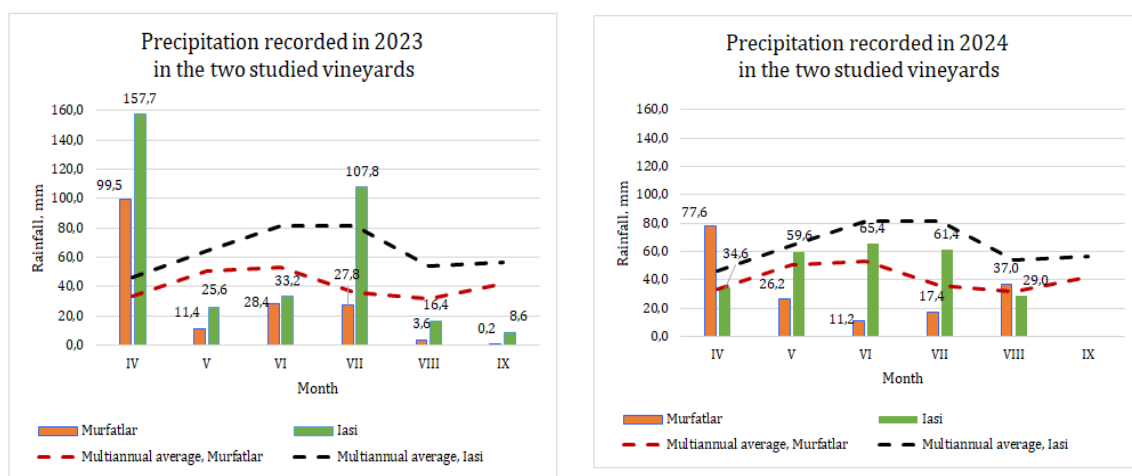


Figure 3. Precipitation for 2023 and 2024 compared to the multi-annual average

The analysis of the experimental data (Table 3), shows that the climatic variability of the two studied years for the two wine regions influenced the synthesis of primary metabolites (sugars and organic acids) in grapes. Thus, at harvest time, the sugar content was significantly higher at Murfatlar compared to Iasi, with higher values for red wine grape cultivars - over 220 g/L, with an average of 8.37% higher compared to Iasi. For white wine grape cultivars, sugars showed similar values, ranging from 200.3 g/L to 212.3 g/L at Murfatlar compared to 200.7 g/L to 206.0 g/L at Iasi. For white wine grape cultivars, sugar levels showed similar values ranging from 200.3 g/L to 212.3 g/L at Murfatlar compared to 200.7 g/L to 206.0 g/L at Iasi. Harvesting took place at Murfatlar from September 13 to September 29 in 2023 and between August 20 and September 16 in

2024. It is noteworthy that the red wine cultivars were harvested before the white wine cultivars. At the Copou Iasi wine center, the white cultivars were harvested from September 23 to September 29 in 2023, and one week earlier in 2024. Similar studies have concluded that with increasing temperatures, the rate of sugar accumulation in grapes is accelerated, forcing wine growers to harvest earlier (Stanfield *et al.*, 2024). Extreme temperatures during grape maturation reduce vine metabolism, resulting in high sugar concentrations and lower total acidity, which affects the taste balance and overall quality of the grapes (Husain *et al.*, 2022).

Regarding the evolution of total acidity, this parameter recorded values ranging from 4.90 to 5.97 g/L of tartaric acid at Murfatlar and from 6.40 to 8.80 g/L of tartaric acid at Iasi, with higher values observed for red wine grape cultivars in this wine center. The most significant increase in pH was recorded for the 'Columna' cv. in 2024, due to reduced photosynthetic activity. Although the years 2023 and 2024 differed climatically, the average pH values fluctuated between 3.380 and 3.480 at Murfatlar and between 3.143 and 3.444 at Iasi, generally moderate values below 3.6 (Table 3), which is favorable for producing stable wines.

Table 3. The chemical characteristics of the musts obtained at the moment of harvest

Wine center	Harvest year	Type	Cultivar	Harvest date	Sugars (g/L)	Total acidity (g/L tartaric acid)	pH
Murfatlar	2023	white	Chardonnay	13.sept	227.8	5.03	3.184
			Sauvignon blanc	26.sept	207.0	4.90	3.607
			Columna	29.sept	202.0	5.66	3.350
		average			212.3±13.7 abc	5.19±0.4 c	3.380±0.21 ab
		red	Fetească neagr-	15.sept	224.3	5.66	3.321
			Pinot noir	17.sept	216.1	5.20	3.653
			Mamaia	19.sept	225.7	5.11	3.253
		average			222.0±5.2 ab	5.32±0.29 c	3.409±0.21 ab
	2024	white	Chardonnay	02.sept	210.8	5.97	3.238
			Sauvignon blanc	02.sept	196.4	5.20	3.420
			Columna	16.sept	193.8	4.90	3.675
		average			200.3±9.2 c	5.36±0.55 c	3.444±0.22 a
		red	Fetească neagră	20.aug	236.1	5.21	3.593
			Pinot noir	26.aug	228.2	5.04	3.360
			Mamaia	02.sept	219.2	4.90	3.486
		average			227.8±8.4 a	5.05±0.15 c	3.480±0.11 a
Iasi	2023	white	Chardonnay	29.sept	228.0	6.40	3.362
			Sauvignon blanc	28.sept	198.0	7.00	3.341
			Columna	23.sept	192.0	7.50	3.301
		average			206.0±19.3 bc	6.96±0.55 b	3.335±0.03 ab
		red	Fetească neagră	12.oct	201.0	7.1	3.114
			Cabernet Sauvignon	10.oct	195.0	8.8	3.235
			Arcaș	06.oct	182.0	8.2	3.081
		average			192.6±9.7 c	8.03±0.86 a	3.143±0.08 b
	2024	white	Chardonnay	06.sept	206	7.0	3.402
			Sauvignon blanc	06.sept	200	6.9	3.339

Wine center	Harvest year	Type	Cultivar	Harvest date	Sugars (g/L)	Total acidity (g/L tartaric acid)	pH
			Columna	09.sept	196	7,1	3,368
			average		200,7±5,0 c	7,00±0,10 b	3,370±0,03 ab
		red	Feteascp neagrp	20.sept	234	6,3	3,562
			Cabernet Sauvignon	20.sept	214	8,2	3,351
			Arcaș	10.sept	217	7,1	3,420
			average		221,7±10,7 ab	7,20±0,32 ab	3,444±0,08 a

Mean values ± standard deviation (n=3). Letters represent significance of $p<0.05$ difference between variants. The difference between any two values followed by at least one common letter is insignificant. Significance: * $p<0.05$, ** $p<0.01$, *** $p<0.001$, ns= not significant.

CONCLUSIONS

Climate change represents a major challenge for viticulture due to the variability of climatic factors (temperature and precipitation) that affect both the physiology and biochemistry of the vine, altering the phenology and composition of grape berries. Research on the impact of climatic factors on vine phenology and grape quality must continue to better understand the relationship between climate and grape development, in order to test the plants' ability to adapt to new conditions and to find the best measures to ensure the sustainability and ongoing success of grape production and the entire wine industry.

ACKNOWLEDGEMENTS

This study was funded by ASAS Bucharest and MADR Bucharest under projects PB-627/01.02.2023: Modern Technologies for Enhancing the Oenological Potential of Romanian Grape. Cultivars to Increase Their International Competitiveness and PB 3310/08.12.2022: Integrated Technological Solutions for Vineyard Cultivation Adapted to Climate Change to Ensure the Profitability of Vineyards in the Northeast of the Country.

REFERENCES

1. Arias L. A., Berli F., Fontana A., Bottini R., and Piccoli P. (2022). Climate change effects on grapevine physiology and biochemistry: benefits and challenges of high altitude as an adaptation strategy. *Frontiers in Plant Science*, vol. 13: 835425, pp: 1:14
2. Ilina-Dumitru A.M., Manolescu A.E., Sumedrea D.I., Florea A.C., and Cosmulescu S.N. (2024). Climate influence on the phenology of table grapes in Stefanesti, Romania. *ISHS Acta Hort.*, 1385: XIII International Conference on Grapevine Breeding, Genetics and Management, 181–188. 1385
3. Fernandes J.C., Cobb F., Tracana S., Costa G.J., Valente I., Goulao L.F., and Amañcio S. (2015). Relating water deficiency to berry texture, skin cell wall composition and expression of remodeling genes in two *Vitis vinifera* L. varieties. *J. Agric. Food Chem.*, 63, 3951–3961.
4. Hardie W. J., and Considine J. A. (1976). Response of grapes to water-deficit stress in particular stages of development. *Am. J. Enol. Vitic.* 27, 55–61.
5. Hussain Shah M., Rafique R., Rafique T., Naseer M., Khalil U., and Rafique R. (2022). Effect of climate change on polyphenols accumulation in grapevine; IntechOpen: London, UK.

6. Kafkas E., Kosar M., Paydas S., Kafkas S., and Baser K.H.C. (2007). Quality characteristics of strawberry genotypes at different maturation stages. *Food Chem.* 100, 1229–1236.
7. Lecourieux F., Kappel C., Pieri P., Charon J., Pillet J., and Hilbert G. (2017). Dissecting the biochemical and transcriptomic effects of a locally applied heat treatment on developing Cabernet sauvignon grape berries. *Front. Plant Sci.* 8.
8. Lorenzo M.N., Taboada J.J., Lorenzo J.F., and Ramos A.M. (2013). Influence of climate on grape production and wine quality in the Rías Baixas, north-western Spain. *Reg. Environ. Chang.* 13, 887–896.
9. Palliotti A., Tombesi, S., Silvestroni O., Lanari V., Gatti M., and Poni S. (2014). Changes in vineyard establishment and canopy management urged by earlier climate-related grape ripening: A review. *Sci. Hortic.*, 178, 43–54.
10. Pillet J., Egert A., Pieri P., Lecourieux F., Kappel C., and Charon J., (2012). VvGOLS1 and VvHsfA2 are involved in the heat stress responses in grapevine berries. *Plant Cell Physiol.* 53.
11. Sweetman C., Sadras V. O., Hancock R. D., Soole K. L., and Ford C. (2014). Metabolic effects of elevated temperature on organic acid degradation in ripening *Vitis vinifera* fruit. *J. Exp. Bot.* 65.
12. Stanfield R.C., Forrestel E.J., Elmendorf K.E., Bagshaw S.B., and Bartlett M.K. (2024). Phloem anatomy predicts berry sugar accumulation across 13 wine-grape cultivars. *Front. Plant Sci.* 15, 1360381.
13. OIV (International Organization of Vine and Wine). Compendium of International Methods of Wine and Must Analysis; OIV: Paris, France, 2019; Volume 1.

The *in vitro* evaluation of the antifungal potential of natural *Capsicum annuum* L. extract and synthetic compounds against the pathogens *Alternaria spp.* and *Fusarium oxysporum*.

A. Din¹, I.D. Sărdărescu¹, D.E. Vizitiu¹, D.I. Sumedrea^{1,*}, D. Vilcoci², G. Cirstea² and C. Mihăescu³

¹National Research and Development Institute for Biotechnology in Horticulture, Stefanesti-Arges, Romania

²Regional Center of Research and Development for Materials, Processes and Innovative Products Dedicated to the Automotive Industry (CRC and D-AUTO), National University of Science and Technology Politehnica Bucharest, Pitesti University Centre, Romania

³National University of Science and Technology POLITEHNICA Bucharest, Pitesti University Centre, Romania

*Corresponding author e-mail: dsumedrea@yahoo.com

ABSTRACT

In the last decade, interest in organic agriculture has been steadily gaining ground, leading to studies on the control and management of phytopathogens using plant extracts and essential oils. This study aimed to evaluate the *in vitro* antifungal potential of the natural extract obtained from the *Capsicum annuum* L. species, as well as synthetic compounds, as a control method against the pathogenic fungi *Alternaria spp.* and *Fusarium oxysporum*. The natural extract was used in four different concentrations (0.5%, 2%, 9%, and 12%) and was characterized in terms of total polyphenol content (TPC) using the Folin-Ciocalteu spectrophotometric method, as well as its antioxidant activity using the DPPH radical scavenging method. The results of this study, in which *Capsicum annuum* L. extract was used, demonstrated high efficacy, showing an inhibitory percentage of 33.33% against the pathogen *Alternaria spp.*, and 21.90% against the pathogen *Fusarium oxysporum*. Regarding the efficacy of synthetic products, it can be said that the mancozeb-based product at a concentration of 0.2% exhibited a strong antifungal effect on both pathogens, with an inhibition percentage of 100%.

Keywords: bioactive compounds, capsaicin, phytopathogens, *Capsicum annuum* L, *in vitro*

INTRODUCTION

Currently, synthetic pesticides are the most effective method for controlling and combating fungal pathogens (Amoako Ofori *et al.*, 2022); however, repeated use of these substances can lead to resistance, as well as the accumulation of their residues in soil and water (Gevao *et al.*, 2000; Garcia *et al.*, 2018).

Organic farming has become increasingly popular, generating a growing interest in studies related to the control and management of phytoparasites using natural extracts and essential plant oils (Tomazoni *et al.*, 2018). However, the current progress of organic

agriculture is insufficient due to two main aspects: the lack of research and evaluation in the various functions of agro-ecosystem services, as well as the difficulty in achieving significant economic benefits due to the lack of market-oriented approaches, large-scale management, specialized production, and marketing strategies (Li *et al.*, 2011). Research on plant compounds with bioactive properties against phytopathogens has garnered increasing interest, as biopesticides represent eco-friendly, sustainable, and potentially less toxic alternatives to synthetic fungicides (Vuerich *et al.*, 2023).

The *Capsicum* L. genus (family *Solanaceae*, subfamily *Solanoideae*) originates from South America (Barboza and Bianchetti, 2004), but both Mexico and Central America are also considered diversity centers for this taxon (Barboza, 2011). *Capsicum annum* L. is one of the cultivated species that exhibits a wide range of cultivars with economic, medical, and industrial importance, being used for food, capsaicinoids, carotenoids, and antioxidants (Olatunji and Afolayan, 2018). This genus includes around 32 species native to the Americas, five of which have been domesticated for consumption: *C. annum* L., *C. frutescens* L., *C. baccatum* L., *C. pubescens* Ruiz & Pav., and *C. chinense* Jacq. (Martínez-Ávalos *et al.*, 2018).

All plants in the *Capsicum* genus produce varying amounts of capsaicin. Additionally, this compound provides a unique characteristic and spicy flavor to each cultivar (Morrine *et al.*, 2018).

In vitro tests have shown that capsaicin extract obtained from *Capsicum spp.* can be used as a preventive method against the pathogenic fungus *Penicillium expansum*, preventing colony development during the 14-day monitoring period (Fieira *et al.*, 2013). According to a study by Xing *et al.* (2006), capsaicin (8-methyl-vanillyl-6-nonenamide) demonstrated antifungal efficacy in tests against the pathogenic fungus *Botrytis cinerea*. Additionally, Buitimea-Cantua *et al.* (2018) demonstrated that *Capsicum* extract exhibited a 50% inhibitory effect on the phytopathogen *Aspergillus parasiticus*. Previous research has demonstrated that natural extracts obtained from *Capsicum spp.*, in addition to being used as biopesticides against insects (Koleva-Gudeva *et al.*, 2013), have also been effective against plant pathogens, showing post-harvest antifungal action against the pathogenic fungi *Alternaria alternata*, *Fusarium oxysporum*, *Verticillium dahliae*, *Rhizoctonia solani*, *Sclerotinia minor*, and *Phytophthora capsici* (Tewksbury *et al.*, 2008; Koleva-Gudeva *et al.*, 2013; Veloso *et al.*, 2014; Rodriguez-Maturino *et al.*, 2015; Pane *et al.*, 2015).

The aim of this study was to test the efficacy of the natural extract obtained from *Capsicum annum* L., as well as synthetic fungicides, in evaluating antifungal activity against the hyphal growth of two pathogens, namely *Alternaria spp.* and *Fusarium oxysporum*.

MATERIALS AND METHODS

The conventional extraction technique was used to obtain the natural extract from *Capsicum annum* L.. The pericarp and seeds were dried and then ground for 3 minutes. The solvent used was made from a binary mixture of distilled water and ethyl alcohol in a 50:50 v/v ratio. The extraction was carried out by immersing 20g of plant powder in 200 ml of solvent and extracting for 180 minutes at a temperature of 70°C. After cooling, the extract was filtered to remove solid particles and stored at +4°C until further analysis and testing. The natural extract was used in four different concentrations (0.5%, 2%, 9%, and 12%)

Antifungal Activity

The pathogens *Fusarium oxysporum* and *Alternaria spp.* were isolated by taking mycelial fragments and asexual fruiting bodies from the collar and infected tomato fruits, then cultivated on Potato-dextrose agar (PDA) medium. Inoculation was carried out using a seeding needle, which was sterilized beforehand by “red heat.” Observations regarding the

growth and development of the pathogen colonies were made at regular intervals: 3, 5, 7, and 10 days post-inoculation. After the colonies developed, about 7 days after seeding, they were replated to evaluate the antifungal potential of the natural extract obtained from *Capsicum annuum* L., as well as two synthetic products based on mancozeb (Dithane M45) and azoxystrobin (Amistar).

Thus, sterilized PDA medium was distributed in each Petri dish, to which synthetic pesticides were added at a concentration of 0.2%, along with the natural extract in four different concentrations: 0.5%, 2%, 9%, and 12%. For an accurate interpretation of the results, three repetitions were performed for each experimental variant. The control variant for the two phytopathogens consisted of PDA medium without the addition of natural extract or fungicides.

The colony diameter was measured in two directions, and the average value was recorded at 3, 5, 7, and 10 days. The percentage of mycelial growth inhibition was calculated using Vincent's formula (Vincent, 1947), where I = Percent inhibition, C = Radial growth in control, C = Radial growth in control, and T = Radial growth in treatment (fungicide).

$$I = \frac{C - T}{C} \times 100$$

Total Polyphenol Content (TPC)

The total polyphenol content (TPC) was measured using the Folin-Ciocalteu spectrophotometric method (ISO 14502-1). In this method, the Folin-Ciocalteu reagent is reduced by the phenolic compounds in the extract, forming a blue-colored complex. The experimental variants were prepared by taking different volumes of the extract, diluting them, and then adding 1 mL of the Folin-Ciocalteu reagent (diluted 10 times) to 200 μ L of each dilution. After 5-6 minutes, 800 μ L of a 7.5% Na₂CO₃ solution was added.

The samples were kept in the dark at room temperature for 60 minutes and analyzed spectrophotometrically at 765 nm. The results were reported as mg gallic acid/g plant (mg GA/g) based on a calibration curve created using concentrations of the standard, with 7 concentration points ranging from 10 to 70 μ g/mL of gallic acid.

Determination of Antioxidant Capacity (DPPH)

The antioxidant capacity of the natural extract was evaluated using the DPPH radical scavenging method (2,2-diphenyl-1-picrylhydrazyl), according to the protocol developed by Shimamura *et al.* (2014), with subsequent adjustments. To highlight the antioxidant reaction of the control (Trolox in this case) and the experimental variants, a DPPH solution and the standard/extract were added to a test tube in a volumetric ratio of 1:7. The absorbance of each sample was measured at a wavelength of 517 nm, after a stabilization period of 5 minutes under UV light, followed by plotting the calibration curve for Trolox. The Trolox calibration curve was generated for 6 different concentrations of the reagent, and the antioxidant capacity of each sample was expressed as TEAC (Trolox Equivalent Antioxidant Capacity), Where: IC_{50} Trolox = half the maximum concentration of Trolox inhibitors and IC_{50} Extract = half of the maximum inhibitory concentration of Extract using the formula:

$$TEAC = \frac{IC_{50} Trolox}{IC_{50} Extract}$$

All analyzed data were processed using one-way ANOVA, followed by Šídák's multiple comparisons test. Processed data were expressed as mean $\pm$ standard error (SE). This analysis was performed using GraphPad Prism 9.0.0.0 software.

RESULTS AND DISCUSSIONS

Dynamics of mycelial hyphae growth on culture medium

Alternaria spp. and *Fusarium oxysporum* were isolated on PDA medium (Figure 1) being suitable for the cultivation and replication of the two pathogens (Din *et al.*, 2022). The dynamics of mycelial growth was fast, with a radial expansion from the point of inoculation observed. At 3 days after inoculation, the diameter of the colonies for *Alternaria* spp. was on average 1.6/1.7 cm, reaching 7/7 cm at 10 days. In contrast, *Fusarium oxysporum* showed a more accelerated growth, with an average colony diameter of 3/3.1 cm at 3 days and 7/7 cm at 5 days after inoculation.



Figure 1. *Alternaria* spp. (a) and *Fusarium oxysporum* (b) on PDA plate

Antifungal Activity of the Natural Extract of *Capsicum annuum* L. and Synthetic Products

Unlike synthetic products, it can be stated that the natural extract obtained from the *Capsicum annuum* L. species exhibited fungistatic effects directly proportional to the concentration used for both pathogens. To establish a notable inhibitory percentage, four different concentrations of the natural extract were set: 0.5%, 2%, 9%, and 12%.

Figure 2 shows the fungistatic action of the natural extract obtained from *Capsicum annuum* L. on the mycelial hyphae of the pathogens *Alternaria* spp. and *Fusarium oxysporum*.

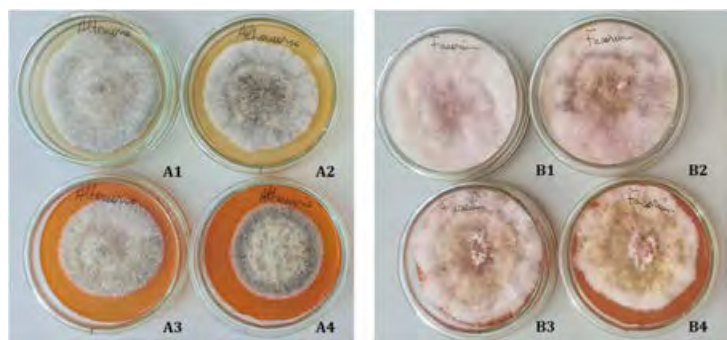


Figure 2. Growth of *Alternaria* spp. and *Fusarium oxysporum* isolate on PDA amended with ethyl extract of *Capsicum annuum* L. (*Alternaria* spp.: A1=0,5%; A2=2%; A3=9%; A4=12% and *Fusarium oxysporum*: B1=0,5%; B2=2%; B3=9%; B4=12%).

The statistical analysis of the results after 10 days is illustrated in Figure 3, where a 33.33% inhibition rate of the mycelial growth of the pathogen *Alternaria* spp. is observed at the maximum concentration used in our experiments (12%), compared to the pathogen *Fusarium oxysporum*, which showed an inhibition rate of 21.90% at the same concentration of the natural extract.

At the concentration of 0.5% for the growth and development of *Alternaria spp.*, the inhibition percentage was 4.76%; at 2%, it was 16.19%; and at 9%, the inhibition percentage was 21.14%. Regarding the growth and development of the pathogen *Fusarium oxysporum* at concentrations of 0.5%, 2%, and 9%, the inhibition percentage after 10 days of monitoring was 0%, which was equal to that of the control variant.

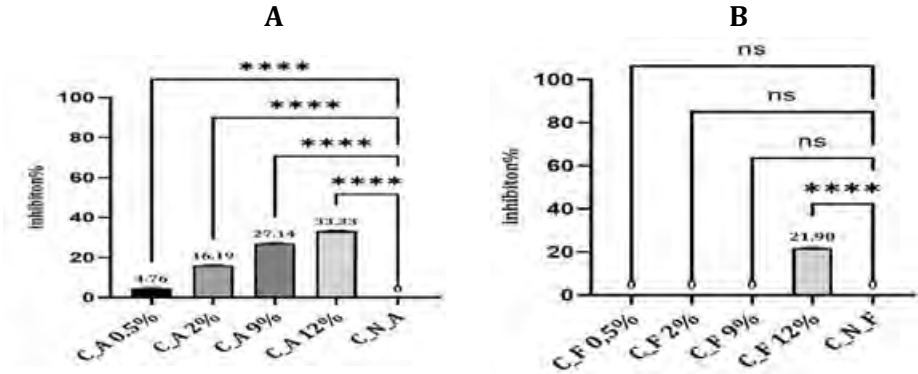


Figure 3. Effect of concentrations of the three types of extracts on the pathogenic fungus *Alternaria spp.* (A) and *Fusarium oxysporum* (B). The data are expressed as the mean ± SD values of three independent experiments performed in triplicate, and the values of p were calculated by the one-way ANOVA method followed by Šídák's multiple comparison test ****p < 0.0001; ns p > 0.9999 (C,... – *Capsicum annuum* L extracts A... – *Alternaria spp.*; F,... – *Fusarium oxysporum*, C_N –negative control).

Prasad *et al.* (2018) studied the effect of the extract obtained from the *Capsicum annuum* L. species against the pathogen *Fusarium oxysporum*, and the results showed a maximum inhibitory effect of 75.7%. Additionally, López-Muñoz *et al.* (2019) demonstrated that the habanero pepper extract (*Capsicum chinense* var. *Habanero*) is effective for controlling *Cochliobolus spp.*, with an inhibition percentage of 82.29%, as well as against the pathogen *Fusarium andiyazi*, with an inhibition percentage of 55.61%.

The sensitivity of these two pathogens to the natural extract of *Capsicum annuum* L. was compared to their sensitivity to synthetic pesticides, using active substances azoxystrobin and mancozeb (Figure 4). The concentrations used for the two synthetic pesticides were 0.2%, according to the manufacturer's instructions.

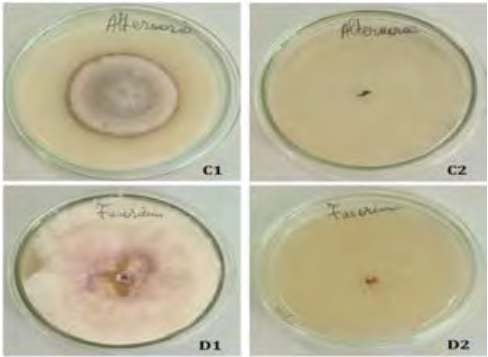


Figure 4. Antifungal effect of fungicides *in vitro* *Alternaria spp.*=series C (C1=azoxistrobin 0,2%; C2=mancobez 0,2%) and *Fusarium oxysporum* series D (D1=azoxistrobin 0,2%; D2=mancobez 0,2%).

In vitro experiments using synthetic products showed that the mancozeb-based product demonstrated an inhibition capacity of 100% for both pathogens, proving very effective in combating and controlling these pests in tomato crops. Regarding the azoxystrobin-based synthetic product, the results were different: for the pathogen *Alternaria spp.*, the inhibition percentage was 43.53%, while for the pathogen *Fusarium oxysporum*, the effects

were not visible, with the inhibition percentage of the fungal colony equal to that of the control variant (0%) (Figure 5).

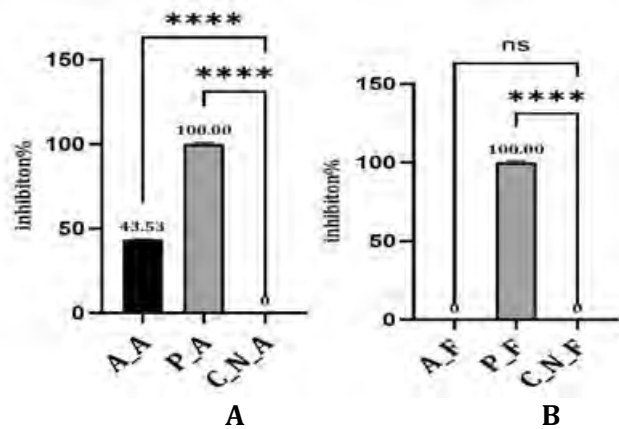


Figure 5. The effect of synthetic pesticides on the pathogenic fungus *Alternaria spp.*(A) and *Fusarium oxysporum* (F). The data are expressed as the mean $\pm$ SD values of three independent experiments performed in triplicate, and the values of p were calculated by the one-way ANOVA method followed by Šídák's multiple comparison test ****p < 0.0001 (A- azoxistrobin, P – mancobez, A-*Alternaria spp.*, F-*Fusarium oxysporum*, C_N- negative control)

Therefore, it was highlighted that the natural extract obtained from the *Capsicum annuum* L. species possesses authentic antifungal qualities that can be exploited industrially in the agricultural sector, representing a sustainable alternative to synthetic pesticides, as the raw material acquisition and production process do not involve high costs.

Analysis of Polyphenols and Antioxidant Capacity

The total polyphenol content (TPC) of the natural extract obtained from *Capsicum annuum* L. showed a value of 70.58 ± 0.46 mg/ml GAE, while the antioxidant capacity (DPPH) was 0.0134 ± 0.0040 , which was correlated with the lowest value of the half-maximal inhibitory concentration (IC50), specifically 2834.6 μ g/mL (Table 1).

Table 1. The total polyphenol content and antioxidant capacity of the *Capsicum annuum* L. extract.

Plant extract	TPC (mg/ml GAE)	IC ₅₀ (μg/mL) (DPPH)	TEAC (DPPH)
<i>Capsicum annuum</i> L.	70.58±0.46	2834.6	0.0134±0.0040

Salamatullah *et al.* (2022) used different cultivars of the *Capsicum annuum* L. species (green, yellow, and red peppers) to obtain natural extracts. The analyses showed that the total polyphenol content varied, with values ranging from 30.15 mg GAE/g for the green pepper cultivar, followed by 28.73 mg GAE/g for the red pepper, and 27.68 mg GAE/g for the yellow pepper.

The same study also determined the antioxidant capacity, which ranged from 72.76% (green pepper) to 70.26% (red and yellow peppers). Additionally, Kavuncuoğlu *et al.* (2021) reported a high polyphenol content of 515.85 mg GAE/g for the yellow pepper extract and 362.48 mg GAE/g for the *Charleston pepper* extract.

CONCLUSIONS

The overall effectiveness assessment of a plant protection product involves analyzing several aspects, including the duration of its biological activity (persistence of action), compatibility with various protection strategies or cultural practices, and ease of application. To evaluate the *in vitro* antifungal activity of the natural extract obtained from *Capsicum annuum* L. against the pathogens *Alternaria spp.* and *Fusarium oxysporum*, four concentrations were used: 0.5%, 2%, 9%, and 12%, which were incorporated into PDA culture media. The highest inhibition rate for the pathogen *Alternaria spp.* was observed at a concentration of 12%, where the inhibition percentage was 33.33%. For the pathogen *Fusarium oxysporum*, the inhibition percentage was 21.90% at the maximum concentration used. Synthetic fungicides demonstrated good antifungal activity against the studied pathogens. The mancozeb-based synthetic product achieved a 100% inhibition rate for both studied pathogens.

Thus, our experimental results suggest that the extract from *Capsicum annuum* L. could be utilized as a control method in managing the phytopathogenic fungus *Alternaria spp.*

ACKNOWLEDGEMENTS

This research paper would not have been possible without the exceptional support of the Regional Center of Research & Development for Materials, Processes and Innovative Products Dedicated to the Automotive Industry (CRC&D-AUTO), (National University of Science and Technology POLITEHNICA Bucharest, Pitesti University Centre).

REFERENCES

1. Amoako O., P., Owusu-Nketia, S., Opoku-Agyemang, F., Agbleke, D., and Naalamle Amissah, J. (2022). Greenhouse Tomato Production for Sustainable Food and Nutrition Security in the Tropics. IntechOpen. <http://dx.doi.org/10.5772/intechopen.105853>
2. Barboza G.E. (2011). Lectotypifications, synonymy, and a new name in *Capsicum* (Solanoideae, Solanaceae). *PhytoKeys*. Vol.2:23–38.
3. Barboza G.E. and Bianchetti L.B. (2004). Three new species of *Capsicum* (Solanaceae) and a key to the Chemin Bässler, H., Recetario pame de San Luis Potosí y Querétaro. *Cocina Indígena y popular* 26. Consejo Nacional para la Cultura y las Artes: México, D.F.
4. Buitimea-Cantúa B.V., Velez-Haro John J.M., Buitimea-Cantúa N.E., Molina-Torres J., and Rosas-Burgos E.C. (2018). GC-EIMS analysis, antifungal and anti-aflatoxigenic activity of *Capsicum chinense* and *Piper nigrum* fruits and their bioactive compounds capsaicin and piperine upon *Aspergillus parasiticus*. *Natural Product Research*. Vol. 32(20).
5. Din A., Vilcoci D.Ş., Cirstea G., Negrea D., Moga S., Mihaescu C., and Mitrea R. (2022). Characterization of 'Cabernet Sauvignon' pomace extracts and evaluation of antifungal potential of *Alternaria sp.* and *Fusarium sp.* *Notulae Botanicae Horti Agrobotanici Cluj-Napoca*. Vol.50(3):12774. <https://doi.org/10.15835/nbha50312774>
6. Fieira C., Oliveira F., Calegari R.P., Machado A., and Coelho AR. (2013). *In vitro* and *in vivo* antifungal activity of natural inhibitors against *Penicillium expansum*. *Ciênc Tecnol Aliment*. Vol. 33(1): 40-46.
7. Garcia T., Veloso J., and Diaz J. (2018). Properties of vanillyl nonanoate for protection of pepper plants against *Phytophthora capsici* and *Botrytis cinerea*. *Eur. J. Plant Pathol*. Vol.150(4): 1091–1101.
8. Gevaio B., Semple K.T., and Jones K.C. (2000). Bound pesticide residues in soils: a review. *Environmental Pollution* 108(1):3-14. [https://doi.org/10.1016/s0269-7491\(99\)00197-9](https://doi.org/10.1016/s0269-7491(99)00197-9)
9. Kavuncuoğlu H., Çapar T.D., and Yalçın H. (2021). Total Phenolic Contents, Antioxidant Activities and Antioxidant Capacities of Some Selected Pepper (*Capsicum Annuum* L.) Pulp and Seeds. *European Journal of Science and Technology*. No. 21:581-586.
10. Koleva-Gudeva L., Mitrev S., Maksimova V., and Spasov D. (2013). Content of capsaicin extracted from hot pepper (*Capsicum annuum* ssp. *microcarpum* L.) and its use as an ecopesticide. *Hem Ind*. Vol.67:671-675.
11. Li W., Liu M., and Min Q. (2011). China's Ecological Agriculture: Progress and Perspectives. *J. of Resources and Ecology*. Vol.2(1):1-7

12. López-Muñoz N.R., Romero-Bastidas M., Arce-Amézquita P.M., and Hernández-Rubio J.S. (2019). Antifungal activity of antioxidants derived from four cultivars of *Capsicum* spp. against phytopathogen fungi. *Ecosist. Recur. Agropec.* Vol.6(18):487-498.
13. Martínez-Ávalos J.G., Venegas-Barrera C.S., Martínez-Gallegos R., Torres-Castillo J.A., Olazarán Santibáñez F.E., Mora-Olivo A., Guerra-Pérez A., Arellano-Méndez L.U., and Garza Ocañas F. (2018). A Review on the Geographical Distribution, Fruit Production and Concentration of Capsaicinoids in *Capsicum annum* var. *glabriusculum* in the Northeastern Region of Mexico. Preprints. <https://doi.org/10.20944/preprints201811.0517.v1>
14. Morrione A.O., Zen-Zi W., Weih G.B., Grant A.H., Kamal D., and David J.B. (2018). Comparative analysis of capsaicin in twenty nine varieties of unexplored *Capsicum* and its antimicrobial activity against bacterial and fungal pathogens. *Journal of Medicinal Plants Research* . Vol.12(29):544-556.
15. Olatunji T.L., and Afolayan A.J. (2018). The suitability of chili pepper (*Capsicum annum* L.) for alleviating human micronutrient dietary deficiencies: A review. *Food Sci Nutr.* Vol.6(8):2239-2251.
16. Pane C., Fratianni F., Caputo M., Parisi M., Nazzaro F., and Zaccardelli M. (2015). Antifungal activity of wild *Capsicum foliar* extracts containing polyphenols against the phytopathogens *Alternaria alternata*, *Rhizoctonia solani*, *Sclerotinia minor* and *Verticillium dahliae*. *Multidisciplinary approach for studying and combating microbial pathogens*, 34-38.
17. Prasad G., Vinay K., and Dwivedi S.K. (2018). "Antifungal activity of some selected medicinal plants against *Fusarium solani* causing wilt and rot in Pearl millet." *AJBS.* Vol.13 (1):21-27.
18. Rodríguez-Maturino A., Troncoso-Rojas R., Sánchez-Estrada A., González-Mendoza D., Ruiz-Sanchez E., Zamora-Bustillos R., Ceceña-Durana C., Grimaldo-Juarez O., and Aviles-Marina M. (2015). Efecto antifúngico de extractos fenólicos y de carotenoides de chiltepín (*Capsicum annum* var. *glabriusculum* en *Alternaria alternata* y *Fusarium oxysporum*). *Rev Argent Microbiol.* Vol.47: 72-77.
19. Salamatullah A.M. , Hayat K., Husain F.M., Ahmed M.A., Arzoo S., Althbiti M.M., Alzahrani A., Bandar Ali M. Al-Zaied, Alyahya H.K., Albader N., Nafidi H.A., and Bourhia M. (2022). Effects of Different Solvents Extractions on Total Polyphenol Content, HPLC Analysis, Antioxidant Capacity, and Antimicrobial Properties of Peppers (Red, Yellow, and Green (*Capsicum annum* L.)) *Hindawi Evidence-Based Complementary and Alternative Medicine.* Vol.2022:737210 <http://doi.org/10.1155/2022/7372101>
20. Shimamura T., Sumikura Y., Yamazaki T., Tada A., Kashiwagi T., Ishikawa H., Matsui T., Sugimoto N., Akiyama H., and Ukeda H. (2014). Applicability of the DPPH assay for evaluating the antioxidant capacity of food additives-inter-laboratory evaluation study. *Analytical Sciences.* Vol. 30(7):717-721. <https://doi.org/10.2116/analsci.30.717>
21. Tewksbury J.J., Reagan K.M., Machnicki N.J., Carlo T.A., Haak D.C., Peñaloza A.L.C., and Levey D.J. (2008). Evolutionary ecology of pungency in wild chilies. *Proc Natl Acad Sci.* Vol.105: 11808-11811.
22. Tomazoni E.Z., Griggio G.S., Broilo E.P., da Silva Ribeiro R.T. Gonçalves Soares G.L., and Schwambach J. (2018). Screening for Inhibitory Activity of Essential Oils on Fungal Tomato Pathogen *Stemphylium solani* Weber. *Biocatalysis and Agricultural Biotechnology.* Vol.16: 364-372. <https://doi.org/10.1016/j.bcab.2018.08.012>
23. Veloso J., Prego C., Varela M.M., Carballeira R., Bernal A., and Merino F. Díaz J. (2014). Properties of capsaicinoids for the control of fungi and oomycetes pathogenic to pepper. *Plant Biol Stuttg Ger.* Vol.16: 177-185.
24. Vincent J.M. (1947). The esters of 4-hydroxybenzoic acid and related compounds. Part I. Methods for the study of their fungistatic properties. *Journal of the Society of Chemical Industry.* Vol. 66:149-155. <https://doi.org/10.1002/jctb.5000660504>
25. Vuerich M., Petrusa E., Filippi A., Cluzet S., Fonayet J.V., Sepulcri A., Piani B., Ermacora P., and Braidot E. (2023). Antifungal activity of chili pepper extract with potential for the control of some major pathogens in grapevine. *Pest Manag Sci.* Vol.79: 2503-2516 <https://doi.org/10.1002/ps.7435>
26. Xing F., Cheng G., and Yi K. (2006). Study on the antimicrobial activities of the capsaicin microcapsules. *J Appl Polym Sci.* Vol.102: 1318-1321.
27. ISO 14502-1. Determination of substances characteristic of green and black tea, Part.1: Content of total polyphenols in tea - Colorimetric method using Folin-Ciocalteu reagent. 2005.

The evolution of the degree of attack by grey rot (*Botrytis cinerea*) on four wine grape cultivars under conventional and organic control treatments, in the Murfatlar viticultural center

I. Dina ¹, A. Ranca¹, A.S. Ene^{1*} and A. Tanase¹

¹ Research and Development Station for Viticulture and Oenology Murfatlar, Romania

* Corresponding author e-mail: ayar_ene@yahoo.com

ABSTRACT

Between 2023 and 2024, in the vineyard of the Research and Development Station for Viticulture and Oenology Murfatlar, the susceptibility of four wine grape cultivars (*Vitis vinifera*) ('Columna', 'Pinot gris 13', 'Mamaia', and 'Fetească neagră') to grey mold (*Botrytis cinerea*) was tested under two types of disease control: organic and conventional. In both disease control systems, four *botrytis* treatments were applied each year, using the same commercial products with the same active substances per hectare. The treatments, both organic and conventional, were applied during the phenological stages when the grapevine is most vulnerable to this pathogen: after flowering, during bunch closure, at the beginning of veraison and during veraison. Thus, a trifactorial experiment was achieved: vintage year x disease control system x grape cultivar. In the two years of study, the two types of disease control were also differentiated by appropriate field and fertilization technologies. The degree of attack by grey rot (*Botrytis cinerea* Pers.) was determined on 100 grape clusters per variant at harvest. For the less affected cultivars, 'Columna' (2.48%) and 'Mamaia' (3.49%), the degree of attack (DA %) was statistically lower under all control systems and compared to the other cultivars. These cultivars showed greater native resistance to grey rot compared to 'Fetească neagră' (3.78%) and 'Pinot gris 13' (4.59%). These results are of interest for organic grey rot control practices, and consequently, the 'Columna' and 'Mamaia' cultivars can be recommended for organic cultivation.

Keywords: wine grape cultivars, conventional and organic control, grey rot, grapes, degree of attack (DA %)

INTRODUCTION

In the current conditions, controlling the main grapevine diseases - Downy mildew, Powdery mildew, and Grey rot - has become a particularly important issue. This is due to the increased virulence and evolution of phytopathogens caused by climate change, intensive grapevine cultivation technologies, and excessive use of toxic phytosanitary products. During the bioactive period, the vine, a perennial plant in monoculture, is vulnerable to a wide range of pathogens that cause various diseases on plant organs. To avoid environmental pollution, there is now a focus on the ecological adaptation of protection measures against grapevine diseases and pests. (Compant *et al.*, 2016)

Grey rot, caused by the fungus *Botrytis cinerea* (De Bary) Fck, attacks all grapevine organs but most frequently affects mature berries near harvest, causing significant yield losses and deteriorating wine quality.

To obtain healthy and stable yields, the protection program against *Botrytis cinerea* (De Bary) Fck includes four treatments applied during the phenological stages when grapevines are most vulnerable to this pathogen: after flowering, during bunch closure, at the beginning of veraison, and during veraison (20 days before grape harvest). (I. C. Alexandrescu *et al.*, 1994 Encyclopedia).

Given the risk of developing resistance to fungicides used to control *Botrytis cinerea* (De Bary) Fck (Fan *et al.*, 2017; Rupp *et al.*, 2017), as this pathogen has a highly pronounced adaptive mechanism to environmental conditions and changing virulence depending on abiotic factors (temperature, relative humidity, canopy moisture, etc.), and the persistence of these pesticides in the final product, it is necessary to justify the use of phytosanitary products in the grapevine protection program.

In this regard, biological control can be a safe alternative and an ecological approach for managing *B. cinerea* disease up to grape harvest, avoiding high doses of chemical substances (Li *et al.*, 2023; Pertot *et al.*, 2017).

All grapevine cultivars are susceptible to grey rot (*Botrytis cinerea* Pers.), with a high incidence during grape ripening when weather conditions are favorable: temperatures between 15 - 23°C, high relative humidity > 80%, generated by abundant precipitation, followed by large day-night temperature fluctuations.

Unlike conventional practices, organic agriculture involves controlling diseases and pests without using synthetic pesticides, herbicides, or fertilizers (Briar *et al.*, 2007) and promotes biodiversity, natural biological cycles, and product quality improvement.

This paper presents a comparison of the biological effectiveness of two grey rot control systems, conventional vs. organic. The level of biological effectiveness of the two systems was expressed by the degree of attack recorded on grapes of four wine grape cultivars grown under field conditions.

MATERIALS AND METHODS

Plant material and cultivation system: Four wine grape cultivars, two white and two red - 'Columna', 'Pinot gris 13', 'Mamaia', and 'Fetească neagră' - were tested during the period 2023-2024 in the vineyards of the Research and Development Station for Viticulture and Oenology Murfatlar, under two types of cultural practices: organic and conventional. Three of the four cultivars have medium resistance to gray rot, while 'Pinot gris 13' is more sensitive (Olteanu *et al.*, 2002).

The experimental plots were located in the plantation at S.C.D.V.V. Murfatlar, Romania, at an altitude of 27-50 m. The vineyard had distances of 2.2 x 1.1 m between rows and plants, and the orientation of the rows is North-South for all variants. The plants were grafted onto the same rootstock, *V. berlandieri* x *V. riparia*, Selection Oppenheim 4. The vine training system was a semi-trunk Guyot with a stem height of 75-80 cm and a bilateral cordon with two fruiting spurs. For each cultivar three experimental plots were organized with the following systems for controlling gray rot: untreated (control), conventional and organic. The treatment schemes specific for the two disease control systems remained identical in both years of the study. No treatment was applied in the control plot. Each experimental plot consisted of 300 plants. The experiment organization (year x disease control system x cultivar) is presented in Table 1.

Table 1. Design of the trifactorial experiment, Murfatlar, 2023-2024

Factor A – Year	Factor B - disease control system	Factor C- cultivar
Year 2023- a ₁	- control- untreated – b ₁	'Columna' - c ₁
Year 2024- a ₂	- conventional treatments- b ₂	'Pinot gris 13' - c ₂
	- organic treatments- b ₃	'Mamaia' – c ₃
		'Fetească neagră' – c ₄

The soil type is a typical calcareous chernozem, with a medium texture, having a humus content of 2-3%. The soil maintenance system under the vine and between rows was black furrow for all variants.

Four phytosanitary treatments were applied in the conventional and organic disease control variants. Conventional treatments consisted of using phytosanitary products with the following composition: Boscalid 50% (Cantus 1.0 kg/ha) after flowering, Fludioxonil 25% + Cyprodinil 37.5% (Switch 62.5 WG 1.0 kg/ha) during bunch compacting, Pyrimethanil 400 g/L (Pyrus 400 SC 1.5 L/ha) at the onset of veraison, and Fenhexamid 500 g/L (Teldor 500 SC 1 L/ha) during veraison.

Organic treatments consisted of spraying with 20% citrus seed extract (Zytron 1.5 L/ha) applied after flowering and during bunch compacting, and 80% Mimosa tenuiflora extract (Mimoten 2.5 L/ha) applied at the onset of veraison and in the veraison stage.

To determine the degree of attack by *Botrytis cinerea*, 100 grapes were harvested from each variant and using a severity scale with seven disease severity levels: 5, 15, 25, 35, 50, 75, and 100% (Caffi *et al.*, 2010), the frequency (F %) and the intensity (I %) of grey rot symptoms on the grapes were determined each year. F % referred to the number of grapes out of the 100 harvested that showed disease symptoms/total number of grapes evaluated, while I % referred to the percentage of the grape affected by rot/total surface area of the observed grape. AD % was calculated using the formula:

$$AD \% = \frac{F \% * I \%}{100}$$

Data on the degree of fungal attack were collected and analyzed using SPSS (Statistical Package for the Social Sciences). ANOVA (Analysis of Variance) was performed in SPSS to determine statistical differences between groups, assessing the significance of differences between means for each measured variable. After conducting ANOVA, the Duncan test was applied as a post-hoc test to identify specific differences between groups. The Duncan test allowed for the comparison of multiple means to determine which treatments (organic, conventional and control) were significantly different from each other in the context of the three variables of interest.

During the two years of study, all specific viticultural technological operations were carried out throughout the growing season, with treatments applied as needed using a 1000 LT tractor-mounted sprayer for grapevines. A series of data on meteorological conditions were recorded and processed, including mean, maximum, and minimum air temperatures, precipitation, relative air humidity, and sunshine duration.

RESULTS AND DISCUSSION

From a thermal point of view, during the two years, the average temperature during the growing season was recorded as 3.2-3.6°C above the normal average (18.3°C). In the Murfatlar wine-growing center, the normal accumulation of precipitation during the growing season is on average 245 mm. However, during the studied years, the precipitation was below the normal value in 2023 (110.2 mm), experiencing an excessive drought during the ripening period of the grapes (August - 3.6 mm and September 0.2

mm) and well above the normal average in 2024 (281.8 mm), with a significant contribution of rainfall between 28 - 31.08.2024 (62.0 mm) and in the first decade of September (62.4 mm), coinciding with the grape ripening phase. Regarding the relative humidity, it is observed that in 2023, it was lower with reduced sunlight exposure compared to 2024. The data are presented in Table 2.

Table 2. Climatic data from the Murfatlar viticultural center during the growing season of 2023 and 2024

Month	Multiannual average	Year 2023						Year 2024					
		Monthly average (°C)	Absolute maximum (°C)	Absolute minimum (°C)	Rainfalls (mm)	Air Higrscopicity (%)	Sunshine (h)	Monthly average (°C)	Absolute maximum (°C)	Absolute minimum (°C)	Rainfalls (mm)	Air Higrscopicity (%)	Sunshine (h)
IV	10.5	10.8	20.3	-1.9	39.5	85.8	159.8	16.2	31.9	3.4	77.6	65.2	167.2
V	16.2	17.4	29.7	1.5	11.4	73.4	227.3	16.6	27.7	3.4	26.2	67.4	186.5
VI	20.4	22.8	33.6	7.9	28.4	64.8	227.5	26.1	37.7	12.5	11.2	53.6	230.8
VII	22.6	26.8	39.1	13.4	27.8	57.6	227.6	28.5	38.8	10.1	17.4	39.1	285.6
VIII	22.6	26.9	36.9	11.5	3.6	58.7	225.5	26.1	36.6	14.5	87.0	58.8	228.3
IX	17.6	22.2	31.4	8.6	0.2	59.5	244.1	21.9	27.5	16.6	62.4	72.4	224.3
Annual mean	18.3	21.5				66.6		22.6				59.4	
Annual sum					110.2		1311.8				281.8		1421.9

In the first part of the growing season, the degree of attack by the fungus *Botrytis cinerea* in all experimental plots was very low. However, with the onset of the grape ripening phenophase, both in 2023 and 2024, the incidence rate of the attack caused by *Botrytis cinerea* increased. In 2024, the frequency and intensity of the attack grew compared to 2023, favored and amplified by the excess precipitation recorded between August 28 – 31, 2024 (62.0 mm) and in the first decade of September (62.4 mm). At harvest, 100 grapes from each variant were evaluated, and observations and assessments were made using the disease severity diagram scale with seven levels of severity: 5, 15, 25, 35, 50, 75, and 100% (Caffi *et al.*, 2010).

As a result of the data analysis regarding the degree of infestation with gray rot over the two studied years, significant differences between the types of treatments are highlighted. Table 3 presents the mean values, standard deviations, and confidence intervals for each variable, along with the statistical significance of the differences between groups. Considering the degree of attack by *Botrytis cinerea* (AD %) for the tested cultivars, all three experimental factors had significant effects in the series of experiments.

Following the statistical analysis, the following significant data regarding the effect of control systems on the degree of fungal attack for the studied cultivars emerged: for the 'Columna' cultivar the mean degree of fungal attack in the control plot was 6.23 (standard deviation 5.83), whereas for the conventional control system, the mean significantly decreased to 0.20 (standard deviation 0.28), and for the organic control system, it was 1.03 (standard deviation 0.34). Similarly, the 'Mamaia' cultivar showed a notable reduction in the degree of attack, with a mean of 9.21 (standard deviation 9.81) in the control, 0.41 (standard deviation 0.42) in the conventional system, and 0.86 (standard deviation 0.74) in the organic system.

On the other hand, 'Fetească neagră' and 'Pinot gris 13' cultivars showed higher susceptibility to grey rot. 'Fetească neagră' cultivar recorded a mean of 9.50 (standard deviation 10.03) in the untreated (control) group, while 'Pinot gris 13' had a mean of 11.35

(standard deviation 12.71). These values indicate a more severe fungal attack compared to the 'Columna' and 'Mamaia' cultivars. Statistics show that both the organic and conventional control systems had a significant effect on reducing the degree of fungal attack, especially for the 'Columna' and 'Mamaia' cultivars, which exhibit higher native resistance to grey rot compared to the other analyzed cultivars, 'Pinot gris 13' and 'Fetească neagră'.

Table 3. Descriptive statistics of fungal attack based on the applied treatment (organic, conventional, and control)

Descriptive Statistics					
Dependent Variable: Degree of fungal attack					
Cultivar	Disease treatment system	Year	Mean	Std. Deviation	N
1	2	3	4	5	6
'Columna'	Control	2023	2.1100	.	1
		2024	10.3500	.	1
		Total	6.2300	5.82656	2
	Conventional	2023	0.0000	.	1
		2024	0.4000	.	1
		Total	0.2000	0.28284	2
	Organic	2023	0.7800	.	1
		2024	1.2700	.	1
		Total	1.0250	0.34648	2
	Total	2023	0.9633	1.06688	3
		2024	4.0067	5.51068	3
		Total	2.4850	3.92185	6
'Fetească neagră'	Control	2023	2.4100	.	1
		2024	16.6000	.	1
		Total	9.5050	10.03385	2
	Conventional	2023	0.2500	.	1
		2024	0.8500	.	1
		Total	0.5500	0.42426	2
	Organic	2023	0.6500	.	1
		2024	1.9200	.	1
		Total	1.2850	0.89803	2
	Total	2023	1.1033	1.14914	3
		2024	6.4567	8.80066	3
		Total	3.7800	6.33296	6
'Mamaia'	Control	2023	2.2700	.	1
		2024	16.1500	.	1
		Total	9.2100	9.81464	2
	Conventional	2023	0.1100	.	1
		2024	0.7100	.	1
		Total	0.4100	0.42426	2
	Organic	2023	0.3300	.	1
		2024	1.3900	.	1
		Total	0.8600	0.74953	2
	Total	2023	0.9033	1.18867	3
		2024	6.0833	8.72462	3
		Total	3.4933	6.25000	6
'Pinot gris 13'	Control	2023	2.3600	.	1
		2024	20.3400	.	1
		Total	11.3500	12.71378	2
	Conventional	2023	0.1300	.	1

Descriptive Statistics						
Dependent Variable: Degree of fungal attack						
Cultivar	Disease treatment system	Year	Mean	Std. Deviation	N	
1	2	3	4	5	6	
		2024	1.5200	.	1	
		Total	0.8250	0.98288	2	
	Organic	2023	0.8900	.	1	
		2024	2.3400	.	1	
		Total	1.6150	1.02530	2	
	Total	2023	1.1267	1.13368	3	
		2024	8.0667	10.63692	3	
		Total	4.5967	7.76021	6	
	Average disease system treatment	Control	2023	2.2875	0.13175	4
			2024	15.8600	4.12562	4
Total			9.0738	7.74172	8	
Conventional		2023	0.1225	0.10243	4	
		2024	0.8700	0.47237	4	
		Total	0.4963	0.50968	8	
Organic		2023	0.6625	0.24240	4	
		2024	1.7300	0.49511	4	
		Total	1.1963	0.67515	8	
Average Total		2023	1.0242	0.97324	12	
		2024	6.1533	7.50311	12	
		Total	3.5888	5.85154	24	

To assess the impact of control systems on fungal attack, ANOVA tests were conducted for the degree of attack. The analyzed grey rot (*Botrytis cinerea* Pers.) control systems were: organic, conventional and control, with each group consisting of 24 variants. The ANOVA results are presented in Table 4.

Table 4. Results of the analysis of variance (ANOVA) for the degree of fungal attack based on the control system (organic, conventional and control)

Tests of Between-Subjects Effects						
Dependent Variable: Degree of fungal attack						
Source	Type III Sum of Squares	df	Mean Square	F	Sig.	Partial Eta Squared
Corrected Model	376.662 ^a	5	75.332	3.300	0.027	0.478
Intercept	309.099	1	309.099	13.541	0.002	0.429
Cultivar	13.679	3	4.560	0.200	0.895	0.032
Treatment	362.983	2	181.491	7.951	0.003	0.469
Error	410.870	18	22.826			
Total	1096.631	24				
Corrected Total	787.532	23				
a. R Squared = 0.478 (Adjusted R Squared = 0.333)						

The ANOVA analysis highlighted significant effects of the control system on the degree of fungal attack ($F(2,18) = 7.951, p = 0.003$, Partial Eta Squared = 0.469). This result suggests that the type of control (organic, conventional, control) significantly influences the severity of the fungal attack, with an explanatory impact of nearly 47%.

Regarding the grapevine cultivars, the analysis did not find significant differences in the degree of fungal attack between experimental plots ($F(3.18) = 0.200$, $p = 0.895$, Partial Eta Squared = 0.032). This suggests that variability in fungal attack severity is not significantly influenced by the grapevine cultivar in the context of the applied control systems. However, descriptive data indicate that the 'Columna' and 'Mamaia' cultivars show a lower degree of fungal attack compared to the other cultivars. 'Columna' and 'Mamaia' had significantly lower mean values for the degree of fungal attack, suggesting greater native resistance to grey rot compared to 'Fetească neagră' and 'Pinot gris 13' cultivars. These observations are supported by Duncan's post-hoc tests, which identified 'Columna' and 'Mamaia' as groups with significantly lower means compared to the other cultivars. To explore specific differences between the organic, conventional, and control treatments regarding the degree, intensity, and frequency of fungal attack, Duncan's test was performed, which was applied after ANOVA to identify significant differences among the studied cultivars (Table 5).

Table 5. The comparison of cultivars regarding the degree of attack based on the control system (organic, conventional and control) using the Duncan test

Degree of fungal attack		
Duncan ^{a,b}		
Grapevine-cultivar	N	Subset
		1
'Columna'	6	2.4850
'Mamaia'	6	3.4933
'Fetească neagră'	6	3.7800
'Pinot gris 13'	6	4.5967
Sig.		0.492
Means for groups in homogeneous subsets are displayed.		
Based on observed means.		
The error term is Mean Square (Error) = 22.826.		
a. Uses Harmonic Mean Sample Size = 6.000.		
b. Alpha = 0.05.		

The Duncan test results for the degree of *Botrytis cinerea* attack show differences among the analyzed grapevine cultivars. The 'Columna' cultivar records the lowest degree of attack (2.485), indicating higher resistance to this pathogen. 'Mamaia', with a mean attack degree of 3.4933, falls into the same subset as 'Columna', suggesting a relatively high and comparable resistance. On the other hand, 'Fetească neagră' (3.7800) and 'Pinot gris 13' (4.5967) have higher mean values, indicating greater susceptibility to fungal attack, with 'Pinot gris 13' being the most sensitive. Although the mean values suggest clear differences between cultivars regarding the resistance to *Botrytis cinerea*, the statistical significance of these differences ($p = 0.492$) indicates that they are not significant at the $\alpha = 0.05$ level. Nevertheless, the results suggest that the 'Columna' and 'Mamaia' cultivars are more resistant to fungal attack compared to 'Fetească neagră' and 'Pinot gris 13'.

CONCLUSIONS

In the two years of the study, the climatic evolution during the growing season was distinctly different. The year 2023 was very dry (110.2 mm) compared to 2024, when

precipitation accumulations reached 281.8 mm, with 124.4 mm recorded during the grape ripening period.

Considering the degree of attack by *Botrytis cinerea* (AD %) for the tested cultivars, all three experimental factors had significant effects in the series of experiments. According to the statistical calculations, the type of control (organic, conventional) significantly influenced the severity of the fungal attack, with an explanatory impact of nearly 47%.

The 'Columna' and 'Mamaia' cultivars had significantly lower average fungal attack degrees under all control systems, each year, suggesting a higher native resistance to gray rot (*Botrytis cinerea*) compared to the 'Fetească neagră' and 'Pinot gris 13' cultivars.

This native resistance of the 'Columna' and 'Mamaia' cultivars offers an advantage in the selection of future cultivars for managing this disease and may be better suited to organic cultivation systems than other cultivars.

REFERENCES

1. Alexandrescu I. C., Oşlobeanu M., Jianu L. and Piţuc P. (1994). Mică enciclopedie de viticultură., Editura "Glasul Bucovinei, Iaşi , 714 p.
2. Briar S., Grewal P. S., Somasekhar N., Stinner D. and Miller S. (2007). Soil nematode community, organic matter, microbial biomass and nitrogen dynamics in field plots transitioning from conventional to organic management. *Applied Soil Ecology*, 37.3: 256-266.
3. Compant S., and Mathieu F. (2016). (ed.). Biocontrol of major grapevine diseases: leading research. Cabi.
4. Fan F., Hamado M.S., Li N., Li G. Q. and Luo C.X. (2017). Multiple fungicide resistance in *Botrytis cinerea* from greenhouse strawberries in Hubei Province, China. *Plant disease*, 101.4: 601-606.
5. Li R., and Cheng Y. (2023). Recent advances in mechanisms underlying defense responses of horticultural crops to *Botrytis cinerea*. *Horticulturae*, 9.11: 1178.
6. Olteanu I., Cichi, D., Costea, D. C. and Maracineanu L. C. (2002). Special Viticulture. Ed. Universitaria, Craiova, 473 p.
7. Pertot I., Giovannini O., Benanchi M., Caffi T. and Rossi V., (2017). Combining biocontrol agents with different mechanisms of action in a strategy to control *Botrytis cinerea* on grapevine. *Crop Protection*, 97, 85-93.
8. Rupp S., Weber R. WS., Rieger D., Detzel P. and Hahn M. (2017). Spread of *Botrytis cinerea* strains with multiple fungicide resistance in German horticulture. *Frontiers in microbiology*, 7: 2075.
9. Caffi T., Rossi V. and Bugiani R.. (2010). Evaluation of a warning system for controlling primary infections of grapevine downy mildew. *Plant disease*, 94.6: 709-716.

Issues which pertain to the vine plants intrinsic features as well as to the progressive and further civilizing touch held by its culture as it has been currently practiced upon the actual territory of Romania

A. Popa¹ and F. Tuțulescu^{1*}

¹University of Craiova, Horticulture Faculty, Romania

*Corresponding author email: felicia.tutulescu@edu.ucv.ro

ABSTRACT

Archaeological research conducted over time has proven that on the territory of present-day Romania there has existed since ancient times a highly civilized human society that recorded slow but progressive accumulations generated by the natural environment of the territory endowed with a wide cultivar of forms that supported the free development of plants. The perceptible existence of wild but fruitful vines has been attested in two hundred and thirty localities. The attractive taste which he had at first occasionally discovered in the fruits mentioned above had certainly aroused the well-deserved attention of the primitive human being, and had therefore led him to deliberately take successive but strictly local actions to remove the other surrounding plants which might have become obstacles to the undisturbed development of the vine, applying to them at that time the so-called "paralysis procedure". The natural and extensive territorial progress of the vine has always been due to its intrinsic characteristics, namely: minimum requirements for environmental factors; easy rooting; vegetative propagation. This was therefore the main reason why vines were among the first plants taken into farms. Our ancestors dedicated special attention to vine cultivation and wine technology, as evidenced by the numerous vessels and utensils discovered throughout the country as well as the large number of localities that have names derived from vine culture.

Keywords: *archaeological research, Vitis sylvestris, clearings, history, vineyard*

The history of the vine in the Romanian

Archaeological investigations performed until the present day have proven with no possible doubt ever able to arise that the actual territory of Romania successively appeared during the so-denominated *Polished Stone Era* (that is to say from about 6500 b. J.-Chr. until around 2500 b. J.-Chr.) some among the most intensely flourishing, original and spiritually advanced types of human civilized societies which have ever been known to have existed throughout the whole territorial extent going from the Gibraltar Straits until the Ural Mountains. The respective achievements of efficiently attending to and of furthermore successfully sustaining a high civilizing development level had been rendered possible as being a direct consequence of some quite slow yet unavoidably progressive accumulations the starting point of which had effectively occurred even prior to the earliest thus pre-Antique so-denominated *Carved Stone Era*. These rather slow yet gradually progressive accumulations had been generated by the above evoked territory's natural environment which not only has been endowed with an intrinsic and quite prodigal asset of cultivar but which had also been supportive in regard to the free

development of the vegetative plants, to the more or less consequent from the existence of the living animals and – last but not least – to the step by step socially organized lives carried on by the primitive human beings for whom it had eventually become most useful as well as even pleasant to creatively assimilate the various influences he had encountered while making the immediate acquaintance of the other regions which did naturally surround one of his origins. In this context, a particular significance had been respectively held by the generally favoring local micro-climates as well as by the multitude of (and averagely rather large) extents detained by most of the by then existing *sylvan plots* throughout which *the climbing lianas* had by then been abundantly frequent - as by the way they also used to be at that time throughout the *Caucasus* region (*T. Antonescu, 1901*). While he had not yet chosen a sedentary settlement of his own the human being could have strolled on from here to there in his search for animals to hunt; from those he got various skins and furs which enabled him to protect himself while confronted to whatever naturally supervened misfortunes; he had around himself plenty of wood out of which he could however build up some temporary shelters; of course he had also disposed of fire through the action of which he could warm himself up and roast his captured venison meat; he could as well most easily spot honey and/or pick up various fruits among which especially grapes since at that earliest of times the wild yet fruit-bearing vine plants could have been most frequently encountered; as evidence able to support this statement let us therefore mention its presence upon the actual territory of our country under a still spontaneously generated status and this fact does nowadays occur even after that many flown millennia; more precisely speaking the perceptible existence of the wild but fruit-bearing vine has been attested in a number of not less than two hundred thirty Romanian localities – see Figure 1 below (*E. Pop, 1931*).



Figure 1. Ascertained presence spots of the vine species *Vitis sylvestris* (Gmel.) as it could be encountered in Romania (*apud E. Pop, 1931*)

Vitis sylvestris (Gmel.) could be quite recently encountered throughout the so-denominated Stârmina Woods which are situated in the actual Romanian department of Mehedinți (2018). The attractive taste he had at first occasionally discovered at the above mentioned fruits had of course arisen the well-deserved attention of the primitive human being and had therefore determined him to willfully take the respective actions to not just

support the natural development of but furthermore even to protect the respective trees, shrubs and climbing lianas which could offer him those good fruits; he thus had chosen to remove the surrounding other plants which could have become obstacles in respect to the formers untroubled development by at that time applying to them the so-denominated and should we so define it *pre-agricultural "crippling procedure"*¹. Through such successively taken yet strictly local actions some so-denominated "*runcuri*" [*clearings*] had come to appear as (still minimal) free plots into the by then virgin woods; however, the primitive human being had succeeded in the establishment of such tree-free small areas of his *first-ever gardens* (though even by then these plots had not yet been *cultivated* in the future and proper sense of this term).



Figure 2. Gorj Cleanups (2020)

Their chosen places had been in most cases determined by the (prior and naturally supervened) presences of some wild vine fruit-bearing logs or either of some trees bearing wild but tasty fruits. It must have been at first around those in this most ingenious way realized *anticipations of agriculture* that the primitive human being might have been - for the first time throughout human history - tempted to settle down (even if at that time he could have understood his own still rather foggy yet intensely luring intentions as being only temporary). It is therefore within such should define them as *innovative sites* that the first ever *pit houses* by then most appropriate to dwell into might have appeared (I.C. Teodorescu, 1964). From those above mentioned vine logs some larger amounts of grapes might certainly have come to be gathered and it must have been due to their excessive amounts themselves that some of them had (eventually because by then still unavoidably!) come to be crushed (while at the very first pre-historical time it had truly not mattered at all should this action have been fortuitous or either willfully exerted). From these crushed fruits a - delicious of course! - juice had dropped which was beyond whatever doubt

¹ The above mentioned "*crippling procedure*" had by then consisted in the extraction from the concerned tree of a circular roundabout bark ring. Through the manual execution of this operation their unavoidable withering could have been provoked. It could have been most easily performed through the use made of a simple stone which could have been found inside of river beds and previously carved in order to fulfill this purpose. The existence and efficiency of this most ingenious intervention operated by the human being during his earliest ever thus primitive lifetime upon the territory of the Romanian historical province of Moldavia have been scientifically demonstrated by Mr. Constantin Mătase – at that time Director of the Archaeology Museum which had been founded in the Romanian city of Piatra Neamtz.

worthy to be collected; or it had then been at this precise moment that under the amazed eyes of that time's coeval people a miraculous phenomenon had most unexpectedly occurred: after a by then still unevaluated time interval the still not sipped thus remaining liquid amounts of those fruits stored juice *had started to coldly boil!* (In spite of the fact that it does appear as a contradictory statement when simply understood under the strict terms made use of by logic the present figure of speech does hereby mean with no fire at all below its containing recipient which might have been consequently able to warm the involved juice up). In our opinion, it had been through this (understood at that time as a *quasi-magic modality*) that the primitive human being had come to be revealed the (from then on most dearly cherished!) *secret of wine*. Beyond whatever doubt it had precisely been due to this of course naturally occurred circumstance *but which at that pre-historical time had however been impossible to explain* that the earliest appeared among human beings have not just eventually but most intensely to become convinced that the newly discovered beverage should certainly have been taken into their entirely due consideration *as an entitled to be worshipped "gift"* which could have been most graciously imparted to them by the effectively active divine forces. Should the primitive human being have consumed it *he could therefore and unmistakably consequently be able to enter in immediate contact with them*. While being situated under this deliciously powerful impression most strongly caused by this newly revealed beverage he could thus only have been led towards the logical next effectively made decision which had been the one to take into the highest ever of his nourished considerations *the future multiplying of the vine* thereby endowing it with *the statute of a major concern* among his current preoccupations. The natural and quickly extensive territorial progress of the vine had always been due to its intrinsic features namely: - holding a rather small number of necessary requirements; - rooting quite easily; - being also able to rather quickly multiply itself through various vegetative modalities. This has consequently been the main reason why vine had figured among the first ever plants to be taken by human beings into serious consideration given its future *agricultural exploitation*. In regard to his own already foreseen, sedentary settlement one among the primitive human being's essentially favoring thought about motivations had as well been the vine's above-mentioned capacity of rapidly multiplying itself under the local and simply natural conditions provided by its environment. In virtue of its quite strong root system (which is usually able to most efficiently explore some considerable soil masses) as well as of its remarkably long life duration vine thus required human being a permanent attention while in the meantime through personal satisfactions it had offered him in exchange for it vine had gradually achieved to acquire the intrinsic *statute of a highly precious asset* which above all other considerations *could have been left as a legacy to the expected future generations*; yet in order to successfully operate such a transmission the primitive human being had necessarily at first to settle down by his own will and for a long time duration in the concerned vine plants close vicinity. This fact once accomplished later on he had through some rather little yet eventually decisive steps come to creatively assimilate the vine's initial example by further applying it to some other plants around him that is to say by at first simply picking up and then by gradually but this time by his own will sowing into the soil the respective spotted seeds of some most valuable cereals (A. Vasiliu, 1976). In a slightly ulterior parallel but similar indeed movement he had eventually concluded that for himself it could have been rather rewarding to apply in regard to the animals he could find around him the same – even for these earliest of times we could already so denominate it – *strategy* he had once chosen in respect to the vegetal *regnum* surrounding him; from that moment on he had therefore struggled in order to domesticate and further breed around himself the animals about which he had thought of as being the most useful in respect to his own goals. This had been the very former and the most ancient era in time – the one during which upon the

actual territory held by our country had appeared the first ever zones into which the transition towards an effectively productive economic regime had been initiated – in this respect see Figure 3. Such genuinely appeared territorial zones had each of them been respectively defined (even throughout that *prior-to-Antiquity era!*) by some quite promisingly various handicraft and thereby social profiles since there had been: - communities profiled on hunting; - zones where the chosen profile had been the one of plants cultivation in its already agricultural meaning; - zones mostly profiled on the locally encountered animals domestication process; - specialized settlements where *the first ever human handicrafts* had come to be practiced within some still minimal yet most efficient workshops where various tools were manufactured such as hammers, hoes, various types of spikes respectively suitable for arrows, spears and/or pikes (while all of these specialized spikes had by then been made of carved little stones and/or of some most adequately sharpened animal bone small fragments) or then even vases made out of burned-up clay, etc. As time got by those lucrative interests could only have extended their productive impacts and this had been the main reason why the number of those even since that most ancient human era so many diversely specialized territorial zones could only have increased.

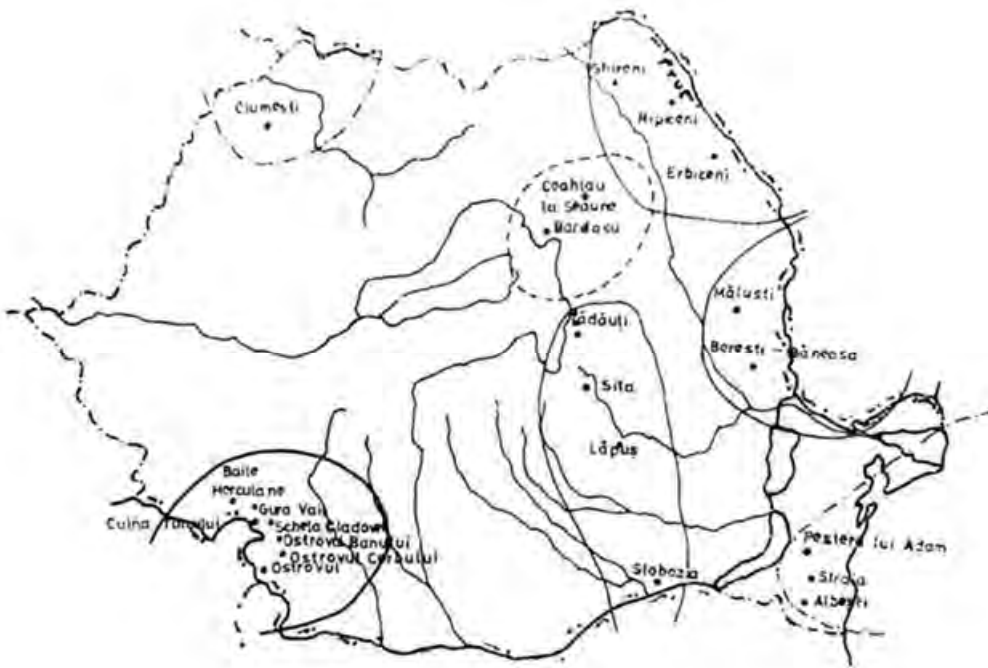


Figure 3. First ever transition zones towards the efficiently productive economy's regime as these had appeared within the space geographically situated in-between the Carpathian Mountains, the Danube Stream, and the Black Sea (later and first ever known as the *Pontus Euxinus*) before the IV-th millennium b.-J.-Chr. (*apud* the History Museum of Romania). *Legenda:* - Zone profiled on the locally encountered animals domestication process; - Zone of the human communities intensely profiled on hunting skills; - Zone vowed to the first ever founded sedentary handicraft workshops – Transition zone towards the plants cultivation process *already understood in its properly agricultural sense*.

A certain fact does as well exist that we ought to not at all ignore: precisely the one that the actual territory of Romania is the geographical space which does dispose of one of the *greatest reserves of salt* throughout Eastern Europe; or in our opinion the fact is beyond whatever doubt that the current use made of this essential naturally provided resource –

not just for its most commonly encountered part held into the human's daily alimentation process but above all other matters for its role in safely preserving the highly precious hunted meats – has as well brought one among the greatest of the gradually accumulated contributions which had eventually determined the primitive human being to progressively manifest *a gradual intentionality trend towards his own life's stability* in respect to his established and therefore inhabited settlements once these had come to be created (E. Linda, 1985). As a matter of fact to the primitive yet most cunningly intelligent human being it had not at all taken a long time to become aware of the fact that due to the intrinsic fragility status which is normally held by the vine grapes it could not have been possible to transport the grapes harvests on some averagely longer distances without having to expect an unavoidable as well as quite considerable amount of accidental losses usually supervening through the grapes deterioration processes no matter should these have been accidentally fortuitous or either due to some naturally degenerative respectively undergone evolutions. The wine's preparation process ought therefore to be necessarily performed in the closest possible vicinity of the vine plantation itself. From these immemorial times until the present day, the vine plants had come to eventually oblige human beings to settle down as nearby as they could around them. This decision had to be taken in virtue of the not just arithmetically rather numerous but furthermore considerably diverse through their specific purposes caretaking agricultural works which ought to have been compulsorily applied to the concerned vine plants. As he had succeeded in preparing his quantity of wine this earliest among the successive vine cultivators had also been able to step forward towards another essentially important direction: the one of *becoming a producer*; it had still been true that he could not yet willfully generate whatever oenological-related and/or involved *raw material* but he had however succeeded his accession to a superior stage of economic development insofar could have been concerned *the wine understood as a finite product* (which by the way had happened to be – at that time as well as nowadays – a most highly valuable one). Seen as merchandise wine had therefore come to assume a due place among the primitive human being's' held *goods to successfully trade with*; or so it had happened that this by then quite newly invented practice had afterward and most quickly become the easiest as well as most convenient modality through which he could have acquired by exchanging wine for them a lot of other objects of various natures that he had effectively needed in order to help himself in his search for some generally better life conditions which might have rendered him able to carry on throughout some safer and/or *even happier common living standards*. It had therefore been pretty easy for him to consequently deduce the fact that should the wine eventually become more empirically attractive through its respectively perceptible quality level this could only have been achieved by at first reaching for some *more intensely ripened as well as sweeter by their taste vine grapes*; this had therefore been the main reason why he had gradually become always more attentive in respect to the specific as well as prodigally various intrinsic assets owned by the vine kinds he had by then stumbled upon and which had been respectively reflected through their grapes respective tastes; the following step to be taken had logically been the one of distinctively marking in order to further multiply them the vine kinds which in his own eyes did seem to present the greatest number of empirically thus immediately perceptible qualities. The naturally resourceful opportunity he had become the fastest aware of had precisely been the spots where the chosen vine kinds were cultivated and by starting from this insight he had further remarked among them the plots that happened to be the most supportive regarding the new decision taken about the most promising foreseen multiplying procedures to be applied. As he had met the recently discovered beverage which later was going to be known as *wine* of which one of its first ever and also for that time rather spectacular exerted influences had been the one to therefore determine the primitive

human being to genuinely experience the need of coming closer towards some other fellow people he could have met around himself as due to the wine he had as well felt the inner impulse of *enjoying togetherness*; or the most appropriate occasion for achieving that brotherly goal could have precisely been the celebrations held in respect to the god which through its strength had manifested the benevolence of imparting to the earliest of the human people the gift of wine and to which they of course had wished to most warmly thank for it. The largest grounds throughout the world itself do support the hypothesis of taking into consideration as a fundamental starting point the first ever invitation to share a meal that had been advanced towards someone: "(...) the most emotionally significant gesture ever made throughout the human history has been the first ever invitation to share a meal addressed to the first ever encountered stranger of whom until that very moment an irrepressible fear had kept him separated. Without this first step taken towards the other one, nothing would have ever happened of his wonderful story" (I. Matheescu, 1984). While facing the many difficulties he had necessarily to encounter around himself the primitive human being had step by step come to increase his awareness capacity, to successfully maintain a lot more steady confidence ones; activity as he could imagine it given the future while as a whole, comprehensive and ultimate result of these gradual evolutions he had become more finely sensitive throughout his insights. The main proof attesting that the general progress of his intellect does consist in the very fact that he had become able to realize by handicraft not only some strictly useful objects as he had done before but above those formers he had become from a certain moment on able to further imagine by himself some *artistically intended representations*. Those were some not quite large recipients yet of various sizes made out of burned clay and at first had presented only incisions or either excision; later on, these recipients could present upon them some paintings respectively related to his own by then nourished religious beliefs or to a large indeed diversity of magical practices. These objects could have taken a lot of forms should these have been truly imitating nature or then if not could have been *zoo-morph* or *anthropo-morph* ones; of course the assumed attitudes which for example could indicate *hieratical solemnity*, *affectionate feelings*, *intense meditation*, *laughter* or even to *accentuate the presence of sexual elements* etc. As a matter of fact, through their own *finesse* and quality perfection some of these objects do seem to have acquired a *unique artistic statute* should we have surveyed in order to compare them with the entire territory of Europe: such are for example *The Altar* – a great artifact made out of burned clay discovered upon the territory of the locality Trușești in the department of Botoșani and scientifically about the so-denominated *Cucuteni Culture* (3000 – 2500 years b. J.-Chr.); *Gânditorul* [*The Cogitator*] – a statuette discovered upon the territory of the locality Tchernavodă in the department of Constantza and scientifically about the so-denominated *Hamangia Culture* (around 4000 years b. J.-Chr.). In a lot of other places situated in Romania have also been discovered exhibits sometimes bearing a symbolic significance such as *rhytons* – *small vases made use of for ritual purposes*, *mugs*, *jugs*, *cups*, *ornamentally destined jewels* or *toys*. All of them apart from their great number are as well exquisitely diverse insofar as could be concerned their respective forms and *finesse* degrees. These are situated in time from 4000 until 1000 years b. J.-Chr. (see Figures 4 to 15).



Figure 4. *Rhyton* – a cup made out of burned clay and used for ritual t of Doljpurposes ornamented through incisions and pricking discovered upon the territory of the locality Oltenitza in the department of Ilfov and scientifically of the so-denominated *Gumelnitza Culture* (4000-3000 years b. J.-Chr.). It is the so-called ancestor of the *Rhyton* made out of silver and on its outside covered with gold which had been later discovered upon the territory of the locality Poroina Mare in the department of Mehedinti and respectively evaluated as on the III-rd century b. J.-Chr.



Figure 5. Unique vase made out of burned clay scientifically of the so-denominated *Gumelnitza-Căscioarele Culture* (4000-3000 b. J.-Chr.). It had two mouthful apertures through which most probably two different persons had by then used to simultaneously drink after having fulfilled the so-denominated *brotherhood sacred ritual*.



Figure 6. Cup made out of burned clay endowed with polychromatic decorations discovered upon the territory of the locality Frumusica and scientifically of the so-denominated *Cucuteni Culture* (4000 b. J.-Chr.)



Figure 7. Vase made out of burned clay was discovered upon the territory of the locality Sălcutza in the department of Dolj (4000-3000 b. J.-Chr.).



Figure 8. Overlapping vessel of burnt clay with polychrome decorations. Cucuteni culture, jud. Neamt, b. J.-Chr



Figure 9. Vase made out of burned clay was discovered upon the territory of the locality Verbicioara in the department of Dolj and scientifically on the Bronze Age.



Figure 10. Little mug discovered upon the territory of the locality Cârna in the department of Dolj and scientifically of the so-denominated *Culture of Gârla Mare* (XIV-th century b. J.-Chr.).



Figure 11. Goblet-type vase made out of silver was discovered upon the territory of the locality Agighiol and scientifically of the Getho-Dacian civilization (IV-th century b. J.-Chr.).



Figure 12. Vase made out of burned clay discovered upon the territory of the department of Neamt locally denominated as *Calu* [*The Horse*] and scientifically of the so-denominated *Cucuteni Culture* (4000 b. J.-Chr.).



Figure 13. Supporting vase made out of burned clay endowed with polychromatic decorations combined with grooves discovered upon the territory of the locality Draguseni in the department of Botosani and scientifically of the so-denominated *Cucuteni Culture* (4000 b. J.-Chr.). It had most probably been within it that were by then placed in a close togetherness the ritually purposed cups of the above mentioned two persons which had used to simultaneously drink within the frame of the by then fulfilled *sacred brotherhood ritual*.



Figure 14. Hedging bills discovered upon the territory of the actual department of Gorj. Following the scientific opinion expressed by N. Al. Mironescu



Figure 15. Type of hedging bill which nowadays is still currently employed and which is most closely similar to the ones that in their times had been made use of by the Dacians.

The history of the vine in the world

Raymond Billiard has therefore cited *Perdrizet* who in 1910 had been the first ever scientist able to effectively assert the hypothesis in accordance to which the Antique Greeks had been during their lifetimes fully aware of the fact that their own later entirely

assumed *myth embodied through Dionyssos the God* had traveled towards them precisely from Thracia; however, they could in no way at all answer to the hereby following questions: at what exact moment in time? by whom's intermediating action had it arrived to them? From this point onwards let us thus invite *Homer* himself to utter: (...) From out there were daily coming ships loaded with wine for the Greek troops which were by then besieging the fortress of Troy; it had [also] been from out there that *Odysseus* had obtained from *Maron* - *Apollo's* priest from *Iamaria* - the exquisitely enchanting beverage through the means of which he had come to get stiff drunken *Polyphemus the Cyclope* (...) (*G. Hagi*, 1925). It had therefore perhaps been right here - on the Northern side of Christ - that was born the cult vowed to the *god of vine and wine*. In 1913 *Raymond Billiard* had by the way issued the respective statements that its propagation had been realized through the successive conquests of the so-called *ancient countries* and that these victories had occurred in the following order: at first *Asia, Thracia, Greece* and *Italy* while from all of them on it had spread further into the other countries. Yet in the eventual reality of the effectively occurring facts the current culture of the vine itself as well as the currently exerted practice of wine-making had followed a rather slow yet slightly evolving thus progressive path. Given this cult's adoption as a genuinely honored faith, it should have been in the first place necessary to get the people accustomed to a god who had come to them already endowed with his patrimony of duly established legends, rites and specific features; or all of these most recently encountered assets had to further undergo the respective as well as successive stages of *insightful awareness, willful assimilation* and then *further on transmission*. The above-mentioned author continues his story the following way: "(...) among all of Europe's [distinct] parts *Thracia* had been the earliest known by the human being; as for its wines it had been the most highly respected in their virtues and it had as well preserved this prestige for the longest of times." On their turn the *Antique Greeks* who at their best were some equally skillful navigators and traders had been the first European people who in their own living times had dared to follow the example of the *Phoenicians* and therefore had spent centuries long in the sought for the achievement of founding as many *harbor-profiled cities* as they could have upon the coast of the *Pontus Euxinus [Black Sea]* and along the bed of the *Danube Stream*. The Greek's current trading practice had by then been the one to offer some quite useful handicraft products created within their workshops as well as amphorae filled in with their sweet wine (for the creation of which they had at first made use of *Corinth-type grapes* then later of *concentrated grapes must*) so that in exchange they could obtain living animals, skins, furs, honey and above all other things *the locally produced wine* which had been brought towards them by the *Thracian* inhabitants in rudimentary leather bags which had been carried upon horseback or either within small boats or rafts which at that time had been very quick indeed to float upon the streams of the many rivers flowing towards the *Danube* and therefore towards the sea. The Greeks had brought upon their great ships some quite large amphorae made out of burned clay into which they consequently emptied the local leather bags; then the so purchased wine was either carried back into their respective city-shaped countries or if not then sold into other harbor cities encountered along (among which the *Scythes* happened to be the most intensely attracted wine lovers). Because of the essential fact that during those most ancient times *once produced the wine was and always remained entirely natural* it had therefore been taken into the highest consideration as a *sacred liquid matter* while being ranked together with *water, blood and milk*. These four liquid matters were thus thought of as worthy enough to be *offered to the local gods as sacrificial tokens*. Among them wine had been the only as well as *earliest entertaining beverage*; it could therefore help the primitive human being on the respective occasions represented by certain rather outstanding moments in expressing *solemn attitudes, gestures, or feelings* should these have respectively meant

adoration, oblation, homage, addressed best wishes, taken oaths, elevated prayers, penitential taken vows, expressed friendships or either pious reminders. Such morally special formulations had been respectively dedicated to the dearest ones, to the momentarily celebrated ones, to the most reverentially evoked late people, to conquering warriors, to sportive contests winners, and last to be hereby mentioned but not at all the least of course and above all to the local gods.

Documents and archaeological objects attesting to the culture vine in Romania

Throughout our country (in many actual vine cultivated regions and centers) a lot of archaeologically discovered exhibit items do stand as supportive evidence therefore sustaining the effective existence upon the actual territory of Romania of currently carried on vine cultivating and wine-making activities during the Primitive and Antique Eras. Let us mention among these objects: - grape-crushing presses made by then out of most attentively chosen stones; - vases made out of burned clay respectively designed to assume various sizes and forms. These vases might also have happened to be ornamented with *drawings* (no matter should these have been performed through painting or either by executed incisions) or *prominent outside-shaped relief forms* which comfortably often represented *vine leaves* or *grape clusters*. Later on (that is to say respectively about the *Bronze* and *Iron Ages*) had as well been found: - some *hedging bills under their genuine Dacian-type employed form*; - *amphorae made out of burned clay and bearing Dacian seals thus consequently by them manufactured*; - *vine seeds as early created as these eras*; - decorative elements as well as some simply nominal mentions carved upon the most ancient ever among the archaeologically retrieved tombstones; - even *coffins* upon the outsides of which various ornamental elements do remind to the people able to see them of the existence of vine and/or wine. As for the *possible written proofs* supportive of the above-mentioned hypothesis there has been at least until the present day only one attested to exist. It is the treatise *Geographia* elaborated by the eminent Antique historian, geographer, and ethnographer *Strabon from Amaseia* (63 b. J.-Chr. – 19 A.D.) through which he does brings some very valuable precisions: the Dacian King Burebista (who around the year 70 b. J.-Chr. had successfully achieved the constitution of the first-ever simultaneously unitary and independent Getho-Dacian state) while being advised by *Daecaenaeus* – the highest moral authority of the country as he had held the religious dignity of a *Great Priest* and at that time was *de facto* the King's wisest counselor – had issued the order to wipe out all the vineyards which had by then existed throughout his country. The fact is therefore proven that here the culture of vine not only had existed but furthermore had become considerably important – and this had been true for a long time before 106 A.D. (the exact year when the Romans had perfected the military conquest of Dacia). As a matter of fact, on this occasion, a *jubilee medal* had been issued in order to honor *Trajan the valiant Emperor* which had been denominated in no other words but *Dacia Felix*.



Figure 16. Copy coin of the *Dacia Felix* Roman jubilee medal. Its original coin is to be found within the frame of the *British Museum* in London.

On one of the sides of this medal (while the other is of course dedicated to the Emperor) is the representation of a woman sitting upon a rock while two children are respectively offering to her as devotion tokens *a wheat ear* and a *grape cluster* - both understood as the main most precious assets which at that time were produced by the newly conquered country (I.C. Teodorescu, 1964).

Table 1. Vine sorts were traditionally cultivated in Romania until the appearance of the phylloxera and their respective denseness indices by then currently held for a hectare. Cutting systems and guidance patterns made use of (apud I. C. Teodorescu, 1939).

Vineyard	Vine Sort	Plants' denseness (logs/hectare)	Cutting systems and guidance forms
Odobesti	Galbenă 2/3 Plăvaie+Poamă Verde+Poamă Pârce	3500-4500	
Orevitza and Golul Drâncei	Corb (Negru Vârtos) 1/3 Seină Neagră (Negru Moale) 1/3 Berbecel (Aurel) 1/3	around 7000	
Dragasani - around 7000	Cârlogancă (Crâmpoșie) 1/3 Braghină 1/3 Gordan+Tămâioasă Românească 1/3		
Cotnari	Grasă 1/3 Fetească Albă 1/3 Frâncușă 1/3 Tămâioasă Albă (Busuioacă) from Moldavia 1/6		
Dealul Mare	Gordan 1/3 Bășicată 1/3 Tigvoasă 1/6 Mingled Tămâioasă+Coarnă Albă+Verdea Negru Vârtos 1/3 Negru Moale 1/3 Coarnă Neagră 1/3 Tămâioasă Neagră 1/6	4000-6000	
Banat (Minis)	Lime Tree Leaf Cadârcă Bacator (Ruița) Abutz Fetească Albă Furmint Mustoasă Păsărească (Aprofehér)	9000	

Vineyard	Vine Sort	Plants' denseness (logs/hectare)	Cutting systems and guidance forms
	Fetească Albă (Leanca) - the local wines'king throughout the region Italian Riesling Furmint mingled up with Grasă (sometimes) Traminer Pinot Gris Iordană Opporto Muscat Ottonel Sylvaner Cabernet Franc Vine kinds creating table grapes: Hamburg Muscat Chasselas (white and red) Millennium Passatutti Muscat etc.	7000 - 10000	

It does indeed seem that our ancestors had therefore dedicated some amazing attention and inventive capacities in order to most efficiently manage the vine's cultivation process; or the effective presence of the same rather unexpectedly high degree of insights' *finesse* has as well been demonstrated through the *subtleness level* that they had also proven in the correctness of the interpretations they had respectively given to the influences exerted upon the vine plants by the various elements of their local environment; or in virtue of those still empirical evaluations they had most accurately deduced the right technical orientation to be chosen given removing whatever inconvenient these naturally occurred elements might have caused or on the contrary of making the best use they could have of the possible advantages these might have offered to the vine. We could be able to duly notice the efficiency of their above mentioned intuitive skills should we take an attentive look to respectively: - the great number of autochthonous kinds all endowed with some as valuable as diverse intrinsic assets the creations of which they have come to realize by applying the selection procedure to the naturally occurred vine's mutations which they eventually had come to take into consideration as being of an immediate use; - the both original and prodigally diverse technical modalities of cultivation insofar the plants' denseness could have been concerned for the approached individual case of each plot; - the vine strings respective cutting and orientation systems which had as well been diverse in order to most adequately suit the distinctive peculiarities held by each of their local and hosting micro-climates; - the associations of vine kinds into *sorts* which had been elaborated aiming to perform such a judiciously balanced and exquisitely delicate touch of oenological *savoir-faire* that they could effectively accomplish not just the day-by-day goals related to economical profit and/or to technological efficiency but in certain cases even the compensation of *some floral deficiencies they had remarked*; until the disaster caused by the phylloxera all of those *virtuose improvements* had been of course most carefully preserved. Above all other considerations, we do also have *geographically positional evidence* able to attest to our ancestors' empirically developed skills which consist in *the various vineyard'situations themselves throughout our country* all of which are so efficiently premeditated. These could be divided in two main

taken options: - their situation within the so-denominated *hillock-shaped zone* or – their situation *closely nearby to the Black Sea*. Apart from its immensely valuable contribution which is so happily reflected through the resulting wines' respective chemical compositions and oenological quality upheld levels this settlement does as well represents *a most favoring amidst-laid position*. As far as effectiveness could ever have been involved the above-mentioned choice does bring together the advantages that the human being could therefore have taken from the mountain and/or sub-montane zones (that is to say grazing fields, wood, venison etc.) with the ones he could have obtained from the *cereals-profiled plain zones*. In the meantime human beings had also managed to avoid an excessive distance with for the greatly important *fairs* where most of the trading exchanges used at that time to be accomplished and all of them were of course related to the Danube Stream and the Black Sea. On the other (historical) side we do as well find ourselves entitled to state that these above evoked hillock-shaped and sub-mountainous counties had for centuries long (that is to say for as long as the migratory people had by those times taken their respective times to cross over throughout our country) offered to the local genuine inhabitants the best refuge shelter they could ever have had because these zones were hard indeed to get at by their successively invading enemies. It had been therefore within them that the Romanian nation had always found its most ancient as well as strongest resilience hearths to rely upon. Of course, our vine cultivated regions had been constituted with for a lot of other concomitantly existing elements. First of all, we ought to mention the intense feeling of love that common people had always nourished for the vine plants. We might find it quite hard to choose should we be asked: what other vegetative plant could ever have "tied up to itself" the human being as strongly and deeply as the vine has succeeded in doing so? As a vine plantation could be inherited for several generations – from father to son – both its perennial endurance and the most respectably important charge of work to be performed in its regard that ought to be successfully carried out throughout almost the whole year had eventually determined the "by now" at that time already more or less primitive human being to settle down in its closest neighborhood. These essential circumstances might have perhaps counted for him almost as heavily as could have weighed downside the usefulness and the pleasure provided to him by the consequently generated fruits it had brought to him which were grapes and their result the wine. As a matter of fact, in Romania even in our days the number of localities the respective official denominations of which are etymologically speaking related by their origins to the vine cultivation and wine-making disciplines is quite impressively high. Yet insofar the Romanian people could ever have been concerned we are due to state that in our opinion there also has existed a distinctively particular element able to intervene in its case and which has directly contributed to the deepening of the Romanians' affectionate feelings respectively nourished in regard to the vine plant. It has been the vine plant's own naturally adopted behavior (that is to say as it had to face some that strong forces so it could in no way at all confront them immediately nor straightly). This has been therefore the main reason why the vine as a plant could be able to stand the mutilation and then even burial procedures applied upon it while remaining still alive so that finally once the spring had come it could triumphantly rise again into the sunlight. Vine has always thus been in the eyes of our ancestors a living example very close to them as well as good to look at in order to consequently become inspired by its excellently proven inner strength so that in their turn they could willfully adopt it. During the centuries of their own endured historical suffering, our ancestors had had therefore right in front of them the rightful example which could for sure enable them to assume all of their misfortunes due to a perpetually nourished personal confidence which had undefectibly concerned the arrival of their longtime sought for and final victory. We, Romanians have always been deeply indebted to the vine plants for having offered to us right at hand the most supportive example we have ever needed for ourselves, and the proofs of its recognition as a best of

friends when in need are therefore constituted by the multiple representations of the vine plants – should these be retrieved throughout many among our most ancient religious books, in the paintings which do exist upon the walls of our Orthodox churches as well as in the exquisitely extensive cultivar represented by the various Romanian Orthodox icons.



Figure 17. Detail painting Tismana monastery, Gorj (2018)



Figure 18. Painting in pronaos, Horezu Monastery (2012)



Figure 19. Gate detail in Arges (Golești Wine Museum)



Figure 20. Dragoș Vodă Church, Putna



Figure 21. Medal of the Contest for Agriculture and Industry (Bucharest, 1881)

REFERENCES

1. Antonescu T. (1901). Dacia, patria primitivă a arienilor. Volumul Lumii unite, Iași
2. Billiard R. (1913). La vigne dans l'antiquité, Lion
3. Hagi G. (1925). Ilustrate Flora Mithel-Europa, LV, Munchen
4. Linda E. (1985). (Universitatea Harvard, Cambridge, Massachuseths) Cultura Cucuteni unică în Europa (II). Magazin istoric, nr.5 (218). Serie Nouă, nr.6, vol XXV, București
5. Matheescu I. (1984). Pe frunza de viță de vie. Ed. Litera. București
6. Pop E. (1931). Vitis silvestris Gmel în Romania. Bul. Grăd. Bot. Cluj. LXI, nr.3-4
7. Popa A., Giugea N., Genoiu T. C. (2015). Oltenia. Mica Românie viticolă. Ed. Aius, Craiova
8. Perdrizet I. (1910). Cultes et mythes du Pangee. Annual de l'Eest Paris-Nancy
9. Teodorescu C.I. (1939). Tratat de Viticultură. Manuscris
10. Teodorescu C.I. (1964). Pe urmele unor vechi podgorii ale geto-dacilor. Ed. Agrosilvică, București
11. Teodorescu C. Șt., Popa A., Sandu Gh. (2021). Oenoclimatul României, ed. a II-a. Ed. Aius, Craiova
12. Vasiliu A. (1976). Istoria științelor agricole în Romania. Ed. Ceres, București

Climate change a necessity in the process of improvement and cultivation of the grapevine

E. Alexandrov

The Institute of Genetics, Physiology and Plant Protection of the USM, Republic of Moldova

*Corresponding author e-mail: alexandrov.eugeniu@gmail.com

ABSTRACT

Climate change manifests itself not only in high temperatures, but also a distribution of rainfall during a vegetative year, extreme weather events (waves of heat, heavy rains, hail, frost, and strong winds) and prolonged drought, which must be taken into account in the complex, in the process of plant growth and development. Climate change solutions can be not only profitable, but can affect the level and quality of life of the population while protecting the environment. To improve the current situation, it is necessary to take actions that contribute to reducing emissions, a adapting to the consequences of climate change, and financing the necessary adaptation measures. Evaluating the climatic conditions of the Republic of Moldova, we find that in the past the climate was of a temperate-continental type, as a result of the evaluation of these conditions over the years, a trend of change was found, towards the creation of arid conditions. Climate change requires the creation of genotypes with increased adaptability and the revision of agricultural plant cultivation technologies. Climate change requires the creation of genotypes with increased adaptability and the revision of agricultural plant cultivation technologies

Keywords: climatic changes, creation, genotype, grapevine, technology, valorisation

INTRODUCTION

Climate change requires revising the strategy for creating plant genotypes, as well as the methods and techniques for cultivating crops, including grapes. The costs of producing planting material and planting, purchasing and using chemicals needed to combat diseases and pests, agricultural work, etc. ultimately affect the productivity and quality of the resulting derivatives, as well as the selling prices of these products. That is, it is necessary to create and select a genotype and technology for cultivating grapes with minimal use and/or exclusion of protective chemicals that contribute to the preservation of biodiversity and the conduct of biological viticulture. The adaptive potential of plants to the environment is a response to climatic factors, simultaneously demonstrating a certain quantitative and qualitative capacity for productivity and derivatives (Gaina B., *et al.* 2023; Nan, R., *et al.* 2021). The purpose of this work is to identify the productive characteristics of grape genotypes in the context of changing climatic factors. Creation and implementation of grape genotypes in relation to changes in climatic factors, thereby contributing to the development of biological viticulture.

MATERIALS AND METHODS

The experiment was organized within the Institute of Genetics, Physiology and Plant Protection of the USM, Republic of Moldova of the USM. The main climatic parameters were evaluated during the period 2002-2023.

The parameters of climatic factors of the last 22 years (2002-2023), such as temperature (average, minimum and maximum), relative air humidity, amount of precipitation (Alexandrov E., 2023a; Nan, R., *et al.* 2021), sunlight, air temperature at the soil surface, average wind speed and soil parameters, annual average in the context of geographical areas using the weather station, were analyzed. PTM-4A. Located at the place of the experiment.

Five varieties (Ametist, Alexandrina, Augustina, Malena, Nistreana) of grapevines served as the study object. Planting scheme 3 meters between rows and 1.5 meters between plants in a row. Sawdust from the vegetable mass of *Miscanthus* sp served as mulch. Electro conductivity, as a method for determining soil fertility, was measured in our experiment both in the version in which mulch was placed and in the one without mulch. The thickness of the mulch layer was about 10 cm, the width of the strip about 50 cm and the length in a row 15-20 meters. In order to measure the parameters, sensors were fixed in the soil, which determine, in the 10-15 cm layer: the temperature in the soil, the temperature at the surface of the soil, the humidity of the soil, the electro conductivity of the soil, as well as the amount of sunlight that falls on the surface of the soil.

The use of soil sensors allowed the determination of air temperature at the soil surface, electrical conductivity, soil temperature and humidity in the 10-15 cm layer. The respective sensors were installed both in the vineyard experiment in which mulch was placed and in the one without mulch.

RESULTS AND DISCUSSIONS

Taking climate changes into account, the promotion of the green economy policy is required by motivating, stimulating through various aspects (economic-financial, technologies, products, etc.) At the same time, plant cultivation technologies have a minimal carbon footprint in the atmosphere. The grapevine varieties created to date meet certain criteria and are valued. However, as a result of cultivation, it is necessary to constantly demonstrate that they meet a number of criteria. Unfortunately, an “ideal” grapevine cultivar adapted to climate change has not yet been created, which cultivated in biological conditions has high quantitative and qualitative productivity, as well as primary and secondary derivative products, which are ultimately very appreciated by consumers.

The increase in consumer demand for products of biological origin creates the preconditions for the further development and expansion of the market for the corresponding products and ultimately contributes to the reducing of the negative impact on the environment, the conservation and use of natural resources, and the increase in the income of the economic agents involved in the production process, etc. The yield of agricultural crops depends on climatic conditions, genotype, technology of growing, storage and processing of culture. As a result of climatic from one year to the next, the cultivation of many perennial species becomes difficult, with negative effects on the growth and development processes, and over time, some of these will disappear.

The Republic of Moldova is located in the temperate-continental climate zone. In the past, the climate was characterized by mild and snowy winters, and the warm summer with more

or less uniform atmospheric precipitation over time (Figure 1. - 4.). The average annual air temperature in the north 8.0 °C and 10.0 °C, and the soil between 10 °C and 12 °C (2002 - 2023). Approximately 10% of the total annual precipitation falls in the form of snow (Mihailescu C. 2004; Atlas. 2019; Ediție enciclopedică, 2009). Based on the temperature indices, there is an increase in the average annual temperature on the territory of the republic by 1.5 °C (Figure 3 and 4.) and an uneven annual amount of precipitation (Figure 1 and 2.). Regarding the amount of atmospheric precipitation during a vegetative year, we note that for a long period of time there is no precipitation, but in a very short time an amount can fall, which far exceeds the norm for that period (Figure 1. and Figure 2.).

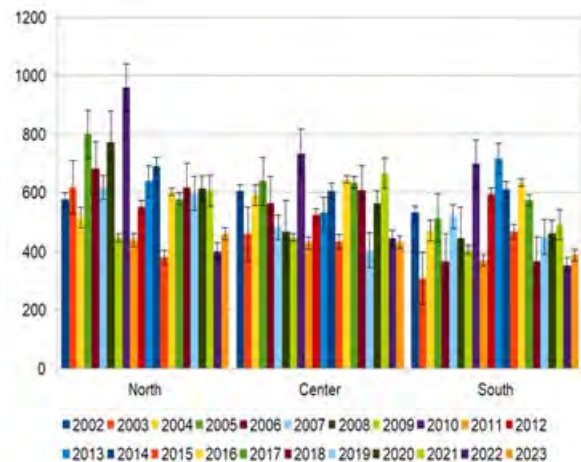


Figure 1. The annual amount of precipitation (mm) in the Northern, Central and Southern regions of the Republic of Moldova (2002 – 2023)

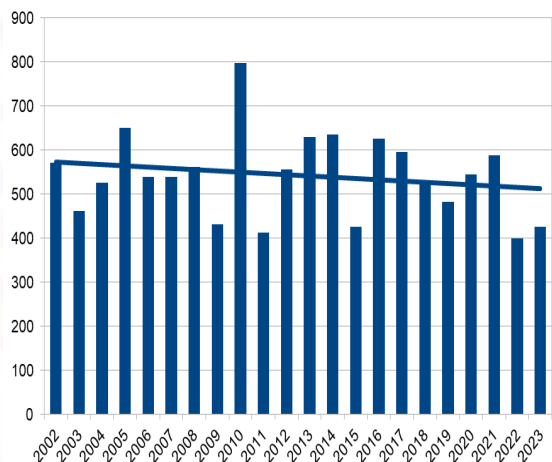


Figure 2. The trend of the annual amount of precipitation (mm). Republic of Moldova (2002 - 2023)

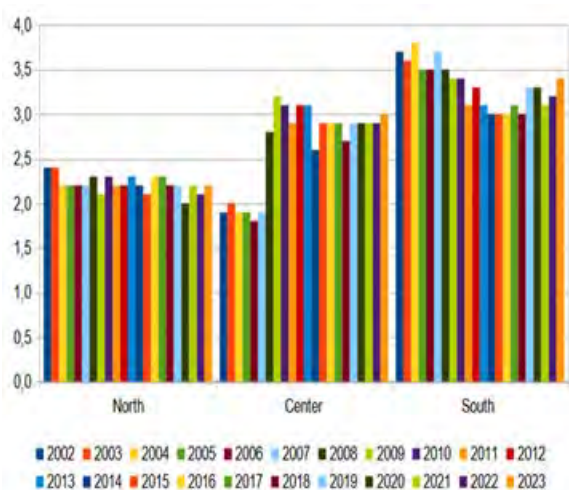


Figure 3. Average annual temperature (°C) in the Northern, Central and Southern areas of the Republic of Moldova (2002 - 2023)

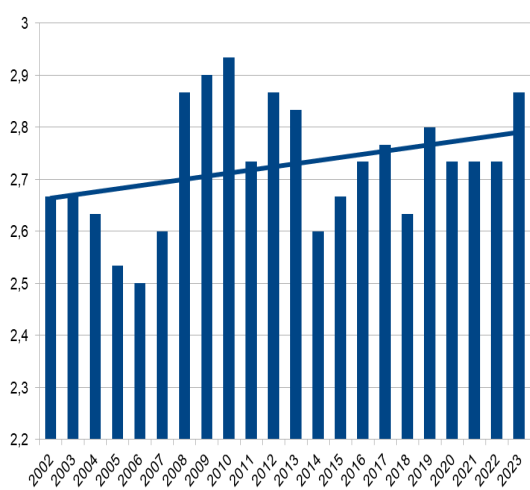


Figure 4. Trend of annual mean temperature (°C). Republic of Moldova (2002-2023)

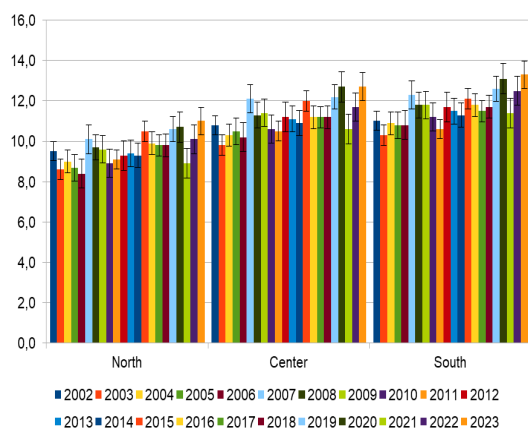


Figure 5. Average annual wind speed (m/s) in the Northern, Central and Southern areas of the Republic of Moldova (2002-2023)

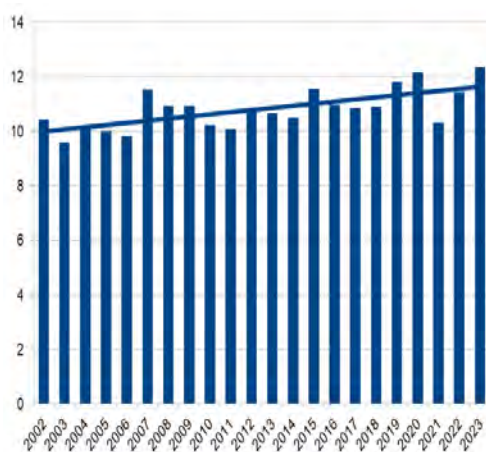


Figure 6. The trend of the average wind speed (m/s). Republic of Moldova (2002-2023)

As a result of the fact that atmospheric precipitation is uneven, it is necessary to take measures to reduce the process of water evaporation from the soil in the technological process of plant growth. Wind significantly influences the process of climate formation and causes a number of geological processes. Finally, the winds can trigger the desertification process. The wind regime is characterized by the predominance of the north-west wind direction on most of the country's territory, and in the southern area – the north direction. The direction and speed of the wind largely depends on the relief, the degree of coverage of the area with forest vegetation. The average annual wind speed varies on the territory of Moldova from 2 to 4 m/s (Figure 5 and 6.) during the analyzed period (2002-2023).

The trend of the factors influencing climate change is highly depend-on the land area covered with forest vegetation. The Republic of Moldova has approximately 450.6 thousand ha of land with forest vegetation (13.6% of the country's territory), including in agroclimatic zones: - 57.7% in the Central area; - 26.6% in the North area and 15.7% in the South area (Hazardurile naturale. 2008; HGRM nr. 864 / 09.12.2020).

During the period May - August 2022 atmospheric precipitation was insignificant. In January there were 14.6 mm of precipitation, February and March 5.0 mm and 6.0 mm respectively, April – 40.8 mm, May and June each 4.4 mm and 3.0 mm, July – 26.2 mm and in August 87.0 mm (Figure 11.).

The soil structure of lands with insufficient water, as a rule, differs essentially compared to the soil structure of lands with abundant water.

The water holding capacity is easily determined due to the electrical conductivity of the soil. The average level of electrical conductivity indicates that the soil has an average structure and, consequently, has an average drainage capacity. Such soils are the most fertile. Because water holding capacity has a major impact on cereal crop productivity.

Electro conductivity is a method of determining soil fertility (granulometric and mineralogical, pH, humidity, absorption capacity, etc.), which allows to determine the need and the necessary quantity of introducing fertilizers into the soil.

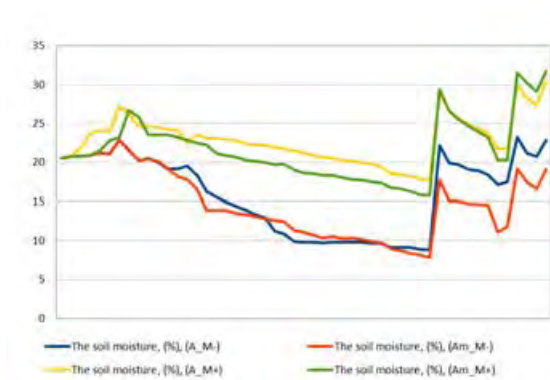


Figure 7. Soil moisture (%), layer 0-15 cm

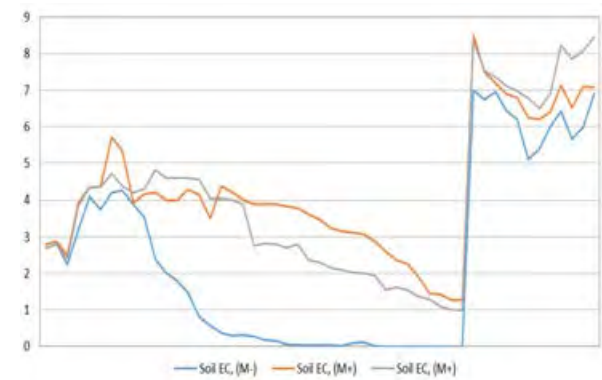


Figure 8. Electroconductivity of the soil, the 0-15 cm layer

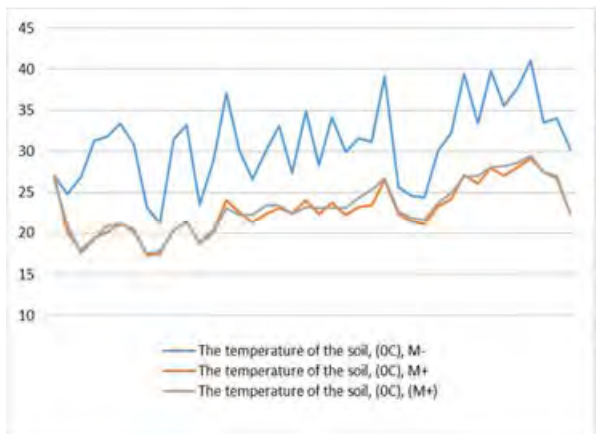


Figure 9. The temperature (°C) of the soil in the 0-15 cm layer.

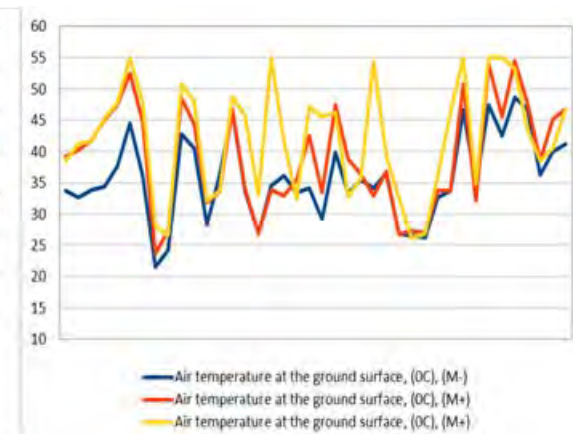


Figure 10. Air temperature (°C) at the surface of the ground

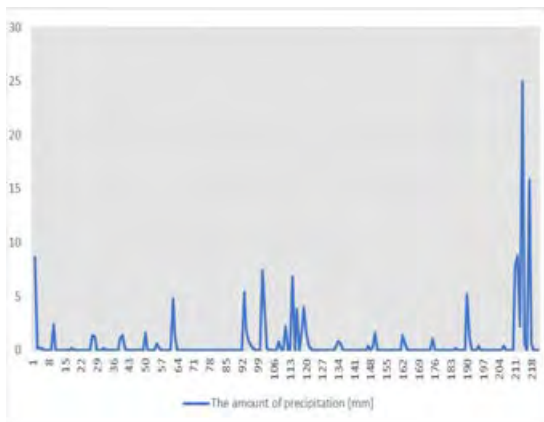


Figure 11. The amount of atmospheric precipitation (mm)

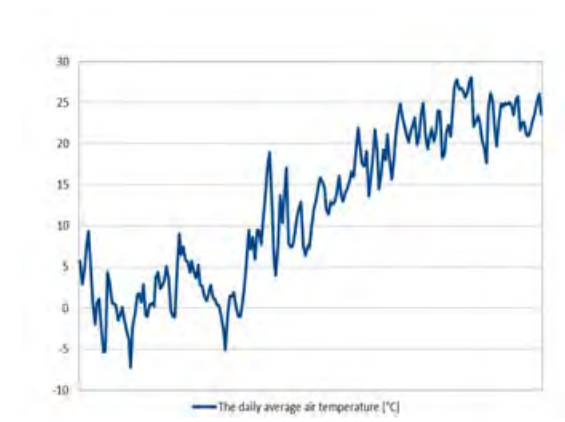


Figure 12. The average air temperature (°C)

Using electro conductivity techniques allows not only determining the potential of soil fertility, but also ensuring the development of sustainable agriculture, determining the ability to provide plants with mineral substances necessary for the development and maintenance of vital processes, determining the level of soil pollution with toxic substances, assessing the cultivation capacity of certain crops on different types of soil, etc. As a result of the mounted experiences, it was found that the electro conductivity of the soil in the case with mulch demonstrates much higher indices than in the case without mulch (Fig. 8).

Analyzing the soil temperature in the layer 0-15 cm from the surface, it was found that in the case where mulch is placed, it is on average 15-18° Celsius lower than in the case without mulch (Figure 9. and 10.)

Thus, by improving soil conditions, mulch has a positive effect on plant development and productivity. So, if the soil was covered with mulch, the annual shoot growth was 12-20% higher and the plant productivity was 25.5-29.5% compared to the control (Figure 7 and 8.). Also, the content of sugars, in the case with mulch, is 2-3% higher compared to the case without mulch.

The financial resources needed to carry out agrotechnical works, related to soil processing, are reduced by approx. 25-35%.

During the experience, it was found that in the case of covering a respective layer with mulch, the growth of grasses is stopped one by one and the process of soil erosion is prevented. According to estimates, it was found that about 40% of agricultural lands are degraded and yield crops lower than their productive capacity.

The continuous degradation of agricultural land drastically reduces the possibilities of obtaining adequate harvests (Atlas, 2021; HGRM nr. 864 / 09.12.2020].

The grapevine cultivars used in the study are rhizogenic interspecific genotypes, which have increased resistance to climatic factors. The climatic conditions of 2023 influenced plant productivity, but still demonstrated successful productivity (Table 1).

From the research results, both the production of grapes and their weight are influenced by the climatic conditions in the year of study. Productions decreased in 2021 by up to 2 t/ha compared to those recorded in 2019, respectively 1.5 t/ha in 2023 for the Amtist cultivar. The same differences were evident in all other cultivars, the climatic year being decisive for the recorded productions. It can be highlighted (table 1) that the lack of precipitation in the vegetative year 2023 and the high temperatures in the summer periods led to a decrease in production, by 1.5 to 2 t/ha, compared to the years favorable to the growth and development of the vine, as well as the analyzed year 2019.

From table 1, it can be seen that, although the number of fruits was not influenced by climate conditions, being more a biological characteristic of the cultivar, the weight of the grapes was higher in 2019 and 2020, years that also recorded the highest production, for all five analyzed cultivars.

Table 1. The influence of climatic factors on the productivity indicators of the analyzed cultivars

Year/cultivar	Production t/ha	grape weight (g)	number of grapes/plant
Amtist			
2019	14	350	27
2020	13	350	25
2021	12	320	26
2022	13.5	330	26
2023	13.5	330	26

Year/cultivar	Production t/ha	grape weight (g)	number of grapes/plant
Alexandrina			
2019	13	450	24
2020	13.5	450	24
2021	11	400	22
2022	12.5	420	23
2023	12	400	23
Augustina			
2019	14	550	24
2020	13.5	560	23
2021	12.5	530	22
2022	13	540	23
2023	12	520	22
Malena			
2019	12	400	22
2020	11	350	23
2021	11.5	350	21
2022	10	320	20
2023	10	320	21
Nistreana			
2019	13	450	21
2020	12.5	350	22
2021	12.5	360	22
2022	12	320	21
2023	11	300	20

CONCLUSIONS

Use of the mulch layer: maintains soil moisture; stops the growth of grassy plants; contributes to the restoration of the fertile layer; - reduces soil temperature; - prevents soil erosion; reduces the financial and human resources for maintaining the plantations by about 25%; plant productivity increases by 25-29%.

Analyzing the amount of precipitation that has fallen, we find that it represents a sufficient amount annually, but dividing this amount over certain periods of time we notice that it is uneven. During several months there is no precipitation, but in a short period of time all this amount can fall. From the research results, the climatic year is decisive for the vine culture for the productions obtained. The lowest productions were evident in the years when the precipitation was reduced and not evenly distributed during the vegetation period and the high temperatures during the summer that led to prolonged drought.

ACKNOWLEDGEMENTS

The research was carried out under the sub-programme 011102. *Increasing and conservation genetic diversity, agricultural crop breeding in the context of climate change.*

REFERENCES

1. Alexandrov E. (2023a). Genotipurile de viță-de-vie în contextul schimbării factorilor climatici. 131 p.
2. Alexandrov E. (2023b). The valorization of viticultural resources taking into account the need to adapt to climate change. In: Romanian journal of horticulture, vol. 4. p. 119-124.

3. Gaina B., and Alexandrov E. (2023). Determination of the spectrum of volatile chemical compounds in the berry juice of interspecific rhizogenic grapevine genotypes. In: Romanian journal of horticulture, vol. 4. p. 141-146.
4. Atlas. (2019). Factorii naturali și antropici de risc. Chișinău, 104 p.
5. Atlas. (2021). Schimbările climatice și starea actuală a peisajelor. Chișinău, 100 p.
6. Hazardurile naturale (2008). Mediul geografic al republicii Moldova. Vol. 3. Chișinău, 160 p.
7. HGRM nr. 864/09.12.2020. Programul de îmbunătățiri funciare în scopul asigurării managementului durabil al resurselor de sol pentru anii 2021-2025 și a Planului de acțiuni pentru anii 2021-2023 privind implementarea acestuia.
8. Mihailescu C. (2004). Clima și hazardurile Moldovei. Evoluția, starea, predicția. Chișinău. 192 p.
9. Nan R., Dobrei A., Alexandrov E., Nistor E., Nan Al., and Dobrei A. (2021) The influence of different soil management and fertilization methods on the grapevine production costs and profitability. In: Journal of Horticulture, Forestry and Biotechnology, Vol. 25(3): 1-7.
10. Republica Moldova. Ediție enciclopedică. Chișinău: Enciclopedia Moldovei, 2009. 734 p.
11. www.meteo.md
12. www.clima.md.

The effect of nitrogen on the growth and development of grafted cuttings from the nursery

D. Dinu¹, D.I. Sumedrea^{1*}, A. Florea¹ and C. Ciobotea¹

¹National Research and Development Institute for Biotechnology in Horticulture,
Stefanesti- Arges, Romania

*Corresponding author: dsumedrea@yahoo.com

ABSTRACT

The complex of agrotechnical measures that ensure a high yield of standard planting material and a favorable impact on the growth and development of woody plants is represented by both the grafting technique and the nutritional elements applied in the nursery. This paper studies the effect of nitrogen on the growth and development of grafted cuttings of four table grapes cultivars 'Argessis', 'Norocel', 'Memory', and 'Ștefănești', grafted on SO4-4 rootstock, planted in the nursery located on a specific soil of Stefanesti Arges viticultural center. After forcing, the grafted cuttings were paraffined with silver paraffin planted in a protected (solar) space, and subjected to 2 fertilization treatments with increased doses of nitrogen: 100 of the Optimal Dose and 75% of the D.O. Increased amounts of nitrogen positively influenced vegetative growth but inhibited root development, regardless of the cultivar. The length of the main shoots oscillated between 43.36 cm when applying 100% DO fertilization to the 'Argessis' cultivar and 17.88 cm for 75% D.O. fertilization, to the 'Memory' cultivar. On the other hand, the total 100% D.O. fertilization led to a decrease in the diameter of the roots, for the first 3 main roots measured.

Keywords: nursery, vine, grafting, rootstock SO4-4, local cultivars

INTRODUCTION

Fertilizers are used to supplement certain soil nutrients when native nutrient levels and soil processes are not sufficient to supply those nutrients for optimum plant growth. N, P, and K are the three major elements used in plant growth. Their utilization is described below. Nitrogen (N) – Of all the essential elements, nitrogen is often the one most limiting for optimum plant growth. Adequate nitrogen produces vigorous growth and a green color. Most of the nitrogen in soils is present as part of the organic matter and becomes available for use by plants as organic matter is decomposed by soil microorganisms. The amount of nitrogen provided by these natural soil processes is generally not enough to maintain the vigorous growth of most plants, therefore, supplemental nitrogen from fertilizer is usually required. One of the main conditions for obtaining high-quality planting material, along with ensuring moisture, is a sufficient amount of nutrients in the soil. Verzilin *et al.* (2020), recommend for soils with a low supply of nutrients the application of 75-95 t/ha of organic fertilizers which must be applied at the same time with plowing in the plantation, 750-950 kg of potassium and 650-850 kg of phosphorus fertilizers. Grigoreva *et.al.* (2018), along with the main fertilizers, recommend foliar feeding with manganese, boric acid, zinc sulfate, and ammonium sulfate. Recent studies (Romanenko, 2020; Volkova *et. al.*, 2020; Duong and Pushkareva, 2020; Venkitasamy *et. al.*, 2019)

recommend a 0.005% sodium humate solution for both root and foliar application, noting not only an improvement in product quality but also an increase in soil fertility. In a study on obtaining a high yield of vigorous grafted vines with a well-developed root system and mature shoots, Grighel and Dadu (2014) recommend the application of organic fertilizers (20–60 t/ha) before planting and minerals $N_{140}: P_{100}: K_{80}$ applied after planting the grafted cuttings throughout the growth and development period, depending on the type of soil. In addition, they recommend additional nutrition for irrigation - manure juice (1:10) or chicken manure (1:3-5), plus nitrogen fertilizers, in the first half of the growing season - fertilizers with phosphorus and potassium (10 - 30 kg/ha s.a.). Our study evaluates the effect of nitrogen fertilization on the yield and quality of grapevine cuttings in nurseries.

MATERIALS AND METHODS

The experiment was carried out at INCDBH Stefanesti, in the Laboratory of Ecology and Multiplication of Horticultural Species aiming to improve the production technologies of viticultural propagation material, in the current context of climate change. Four local cultivars were chosen for table grapes ('Argessis', 'Norocel', 'Memory', and 'Ștefănești'), grafted on the SO4-4 rootstock. The grafting was done at the table, towards the end of February. To be used for grafting, the grafted eyes were moistened for 24 hours at a temperature of 30°C, for 30 hours at a temperature of 30°C, and in the grafting process, paraffin with stimulators was used. After fortification, were analyzed the following parameters: the starting in vegetation, the length and diameter of the main roots and the callus formation.

Before planting in the nursery, for better protection, a paraffin treatment was applied, using silver color paraffin. Paraffin before forcing, which was performed immediately after grafting, aimed to prevent the negative effect of some environmental factors in the process of callus and attachment to grafting. Paraffin the grafted vines before planting in the school of vines was aimed at avoiding dehydration and protecting the young shoots from solar radiation, to increase the yield of grafted vines and raise work productivity. The rooted cuttings were planted in raised earth mounds covered with polyethylene film, the planting system in double rows (with a width of 20 cm), resulting in 3900, in a protected space of 300 m². During the plant development period, two fertilizations options, using N:P:K (30:10:10), were applied weekly for 8 weeks, by fertigation: V1 100% of the recommended optimal dose (D.O.) and V2- 75% of the D.O. The amounts of fertilizers calculated for the 3900 vine cuttings were 200 kg N:P:K (30:10:10) in case of V1 and 150 kg N:P:K (30:10:10) in V2, respectively 60 Kg N, 20 Kg P₂O₅ and 20 kg K₂O, applied in V1 (optimal recommended dose) and 45 kg N, 15 kg P₂O₅ and 15 Kg K₂O, applied in V2.

During the entire growth period of the grafted cuttings, drip irrigation was applied. In October, the following indicators were monitored: shoot length (cm), shoot diameter (mm), number of roots, length of the 3 main roots, and diameter of the 3 main roots.

To highlight the differences between the effect of nitrogen on the indicators analyzed during the study was applied the Duncan test (test with multiple intervals) for a statistical assurance of 5% as well as simple descriptive statistics that show the minimum and maximum values recorded for each indicator depending on the applied fertilization dose.

REZULTS AND DISCUSSIONS

The management of temperature and air humidity during the forcing period

In the experiment, from the beginning of the forcing process, the temperature was 30°C during the first three days, after which it decreased, remaining constant until the end of the forcing period, namely the 11th - 18th day (Table 1).

Table 1. Evolution of temperature and air humidity during the forcing cycle

Day	1	2	3	4	5	6	7	8	9	10	11	12	13	14	15	16	17	18
Temperature °C		30				29	29	28	28	28	27	27	27	27	26	26	25	25
Air humidity %						85-95					85	85	85	80	80	75	75	75

A good percentage regarding the start in vegetation was registered in case of 'Argessis' cv. (100%), having 10% higher start in vegetation compared to the 'Stefănești' cv. The 'Norocel' and 'Memory' cvs. (table 2) also stood out with good results regarding the start of vegetation, under the conditions of keeping the environmental factors presented in Table 1. After forcing, the 'Argessis' cultivar showed the highest shoot growth (12.7 cm), compared to only 6.24 cm, 6.02 cm, respectively 4.28 cm as recorded in the other cultivars (table 2). Regarding the diameter of the roots, it was higher in case of 'Memory' cultivar (1.4 mm), followed by the 'Argessis' cultivar (1.1 mm).

Table 2. The influence of physical processes on the grafting process

Cultivar	Callus width (cm)	Callus percentage (%)	Percent start in vegetation (%)	Shoot length (cm)	Root diameter (mm)
Argessis	1,26	82	100	12.7±1.11	1.1±0.19
Norocel	1.14	60	95	6.24±0.48	0.87±0.12
Memory	1.08	87	96	6.02±0.22	1.4±0.24
Stefănești	0.96	40	90	4.28±0.21	0.6±0.33

The effect of fertilization on the growth of grafted cuttings from the nursery

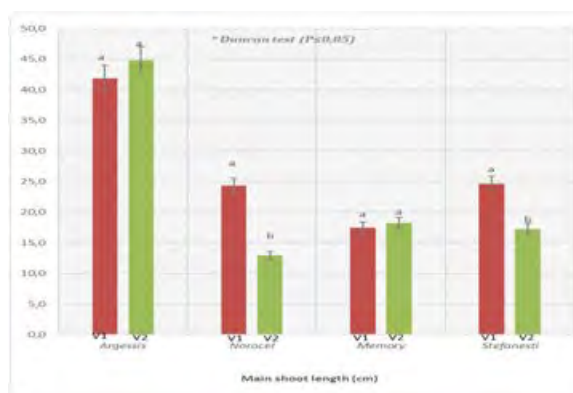


Figure 1. The influence of fertilization on shoot length (cm), depending on the cultivar

*Letters in the graph that are not common represent statistical differences according to the Duncan test

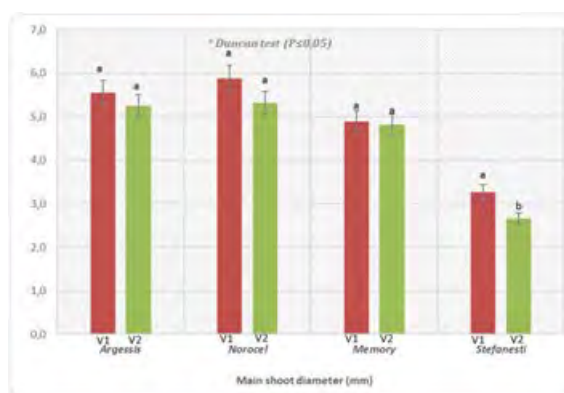


Figure 2. The influence of fertilization on the diameter of the shoots (mm), depending on the cultivar

From Figure 1 it can be seen that fertilization influenced the growth of the shoots in the nurseries, regardless of the cultivar. Thus, the greatest growth of shoots was recorded in V1, the differences being significant. Except for 'Argessis' cv., where the highest increases were evident in V2 (75% D.O) with 44.84 cm, compared to 41.88 cm, as recorded in V1 (100% D.O nitrogen), in all other cultivars the largest increases were evident in the case of applying the optimal fertilization with nitrate (V1). Regarding the diameter of the shoots, it was higher in V1 (100% D.O nitrogen), even if the differences are not significant (Figure 2). The highest values were evident in the 'Norocel' cv. (5.8 mm) in V1. The same significant differences are also observed regarding the number of roots. It was higher in the case of V1, regardless of the cultivar, the differences being significant (figure 3)

For the 4 cultivars of table grapes grafted on the S04-4 rootstock and planted in the nursery in the protected space, the average values of the length of the vegetative growths (shoots) were 25.23 cm and the maximum values for the whole experiment were 66.20 cm. Also, for the diameter of shoot growth and the total number of roots, the minimum values were 2.52 and 5.0, and the maximum values were 2.67 mm and 30 roots (Table 3). It can be seen that by applying fertilization, the histogram regarding these indicators is much different, the sample is no longer homogeneous due to the effect of the experimental variants (Figure 4). The highest shoots growth was recorded in 'Argessis' cv., while 'Stefănești' cv. recorded the highest number of roots (figure 5), in response to the application of D.O. with nitrogen (V1), figure 4.

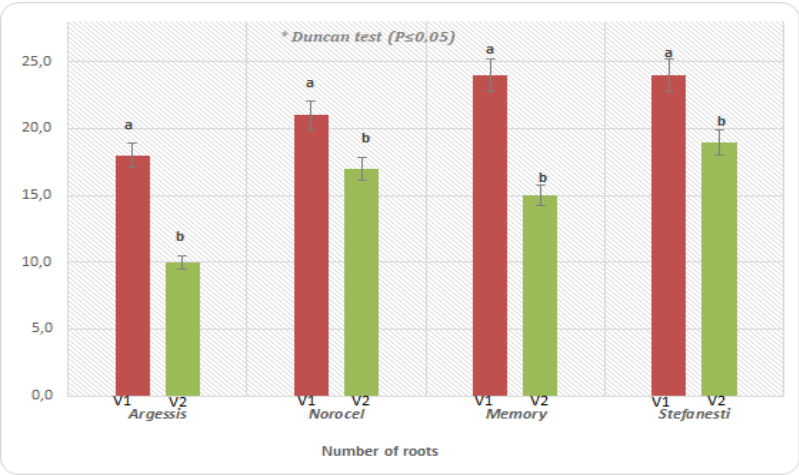


Figure 3. The influence of fertilization on the number of roots depending on the cultivar
**Letters in the graph that are not common represent statistical differences according to the Duncan test*

Table 3. Statistical indicators of growth vigor indicators: shoot length (cm), shoot diameter (mm), number of roots in the analyzed cultivars, from the nursery in the protected area, 2024 at INCDBH Stefanesti

Statistic indicator		Shoot length (cm)	Shoot diameter (mm)	Number of roots
N	Valid	40	40	40
Mean		25.2275	4.7190	18.3750
Median		20.5000	4.9050	19.0000
Mode		14.80(a)	2.52(a)	18.00
Std. Deviation		12.95410	1.34116	6.12974
Skewness		1.500	.086	-.325
Std. Error of Skewness		.374	.374	.374
Kurtosis		1.903	-.298	-.230
Std. Error of Kurtosis		.733	.733	.733
Range		54.90	5.15	25.00
Minimum		11.30	2.52	5.00
Maximum		66.20	7.67	53.00

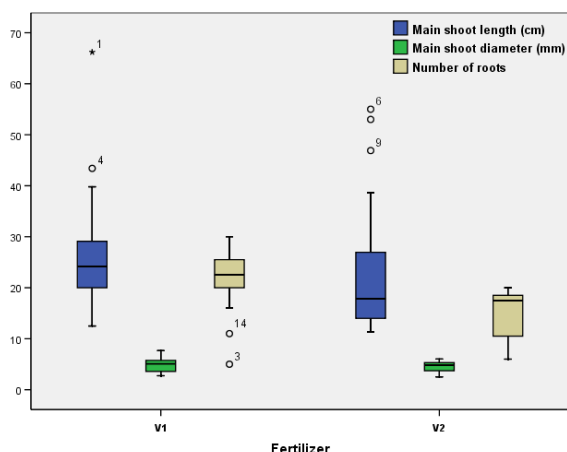


Figure 4. Box-plot, with the distribution of the values recorded on vegetative growth (minimum, maximum and average) and the influence of the fertilization regime

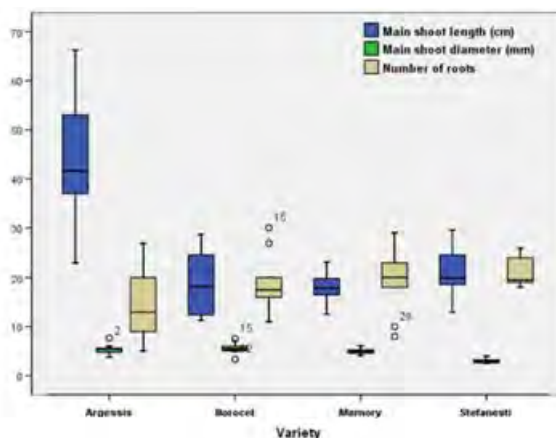


Figure 5. Box-plot, with the distribution of the values recorded on vegetative growth (minimum, maximum, and average) and the influence of the cultivar

The optimal dose of nitrogen application (V1) inhibited the growth of the main roots, leading to significant differences in the length of the 3 main roots in the 'Argessis' and 'Memory' cvs. (figures 6, 7 and 8). In the 'Argessis' cv. the greatest growth of the main root was recorded in V2 (75% D.O. nitrogen) with 22.14 cm, compared to V1 where only 15.02 cm were recorded. Significant differences regarding the growth of the main root were also evident in the 'Memory' cv., which recorded 22.7 cm in V2, compared to 8.9 cm as was evident in V1, a sign that the optimal dose (100% D.O. nitrogen) negatively influenced the root development, in case of this cultivar. It should be noted that the 'Stefănești' cv. responded positively to the application of the optimal dose of nitrogen (100% D.O. nitrogen), recording the largest root growths of 26.18 cm in V1 compared to 11.38 cm recorded in V2. However, the secondary roots induced the greatest increases in V2, a sign that the optimal recommended dose (100% D.O. nitrogen) inhibited the growth of the secondary roots. (figure 7), except for the 'Norocel' and 'Memory' cvs.

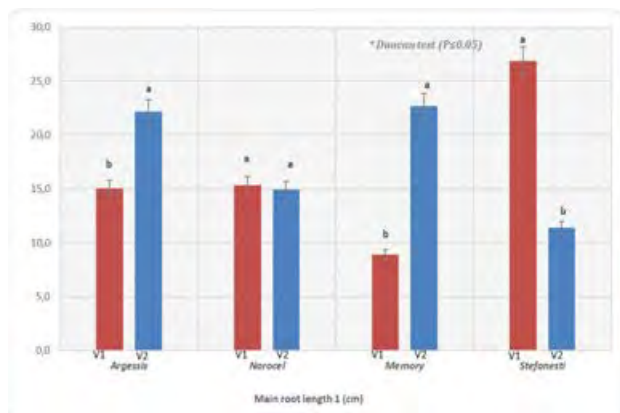


Figure 6. The influence of fertilization on the main root length (cm) , depending on the cultivar

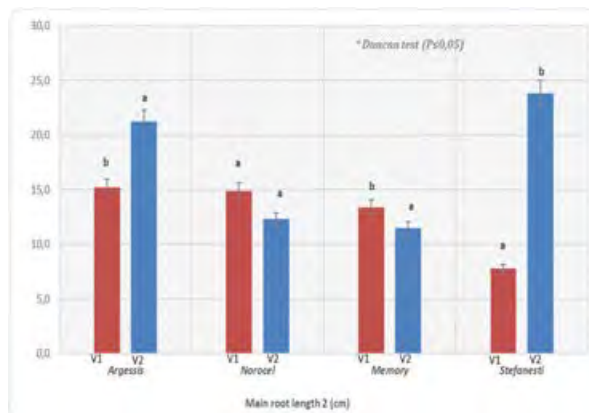


Figure 7. The influence of fertilization on the secondary root length (cm), depending on the cultivar

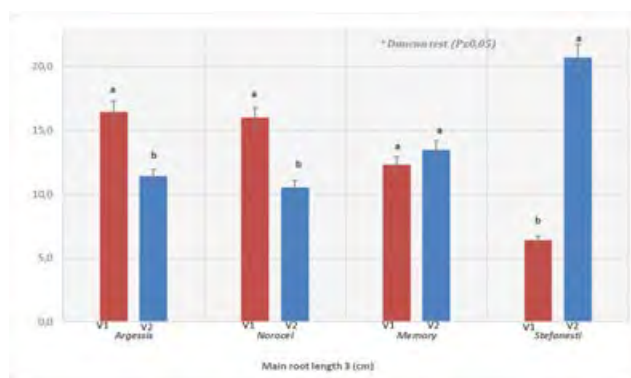


Figure 8. The influence of fertilization on the 3rd root length (cm), depending on the cultivar
**Letters in the graph that are not common represent statistical differences according to the Duncan test*

For all the 3 analyzed roots, the largest diameter was recorded in V2 (75% D.O nitrogen), a sign that high amounts of nitrogen led to the inhibition of growth in diameter, regardless of the cultivar, the differences between the two fertilization options being significant (Figures 9,10 and 11). The largest increases in diameter for the main root were recorded in the 'Norocel' cv. with 3.24 mm, in V2, compared to 2.13 mm as recorded in V1. The smallest increases were recorded in the 'Ștefănești' cv., between 1.86 mm (in V1) and 1.67 mm in V2, the differences being insignificant. (Figure 9). The same differences recorded in V2 were also highlighted in the case of the diameter of the secondary roots, respectively, of the third analyzed root (fig. 10 and 11), regardless of the cultivar.

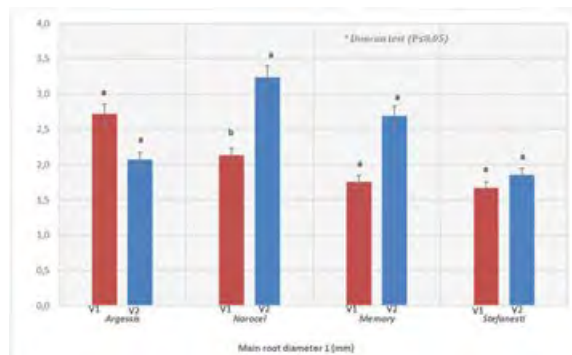


Figure 9. The influence of fertilization on the diameter of the main root (mm), depending on the cultivar

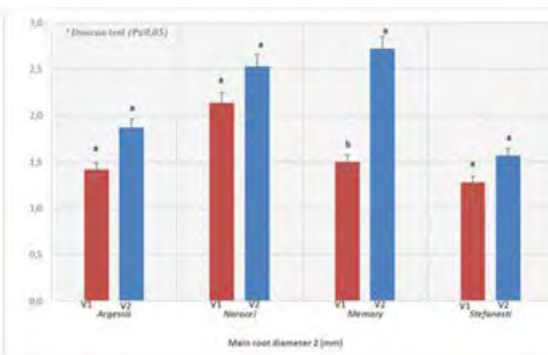


Figure 10. The influence of fertilization on the diameter of the secondary root (mm), depending on the cultivar

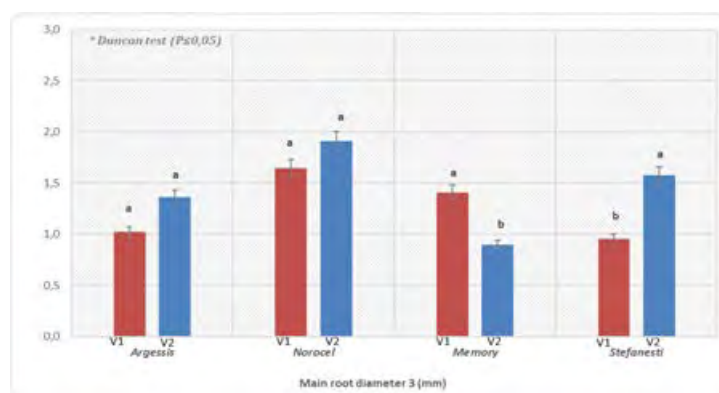


Figure 11. The influence of fertilization on the diameter of the third root (mm), depending on the cultivar

Table 4 shows the maximum and minimum values recorded on the growth of the main roots as well as their diameter. In the figures corresponding to the analyzed values, on the average of the cultivars it can be observed that V2 induced the greatest growth of the main roots, as well as their largest diameter (figures 12,13, 14, 15).

Table 4. Statistical indicators of growth vigor indicators: length of the main root (cm), diameter of the main root (mm), number of roots in the analyzed cultivars, from the nursery in the protected area, 2024 at INCDBH Stefanesti

		Main root length 1 (cm)	Main root length 2 (cm)	Main root length 3 (cm)	Main root diameter 1 (mm)	Main root diameter 2 (mm)	Main root diameter 31 (mm)
N	Valid	40	40	40	40	40	40
	Missing	0	0	0	0	0	0
Mean		17.1575	15.0225	13.3600	2.1530	1.8793	1.3485
Median		15.6500	13.6500	12.2500	2.0100	1.7300	1.4150
Mode		10.30(a)	13.20(a)	9.30(a)	2.12(a)	1.74(a)	0.90(a)
Std. Deviation		6.96205	6.06340	5.44637	0.62526	0.77777	0.51227
Skewness		0.652	0.648	0.945	0.420	1.353	0.183
Std. Error of Skewness		0.374	0.374	0.374	0.374	0.374	0.374
Kurtosis		-0.222	-0.100	1.600	-0.431	3.730	-0.193
Std. Error of Kurtosis		0.733	0.733	0.733	0.733	0.733	0.733
Range		27.50	25.50	27.00	2.68	4.28	2.26
Minimum		8.00	4.80	4.00	0.82	0.51	0.37
Maximum		35.50	30.30	31.00	3.50	4.79	2.63

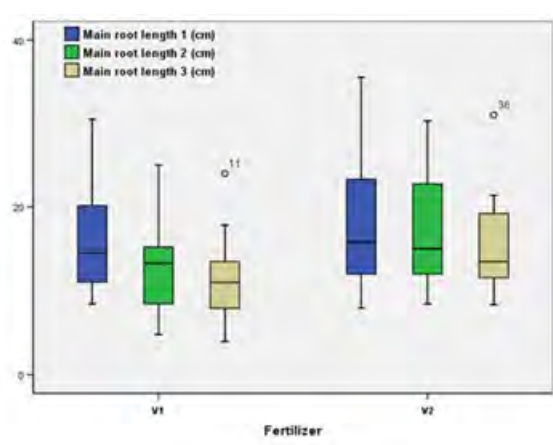


Figure 12. Box-plot, with the distribution of the values recorded on the growth of the main roots (minimum, maximum and average) and the influence of the fertilization regim

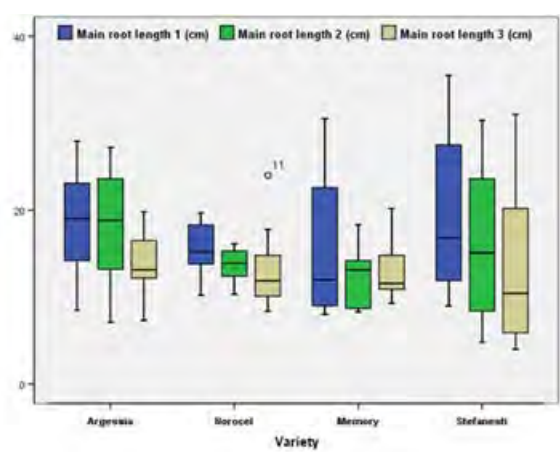


Figure 13. Box-plot, with the distribution of the values recorded on the growth of the main roots (minimum, maximum and average) and the influence of the cultivar

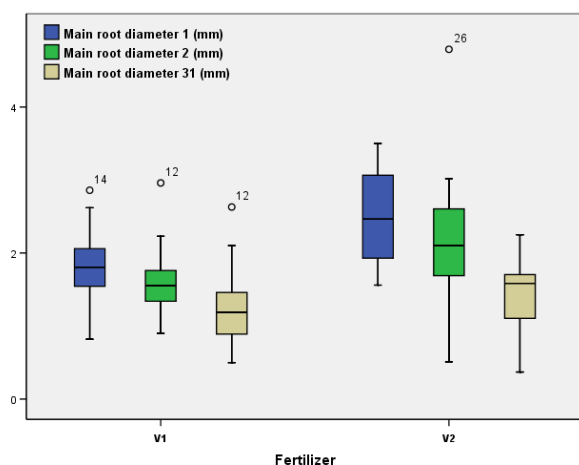


Figure 14. Box-plot, with the distribution of the values recorded on the diameter of the 3 main analyzed roots (minimum, maximum, and average) and the influence of the fertilization regime

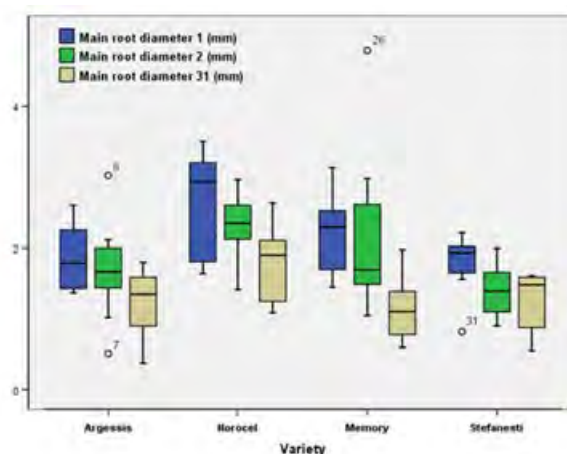


Figure 15. Box-plot, with the distribution of the values recorded on the diameter of the 3 main analyzed roots (minimum, maximum, and average) and the influence of the cultivar

CONCLUSIONS

Research on the influence of the recommended optimal dose (V1- 100% D.O. of nitrogen) on vegetative growth led to improved results in terms of shoot growth and diameter, as well as the number of roots formed. The length of the main shoots oscillated between 43.36 cm when applying 100% DO fertilization to the 'Argessis' cultivar and 17.88 cm for 75% D.O. fertilization, to the 'Memory' cultivar. At the same time, a dose low to the optimal level of nitrogen (75% D.O. nitrogen) improved both root length and diameter. Since there are no studies on the influence of nitrogen on shoot quality in the nursery, further studies are needed over several years, because nitrate is not tightly held by soil particles and is soluble in soil water, and because of the mobility of nitrogen, soil tests for nitrogen are valid for shorter periods than most other elements.

REFERENCES

1. Grighel Gh, and Dadu C., (2014). Fertilizarea plantațiilor mamă altoi, portaltoi și a școlii de vițe. Pomicultura, Viticultura și Vinificația nr. 3 [51]
2. Grigoreva L. V., (2018). Biological growth peculiarities of the cuttings of various rootstocks in a horizontal nursery, International Journal of Pharmaceutical Research, **10(4)**, 632-640
3. Romanenko E. S., (2011). Biologically active preparations with growth-stimulating activity in viticulture. Winemaking and viticulture, **3**, 44 <https://elibrary.ru/item.asp?id=16549657>
4. Venkitasamy C., Zhao L., Zhang R., and Pan Z., (2019). Grapes Integrated Processing Technologies for Food and Agricultural By-Products, 133-163 E3S Web of Conferences **210**, 05003 (2020) *ITSE-2020* <https://doi.org/10.1051/e3sconf/202021005003>.
5. Verzilin AI, Fedulova Y., and Pimkin M., (2020). New biologically pure fertilizers in grape nurseries. E3S Web of Conferences **210**, 05003 <https://doi.org/10.1051/e3sconf/202021005003>
6. Volkova M., Matveikina E., Volkov J. and Stranisheshevskaya E., (2020). Organic viticulture as an important aspect of conserving biodiversity in Crimean agrocenoses. E3S Web of Conferences, **175**, 09004 DOI: 10.1051/e3sconf/202017509004



Floriculture, Ornamental Plants, Landscape architecture

The effect of controlled release fertilizer on growth, flowering, and leaf morphology of *Pulsatilla* species

H.M. Kim ¹, M.S. Roh ², G. Burchi ³ and Y.H. Joung ^{1,*}

¹ School of Biological Sciences and Technology, Chonnam National University,
Republic of Korea

² 4210 Westbrook Drive, Ann Arbor, USA

³ CREA Research Centre for Vegetable and Ornamental Crops, Italy

*Corresponding author email: yhjyoung@chonnam.ac.kr

ABSTRACT

The effect of 14N-6.2P-11.6K controlled release fertilizer (CRF) on growth, flowering, and leaf morphology during vegetative growth of *Pulsatilla tongkangensis* and *P. cernua* var. *koreana* (*P. koreana*) were investigated. *Pulsatilla tongkangensis* flowered earlier (26 to 28 days) than *P. koreana* (50 to 51 days) after natural low-temperature treatment. The highest number of flowers was observed in *P. koreana* (3.6 flowers) and *P. tongkangensis* (1.7 flowers) with 0.9–1.2 g of CRF treatment. The formation of a third rachis and leaflet is a unique characteristic observed in *P. koreana*. Two morphological types of *P. tongkangensis* seedlings, with either long or short petioles, suggests that seedlings with long petioles are considered putative hybrids between *P. tongkangensis* and *P. koreana*. The development of axillary shoots in *P. koreana* seedlings before overwintering, when seedlings received more than 0.6 g of CRF, suggests that the seedlings undergo a phase change from juvenile vegetative growth to mature plants. This is the first detailed report describing morphology of leaves and plants during growth of seedlings and development of *Pulsatilla*.

Keywords: Controlled release fertilizer, juvenility, maturity, Pasque flower, *Pulsatilla cernua* var. *koreana*, *Pulsatilla tongkangensis*

INTRODUCTION

The genus *Pulsatilla* Mill. includes approximately 33 species native to Asia, North America, and Europe (USDA, Agricultural Research Service, National Plant Germplasm System, 2024). *Pulsatilla cernua* var. *koreana* (Y. Yabe ex) Nakai ex T. Mori (referred to as *Pulsatilla koreana*) (<http://www.ipni.org/ipni/idPlantNameSearch.do?id=711999-1>; accessed 22 August, 2023) is distributed across the Korean peninsula, growing in sunny areas with well-drained sandy soil. However, *Pulsatilla tongkangensis* Y. N. Lee et T. C. Lee (<https://www.ipni.org/n/20005221-1>; accessed 22 August, 2023) is growing limited regions in Korea, specifically near calcified rock cavity cliffs and in well-drained areas (Lee *et al.*, 2010; Oh *et al.*, 2014; Yoo *et al.*, 2009).

The application of CRFs such as 14N-6.2P-11.6K Osmocote (<https://iclgrowingsolutions.com/en-us/ornamental-horticulture/products/osmocote-14-14-14-3-4-m/>; accessed on 23 September, 2023) offers a time-efficient alternative to conventional liquid fertilizers (Hauck, 1985). However, previous studies with *P. vulgaris* Mill. grows in

nitrogen-poor soils (Kellner, 1993) have not examined the effect of CRF treatment during the vegetative growth of seedlings or changes in leaf morphology and flowering in the two *Pulsatilla* species studied in this report.

Growth and flowering responses, leaf morphology during seedling stage and leaf analysis for macro- and micro-elements in two species studied in this report has not been reported to our best knowledge. Flowering was accelerated, and the number of flowers was increased in 'Rainier White F1' (*Cyclamen persicum* Mill) Persian cyclamen promoted earlier flowering and increased the number of flowers and buds with treatment of Osmocote 6M (Bosiacki *et al.*, 2021).

Leaf and flower morphology are important characteristics for identifying *Pulsatilla* species (Lee *et al.*, 2010). The formation of the third rachis in *P. koreana* serves as a distinguishing morphological characteristic, to provide a clear distinction between these two species (Lee *et al.*, 2010). During the vegetative phase after germination, morphological changes occur in leaf size and shape, rachis length, and trichome distribution. Identifying vegetative or adult leaves was reported in *Arabidopsis* (Manuela and Xu, 2020). In *Verbascum blattaria* Mill., trichomes present only on seed pods and bracts but absent in leaves at the seedling stage may not be useful for identification or characterization of unknown species (Kim *et al.*, 2023).

The objectives of this research are to (a) examine the morphology of leaves and plants of *P. koreana* and *P. tongkangensis* and variations among seed-propagated *P. tongkangensis* populations and (b) study the effect of CRF treatments on the growth and flowering response.

MATERIALS AND METHODS

Seed germination and transplanting of seedlings: Open-pollinated seeds of *P. koreana* acquired from Mokpo National University in Muan County, Chonnam Province and of *P. tongkangensis* seeds collected from Jungsun, Kangwon-do Province, Korea used in this experiment. On 1 July, 2023, *P. koreana* seedlings developed 3 to 4 true leaves and were transplanted into 1800 square band pots (9.0 × 9.0 × 11.5 cm, Anderson Die & Manufacturing, Portland, OR, USA). On 6 July, 2023, *P. tongkangensis* seedlings with similar leaf development were transplanted into JānorPot 3D containers (9.0 × 9.0 × 10.0 cm, Summit Plastic Co., Mogadore, OH, USA).

Controlled release fertilizer treatments: On 6 July, 2023, CRF was top-dressed at rates of 0, 0.3, 0.6, and 1.2 g per pot for *P. tongkangensis* and 0, 0.3, 0.6, 0.9, and 1.2 g per pot for *P. koreana*. The CRF granules were covered with an additional growing medium to ensure they were not visible. On 6 August, 2023, the pots were spaced at a 9 × 9 cm distance to prevent the leaves adjacent plants from touching. On 25 September, 2023, CRF was reapplied at the same rates.

Observation of leaf and plant morphology: On 17 September, 2023, the leaf morphology of both *Pulsatilla* species was observed when the seedlings had formed 5 to 6 true leaves. The number of leaflets per leaf and the shape of the leaflets were recorded according to previously described (frame A, Fig. 1; Lee *et al.*, 2010). Briefly, the third leaflets and rachis as indicated with solid circle indicated in leaves 2, 3, and 4 in *P. koreana* were observed. However, these was not observed in *P. tongkangensis*. Only the second rachis indicated with a solid arrow in *P. tongkangensis* were formed, while the third rachis indicated with a dotted arrow in *P. koreana* was formed (Fig. 2).

Based on the formation of the third leaflet and rachis, the morphologies of *P. tongkangensis* and *P. koreana*, as well as an intermediate form between these two species, especially in

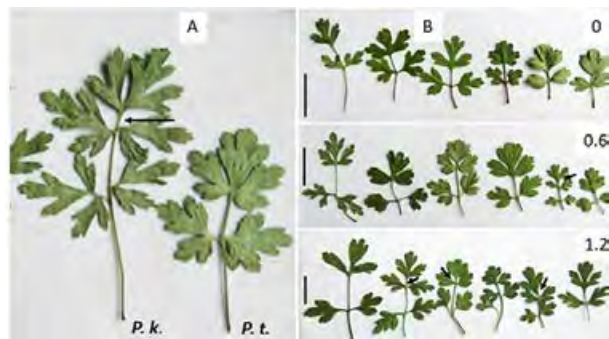


Figure 1. Typical leaf morphology of *P. cernua* var. *koreana* (*P. k.*) and *P. tongkangensis* (*P. t.*); Frame A: formation of (arrow) in *P. koreana* and absence of the third leaflet and rachis in *P. tongkangensis*. Frame B: *Pulsatilla tongkangensis* plants that formed the third rachis (solid arrow) when seedlings were treated with 0, 0.6, and 1.2 g 14N-6.2P-11.6K Osmocote. These leaves were collected for analysis of macro- and micro-elements before overwintering. Bar = 2 cm

P. tongkangensis that are produced, especially when plants received 0.6 and 1.2 g CRF were recorded (frame B, Fig. 1). On 26 October, 2023 the number of plants classified based on leaf morphology was recorded (Table 2). Short and compact leaves developed at the crown of the seedlings of *P. koreana* (C2) and of *P. tongkangensis* (T2) that received CRF treatments were counted (Fig. 4).



Figure 2. Morphological differences of *P. tongkangensis* and *P. koreana* on 17 September, 2023. (A) *P. tongkangensis* leaves exhibit only two leaflets with the second rachis (solid arrow) and leaflet (solid circle), (B) *P. koreana* leaves show a well-developed third leaflet attached to the third rachis (dotted arrow) at the proximal end of the second rachis (solid arrow) in leaves 2 and 3 (indicated in the square box). The third rachis is not fully developed in leaf 4 and is absent in leaves 5 through 8, counted from the youngest leaf (leaf 1)

Experiment design and statistical analysis: Following the overwintering, all pots were completely randomized and designed on a wooden deck 45 cm above the ground until the completion of the experiment. The dates of flowering for the first and second flowers, originating from both the main and axillary shoots, were recorded, and the total number of flowers, was recorded two weeks after the anthesis of the first flower bud from the main shoot. The number of days to flower was counted from 6 February. The monthly average temperatures from sowing in July 2023 to March 2024 were tabulated using data from Weather Underground (<https://www.wunderground.com/forecast/KARB>) (Table 1).



Figure 3. Influence of 14N-6.2P-11.6K CRF on growth morphology of *P. tongkangensis* on 17 September, 2023. (A) CRF was applied at rates of 0 g (1), 0.3 g (2), 0.6 g (3), 0.9 g (4), and 1.2 g (5) per pot on 6 July, 2023. (5-A) Short leaf blade and (5-B) long leaf petiole. Two distinct types of *P. tongkangensis* that received the same amount of CRF. Leaf length was measured from the center of the plant (crown) to the tip of the leaf. (B) The image depicts the shoot apex, including the main (Ma) and axillary (Ax) shoot.

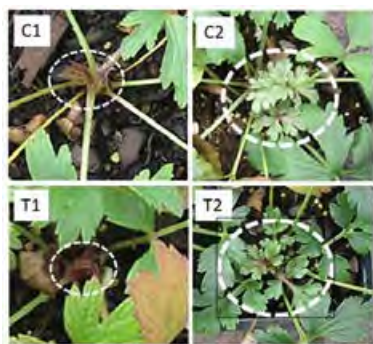


Figure 4. Overview morphology of seedlings prior to overwintering for *P. cernua* var. *koreana* (C1 and C2) and for *P. tongkangensis* (T1 and T2) that formed short and compact leaves. Seedlings received 0g (C1 and T1) or 0.9g 14N-6.2P-11.6K Osmocote (C2 and T2). Photographed on 1 October, 2023

As the temperatures decreased around 15 October, 2023, reaching 1.7 °C at midnight on 31 October (Table 1), the seedlings in the pots were prepared for overwintering. Pots were buried in a ground bed and watered. The seedlings were grown until 20 November, 2023 after which all pots were covered with approximately 5 cm of grass clippings and chopped dried leaves processed with a lawn mower.

Table 1. Monthly high. average, and low temperature (°C) from July, 2023 to March, 2024, and indoor temperature from March to April, 2024

Year	Month	Max.	Ave.	Low.	Indoor
2023	July	26.6	19.1	13.8	
	August	25.9	18.89	13.0	
	September	21.5	14.5	10.1	
	October	13.2	8.3	3.8	
	November	7.9	2.5	-2.8	
	December	5.6	2.2	-1.4	
2024	January	0.5	-2.0	-6.0	
	February	6.8	1.4	-3.9	5.5
	March	10.6	4.6	-0.8	6.1

All pots were lifted from the ground on 6 February, 2024. To protect the seedlings from frost, they were moved to a non-heated garage from 19:00 to 08:30 when temperatures dropped below -2°C during the night. The temperatures were recorded at 08:30. The experiment, including the recording of flower bud development and flower counts, and morphological characteristics was completed on 1 April, 2024 (Table 2). Statistical analyses were performed using the Excel software program (Microsoft Corporation, 2019).

RESULTS AND DISCUSSIONS

Growth and flowering responses: *Pulsatilla tongkangensis* flowered significantly earlier (26 to 28 days) compared to *P. koreana* (50 to 51 days) (Table 2). The days to flowering were unaffected by CRF treatments in both species. Osmocote 6M treatment accelerated the flowering of Cyclamen (Bosiacki *et al.*, 2021). No flowers were produced from the axillary shoots treated with 0 and 0.3 g CRF in both species. Application of 0.6 to 0.9 g of CRF increased the number of total flowers, ranging from 0.8 to 3.6 flowers in *P. koreana* and from 0.6 to 1.7 flowers in *P. tongkangensis*. This increase was attributed to the formation of axillary shoots that produced flowers, producing 1.6 flower buds from the axillary shoot. The flowering percentage exceeded 80% in both species. When seeds were sown in July, 7-month-old plants treated with 1.2 g CRF produced up to four flowers per plant, two flowers each from the main and axillary shoots as observed after overwintering. Osmocote 6M treatment increased the number of flower buds of Cyclamen (Bosiacki *et al.*, 2021). Therefore, enhancing the potential for axillary shoot formation before overwintering (Fig. 3) and continued development over the winter (Fig. 5) could be beneficial for increasing the number of flowers.



Figure 5. Overall appearance of *Pulsatilla* plant treated with 1.2 g 14N-6.2P-11.6K CRF after overwintering. (A) Main shoot (Ma) and axillary shoot (Ax) with long leaves that decayed (L) and short and compact leaves that remain green (S). (B) Appearance of new shoots of *P. koreana* that were not treated with CRF, showing only the Ma. (C) New shoot of *P. tongkangensis* that were not treated with CRF. Photographed on 7 February, 2024. Scale bar = 1 cm

Morphology of leaves and shoot apex: *Pulsatilla tongkangensis* leaves were ternate with palmately lobed leaflets and a cordate base (Fig. 1; indicated by the circle in frame A, Fig. 2). In contrast, *P. koreana* had oddly pinnately compound leaves (Fig. 1; indicated by the dotted circle, leaf no. 5 in frame B, Fig. 2). The formation of the third rachis and third leaflet was observed only in *P. koreana* (dotted arrow, leaf Nos. 2, 3, and 4 in frame B, Fig. 2). The third rachis and leaflet were absent in older leaves numbered 5, 6, 7, and 8, counted from the crown of the *P. koreana* seedlings. Most *P. tongkangensis* seedlings did not form the third rachis and leaflet when they were not treated with CRF (frame B, Fig. 2). This morphological change could signify a phasic shift from vegetative growth to reproductive development influenced by CRF application. Therefore, the presence of a

third leaflet may not serve as a definitive morphological characteristic for identifying and characterizing *P. tongkangensis* potential hybrids with *P. koreana*, and accurate identification should ideally involve molecular markers (Lee *et al.*, 2010).

Pulsatilla. tongkangensis seedlings exhibited significantly shorter petioles (frame A, Fig. 1; Table 2) and wider leaflets (data not recorded) compared to *P. koreana* (frames A and B, Fig. 2). For *P. koreana* seedlings treated with 0.6 g of CRF, the longest leaf measured 7.38 cm, which was significantly longer compared to the control (4.98 cm). However, leaves treated with 1.2 g of CRF were shorter (6.5 cm) compared to those treated with 0.6 g (Table 2). In *P. tongkangensis*, the longest leaf length of 3.77 cm in seedlings treated with 1.2 g of CRF was significantly greater than that of the control (2.43 cm) and those receiving 0.3 g of CRF (2.73 cm).

However, two types of petiole length patterns were observed among *P. tongkangensis* seedlings treated with 1.2 g of CRF: leaves with either a short petiole (5-A in frame A, Fig. 3) or a long petiole (5-B in frame A, Fig. 3) may suggest that *P. tongkangensis* plants with long petiole (intermediate morphology) could result from the hybridization with *P. koreana* and this pattern was only observed in *P. tongkangensis* as two to three plants showed intermediate morphology (Table 2).

In both species, axillary shoot (Ax) before overwintering was not observed when seedlings were not treated with CRF. However, *P. tongkangensis* seedlings that received 0.6 and 0.9 g CRF, and in *P. koreana* was also observed when seedlings were treated with 0.3 to 0.6 g CRF developed an axillary shoot adjacent to the main shoot (Ma) on 17 September, 2023 (frame B, Fig. 3). The formation of axillary shoots, influenced by the application CRF treatments suggests that the shoot apex of the main shoot may undergo reproductive development and similar findings have been reported for *P. vernalis*, where a single application of ammonium fertiliser resulted in increased shoot production and enhanced flower bud formation (Kellner, 1993).

On 1 February, 2024, after overwintering, newly developing leaves of *P. tongkangensis* plants that had received 0.9 g CRF emerged above the ground. The short and compact leaves (S) indicated by a dotted circle (frames C2, T2, Fig. 4) remained semi-green when the pots were lifted (Frame A, Fig. 2). Upon lifting the plants from the ground, the shoot apices of *P. koreana* that had not been treated with CRF (frame B, Fig. 5) appeared smaller and less developed compared to those of *P. tongkangensis*, which showed two well-developed leaves (frame C, Fig. 5). In *P. koreana*, two shoot apices of similar development was observed. However, plants treated with 1.2 g of CRF exhibited more advanced development of both the main and the axillary shoots.

The presence of short and compact leaves developed before overwintering and the formation of axillary shoots may suggest that the seedlings are transitioning towards maturity before overwintering. Therefore, the term 'seedling' is applied before overwintering, while 'plant' is used following overwintering. Flower bud development in young *Pulsatilla* plants may occur later than mid-July and mid-August observed in older plants (Wang and Bartholomew, 2001). Reproductive competence in *Pulsatilla* species may progress in response to short-day photoperiods and low-temperature environments before overwintering. Once flowering occurs in early spring, no further flowering was observed in both *Pulsatilla* species. This suggests that short photoperiods and lower temperatures facilitate the transition from vegetative growth to reproductive development, keeping plants vegetative under long days and high temperatures as reported in June-bearing strawberries (*Fragaria ×ananassa*, Duch.) (Black *et al.*, 2008). Therefore, under natural environmental conditions, floral bud initiation in *Pulsatilla* likely occurs in early autumn when photoperiod (12 – 13.5 hours) and temperature (18/16°C) become conducive for floral induction (Black *et al.*, 2008). Further experiments are required to understand the critical photoperiod and temperature for floral induction and development.

Table 2. Effect of 14N-6.2P-11.6K Osmocote (CRF) on the growth and development of *Pulsatilla koreana* and *Pulsatilla tongkangensis*

<i>Pulsatilla</i> species	14N- 6.2P- 11.6K Osmo- cote (g)	Data collected before overwintering in 2023				Data collected at flowering after overwintering in 2024		
		Long- est leaf length (cm)	No. of plants with Ax ^a	Formati on of mature leaves ^b	Plant type based on leaf morphology ^c	No. of flowers		Flower- ing %
						First / second inflores- cence ^d	Total	
<i>P. koreana</i>	0	4.98	0	5 N	5 K	0.8 / 0	0.8	80
	0.3	6.14	1	3 Y/2 N	5 K	2.0 / 1.4	3.4	100
	0.6	7.38	2	5 Y	5 K	2.0 / 1.6	3.6	100
	1.2	6.50	2	4 Y / 1 N	5 K	2.0 / 0.8	2.8	80
	HSD 1% ^e	1.735	- ^f	-	-	-	1.13	-
<i>P. tong- kangensis</i>	0	2.43	0	na	4 T / 2 I	0.6 / 0	0.6	67
	0.3	2.73	0	na	3 T / 3 I	0.8 / 0	0.6	67
	0.6	3.45	1	na	3 T / 3 I	1.0 / 0.3	1.3	100
	0.9	3.58	2	na	4 T / 2 I	1.3 / 0.2	1.5	83
	1.2	3.77	2	na	3 T / 3 I	1.5 / 0.2	1.7	83
	HSD 1% ^e	0.893	-	-	-	-	0.85	-

^a Refer to Figs. 3 and 5 for the main shoot (Ma) and axillary shoot (Ax).

^b Number of seedlings with mature leaves indicated with an arrow (black solid) and leaves 2, 3, and 4 in solid and dotted circles (Fig. 2). Indicated as yes (Y) or no (N). Mature leaves were not developed (na).

^c Number of *P. koreana* type (K), *P. tongkangensis* type (T), and intermediate type (I) seedlings exhibiting mature/immature leaf morphology characteristics. For example, 4 Y/1 N indicates that four plants formed mature leaves and one plant did not, and "4 K/1 I" indicates that four plants showed *P. koreana* type and one plant intermediate type. Refer to Fig. 1.

^d Number of first / second flowers developed both from the primary shoot and from the axillary shoot apex.

^e Tukey's honestly significant difference (HSD) test at $p \leq 0.01$.

^f Not analyzed.

CONCLUSIONS

The flowering of *P. tongkangensis* occurred earlier (26 to 28 days) than that of *P. koreana* (50 to 51 days); however, the flowering time was not significantly affected by CRF treatment in either species. The formation of the third rachis and leaflet, which is a distinct morphological characteristic of *P. koreana*, implies a transition from vegetative growth to reproductive development, which includes the formation of axillary shoots and compact leaves in response to low temperatures and short-day photoperiods before overwintering. The long petioles observed in *P. tongkangensis* seedlings suggest that these seedlings were hybrids. We first reported on the leaf morphology before and after overwintering and axillary shoot formation in relation to the number of flowers. Formation of third rachis and leaflet in *P. tongkangensis* as influenced by CRF treatment required molecular work to identify whether these plants with long petioles are interspecific hybrids.

Acknowledgements

Germplasm used in this project was provided by J.W. Kang, D.W. Suh, Y.K. Yoo, and M.C. Chung. We express our sincerely thanks for their contributions.

This research was supported by the research grant awarded to Y.H. Joung from the New Breeding Technologies Development Program (Project No. RS-2024-00322366) through the Rural Development Administration (RDA), Korea.

REFERENCES

1. Bosiacki M., Czuchaj P., Szczepaniak S., Walkowiak L. and Abramowicz A., 2021. The influence of slow-release fertilizers on the growth, flowering, and the content of macro-and micronutrients in the leaves of *Cyclamen persicum* Mill. *Agronomy*. 11(11): 2147. <https://doi.org/10.3390/agronomy11112147>
2. Hauck R.D. (1985). Slow release and bio-inhibitor-amended nitrogen fertilizers. Chapter 8 In: Fertilizer Technology and Use. Third Edition. Engelstad OP (editor). ASA, CSSA, and SSSA Books. <https://doi.org/10.2136/1985.fertilizertechnology.c8>
3. Kellner O. (1993). Effects of nitrogen addition on the population dynamics and flowering of *Pulsatilla vernalis*. *Canadian Journal of Botany*. 71: 732-736. <https://doi.org/10.1139/b93-085>
4. Kim H.M., Roh M.S., Yoo Y.K., Yoo K.S., Joung Y.H. (2023). Leaf morphology, genetic analysis and low temperature requirement for flowering of *Verbascum blattaria*. *Plant Genetic Resources*. 21(2): 115-122. DOI: <https://doi.org/10.1017/S1479262123000497>
5. Lee A.K., Yuan T., Suh J.K., Choi D.S., Choi I.Y., Roh M.S., Joung Y.H., Ko J.Y., Lee J.S. (2010). *Pulsatilla tongkangensis*, a natural hybrid swarm population hybridized with *P. koreana* based on RAPD and SNPs of chloroplast DNA. *Horticulture Environment and Biotechnology*. 51: 409-421. <http://www.horticulture.or.kr/english/>
6. Manuela D. and Xu M. (2020). Juvenile leaves or adult leaves: determinants for vegetative phase change in flowering plants. *International Journal of Molecular Science*. 21(24): 9753. <https://doi.org/10.3390/ijms21249753>
7. Microsoft Corporation. (2019). Microsoft Excel, Available at: <https://office.microsoft.com/excel>.
8. Oh H.K., Lee K., Lee Y., Choung Y. (2014). Geographical distribution and ecology in microhabitats of the rare species, *Pulsatilla tongkangensis* YN Lee et TC Lee. *Korean Journal of Ecology and Environment*. 47(spc): 74-82. (In Korean with English abstract). [http://dx.doi.org/10.11614/KSL.2014.47\(5\).074](http://dx.doi.org/10.11614/KSL.2014.47(5).074)
9. USDA Agricultural Research Service National Plant Germplasm System (2024). Germplasm Resources Information Network (GRIN-Taxonomy). National Germplasm Resources Laboratory, Beltsville, MD. Search Taxonomy GRIN-Global (ars-grin.gov). 19 Jan. 2024.
10. Wang W. and Bartholomew B. (2001). 27. *Pulsatilla* Miller, *Gard. Dict. Abr.*, ed. 4, (1136). 1754. In *Flora of China* 6. 329-333.
11. Yoo K.O., Cheon K.S. and Jang S.K., 2009. Environmental and ecological characteristics of *Pulsatilla tongkangensis* habitats. *Korean Journal of Environment and Ecology*. 23(5): 439-446.

Studies on the behavior of some cultivars of *Dahlia* in the Botanical Garden Cluj-Napoca

M. Cantor¹, A. Pui², I. Moldovan¹, A.J. Kozel¹, Zs. Szekely-Varga³ and L. Draghia^{4,*}

¹University of Agricultural Sciences and Veterinary Medicine Cluj-Napoca, Romania

²Botanical Garden 'Alexandru Borza' Cluj-Napoca, Romania

³Sapientia Hungarian University of Transylvania, Faculty of Technical and Human Sciences, Târgu Mureș, Romania

⁴University of Life Sciences Iasi, Romania

*Corresponding author email: lucia.draghia@iuls.ro

ABSTRACT

Dahlias are particularly appreciated due to the elegance of the inflorescences and the long duration of flowering, which gives them multiple uses both in floral art and in landscaping. The research investigates 8 cultivars from the *Dahlia* genus concerning main morpho-ornamental characteristics (height of plants, length of floral stem, number of branches/plants, number of inflorescences per plant, diameter of inflorescence, number of ligules per inflorescence). Moreover were evaluated the determination of the amount of chlorophyll in the leaves and soluble dry matter content in tuberous roots. Based on the data obtained, we noticed a different suitability of these *Dahlia* cultivars, general having a positive significance concerning the morpho-decorative characteristics studied. The cultivars 'Record', 'Tsuki Yori No', 'Josephine' and 'Colour Spectable' present the highest amount of soluble dry matter in the tuberous roots, over 20%, and the chlorophyll content was over 500 $\mu\text{mol}/\text{m}^2$ to the cultivars 'Tsuki Yori No' and 'Colour Spectable'.

Keywords: perennial plant, morpho-decorative characteristics, tuberous root, chlorophyll, soluble dry substance.

INTRODUCTION

The genus *Dahlia* contains approximately 30 perennial herbaceous species with tuberous roots, belonging to the family *Asteraceae* (*Compositae*) that is widely used for ornamental purposes as a potted plant or cut flower (Lord, 2003). It is native to Mexico, where, in addition to its ornamental value, it is a source of food and medicines.

Purple dahlia flowers and roots have potential for use in human food and can be considered good options to improve and diversify a healthy diet (Costa *et al.*, 2022). Its tuberous roots and petals are consumed and used in different gastronomic elaborations (Lara-Cortés *et al.*, 2014) and for medicinal purposes. Dahlia tuberous roots have a high content of carbohydrates, including inulin and fiber, proteins, vitamins, minerals, and bioactive compounds that can benefit human health (Lara-Cortés *et al.*, 2014; Ciobanu *et al.*, 2016). Mejía-Muñoz *et al.* (2020) indicated its tuberous root for diabetic patients because it helps to regulate blood glucose levels, in addition to decreasing cholesterol and triglycerides. Fălticeanu and Munteanu (2006) mention that the *Dahlia* root is rich in

starch-inulin, which can be converted into fructose, a sweetening substance, useful in the diet of diabetics.

This cut flower has very particular ornamental characteristics, so that it has become one of the most beautiful flowers in the world; in addition, it has the largest number of cultivars of any plant species and more than 50,000 have been registered with the Royal Horticultural Society of England (Bye and Linares, 2008; Țurlea (Ciobanu), 2023; Sho Ohno *et al.*, 2011).

In Romania, the production levels of cut flowers still remain very low, the supply does not correspond to the demand although the climatic conditions allow obtaining flowers in the field in the summer with low production costs; however, those possibilities are not sufficiently exploited. In recent years, in our country, *Dahlia* has gained a very significant share in the assortment of summer-autumn cut flowers (Toma and Petra, 2020). The special floricultural qualities of the species of the genus *Dahlia* make these plants suitable for cultivation in gardens and green spaces. They are easy to grow, the decoration period is quite long, and the flowers are numerous and with an impressive colour (Ciobanu (Țurlea) *et al.*, 2021).

This summer-2004, on the occasion of the Olympic Games in Paris, the dahlia decorated the parks and gardens of Paris and even around the Olympic sites. With its vibrant orange-red hue, a hybrid created in the Park Floral de Paris by a city gardener evoked the Olympic flame. This 'Olympic Dahlia' will be produced and planted every year, carving out a wonderful place for itself in the legacy of Paris 2024 (<https://www.paris.fr/en/pages/50-000-new-dahlias-created-for-the-paris-2024-games-27930>).

Dahlias can contribute not only to the decor but also to the flavor of culinary dishes, by adding nutritional value to the human diet. In Brazil, dahlia ligules are used only for ornamental purposes, where there is a wide diversity of types and colors (Costa *et al.*, 2022). Ligules have a long history as edible plant by the indigenous population in the form of small cakes, and even today this species is consumed in salads, in desserts, and as a garnish in many dishes by adding nutritional value to the human diet (Treviño *et al.*, 2007 cite by Costa *et al.*, 2022).

Mlcek and Rop, (2011) reported that while the petals are edible, the other parts of the plant may have an unpleasant taste and can cause allergic reactions.

Dahlias that are suitable for use as cut flowers must possess certain characteristics: vigorous, branching, produce many harvestable stems, good stem length, transport resistance, cut at an image stage, have the ability to grow on. Some of the favorite dahlias as cut flowers are 'Cafe au Lait', 'Hollyhill Black Beauty', 'Crazy for Don' and 'Brookside Snowball' (<https://www.dahlia.org/growing/the-dahlia-as-a-cut-flower/>).

The evolution of dahlia flowers has not ended, botanists and horticulturists who are passionate about these flowers are still trying to enrich this species and obtain blue dahlias (the only color missing from the color range of dahlias), resistant to frost, and also wants to obtain dahlias with a specific smell. The diversity of dahlia characteristics, in terms of plant height, flower shape and size, the multitude of colors, the number of branches on the plants, but also due to the beauty displayed, ranks it among the top flowers used in outdoor landscaping such as parks, gardens, borders etc. (Toma, 2009).

The large number of varieties within the *Dahlia* genus is the result of crosses of several species as: *D. variabilis*, *D. juarezii*, *D. imperialis*, *D. coccinea* and *D. rosea*. Most varieties are known in the crop resulted from the crossing of the species listed above and belong to the hybrid species *Dahlia hybrid* Hort. (Moldovan *et al.*, 2017).

Dahlia is a flowering plant highly appreciated around the globe since the first years of its discovery, this appreciation is growing more and more with the creation of new varieties.

The objective of this research was to investigate the behavior of some *Dahlia* cultivars in the conditions of Transylvania and to present their characteristics and qualities. The growth dynamics of dahlia plants varied according to the characteristics of the varieties.

MATERIALS AND METHODS

The aim of this work was to present the characteristics and qualities as a cut flower of eight *Dahlia* cultivars grown in the Botanical Garden 'Alexandru Borza' of Cluj-Napoca: 'Record', 'Tsuki Yori No', 'Procyon', 'Veritable', 'Purple Gem', 'Colour Spectable', 'Princess' and 'Josephine'(Figure 1).The studies were carried out during the year 2023. The tuberous roots of the cultivars studied were planted in the experimental field on May 09, 2023, at a distance of 60 cm between plants and 30 cm between plants/row.

The environmental conditions in Cluj-Napoca are suitable for the cultivation of dahlias, a temperate climate, with an average annual temperature of around 9.2°C, with average precipitation of 500-600 ml/year. Air currents and winds ensure that the temperature drops during the night, conditions similar to those of the origin of dahlias, and the annual rest period is ensured by cold winters.

The experiment was placed randomly in three repetitions, from each cultivar 10 plants/repetition were planted, total 30 plants/variant. Morpho-decorative observations and measurements were made consisting of the height of plants, length of floral stem, diameter of inflorescence, number of inflorescences per plant, number of ligules per inflorescence, number of branches/plants.

Plant biometry measurements were carried out with: graduated ruler with markings in cm and inches or tape measure (for measuring plant height, flower stem height and plant diameter), and Stainless hardened electronic digital caliper for measuring inflorescence.

In autumn, when the tuberous roots were removed, the soluble dry matter was determined in the eight cultivars using the Zeiss handheld refractometer.

To determine the amount of chlorophyll in the leaf, a test was performed using the MC-100 Chlorophyll Concentration Meter, taking six leaves from each plant: two from the base of the plant, two from the middle and two from the top.

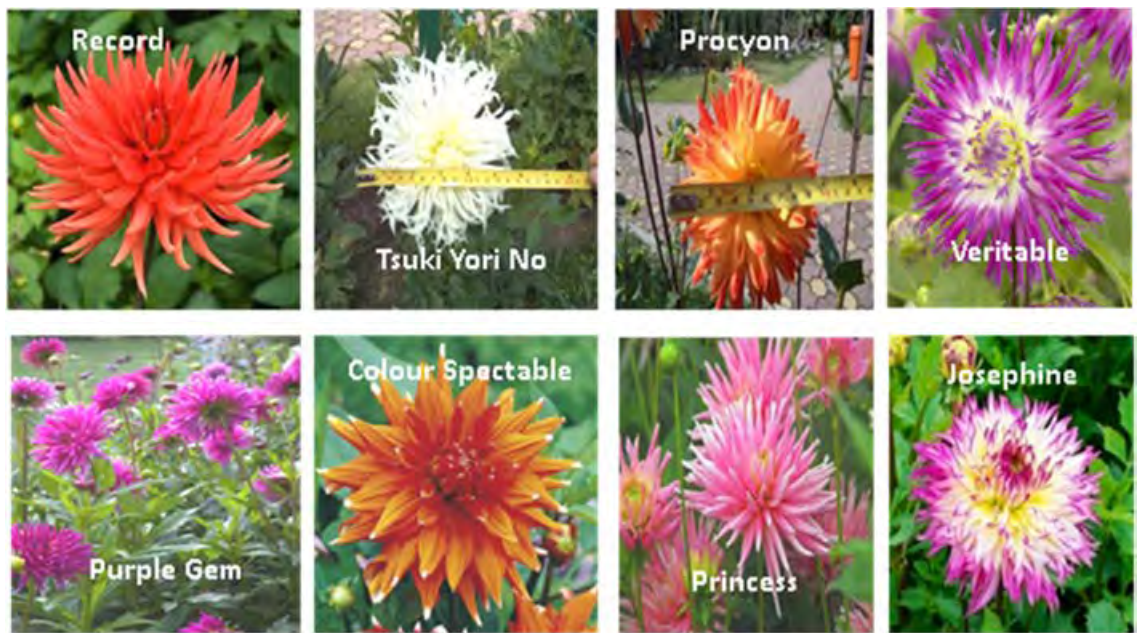


Figure 1. Biological materials of *Dahlia* cultivars

The obtained results were interpreted calculating the average of experience, and the differences between the variants were established by analysing the variance and using the analysis of variance, LSD test and Duncan test (Ardelean *et al.*, 2007).

RESULTS AND DISCUSSIONS

The height of the plant

Analyzing this character (Table 1), we notice two cultivars that stand out with a high waist, 'Colour Spectable' reaching 170.6 cm and 'Veritable' with 140.6 cm, and the lowest height was displayed by the 'Princess' cultivar with 55.2 cm.

Table 1. The height of the plant

No. var.	Cultivar	Average height (cm)	Relative value (%)	± d (cm)	Significance of differences
1	Record	75.8	72.8	-28.34	o
2	Tsuki Yori No	118.3	113.6	14.16	n.s.
3	Procyon	93.5	89,8	-10.64	n.s.
4	Veritable	140.6	135.0	36.46	***
5	Purple Gem	80.3	77.1	-23.84	o
6	Colour Spectable	170.6	163.8	66.46	***
7	Princess	55.2	53.0	-48.94	ooo
8	Josephine	98.8	94.9	-5.34	n.s.
Control	Average of the experience	104.14	100.00	-	-

LSD5% =19.06 LSD1% =28.46 LSD0.1% =36.26

The comparison between the average of the experience, considered as a control (104.14 cm) and the significance of the difference in the analyzed character, highlights very significant positive differences in the 'Colour Spectable' and 'Veritable' cultivars, very significant negative differences in the 'Princess' cultivar, significant negative differences in 'Record' and 'Purple Gem', and the cultivars 'Tsuki Yori No', 'Procyon' and 'Josephine' do not show significant differences from the average of the experience regarding plant height. The results of the analysis recommend placing tall cultivars in less visible green spaces, and short cultivars will be placed in front and in open places.

The number of branches /plant

The ramifications of flowering plants are important both in terms of space occupied in exterior design and for the production of cut flowers. As a result of the data obtained in this research (Table 2), a strong character of branching is observed in the cultivar 'Tsuki Yori No', with an average of 15.33 branches, instead the fewest branches were observed in the cultivar 'Princess', with an average of 3.33 branches per plant.

The results of the analysis regarding the average number of branches per plant show us very significant positive differences in the cultivar 'TsukiYori No', distinctly significant positive differences in the 'Record' and 'Veritable', non-significant difference compared to the average of the experience that is the control, for the cultivar 'Procyon' and 'Josephine', and the negative differences of this character are observed in the other cultivars, namely, distinctly significant negative differences in the cultivars 'Purple Gem' and 'Colour Spectable', and 'Princess' shows very significant negative differences of this character

analyzed compared to the average of experience, considered as a control. We can say that from the point of view of the number of branches, the recommended cultivars are 'Tsuki Yori No', 'Record' and 'Veritable'.

Table 2. The number of branches /plant

No. var.	Cultivar	Average no. branches	Relative value (%)	± d (no.)	Significance of differences
1	Record	12.67	136.410	3.38	**
2	Tsuki Yori No	15.33	165.128	6.04	***
3	Procyon	9.00	96.923	-0.29	n.s.
4	Veritable	12.67	136.410	3.38	**
5	Purple Gem	6.00	64.615	-3.29	oo
6	Colour Spectable	6.00	64.615	-3.29	oo
7	Princess	3.33	35.897	-5.96	ooo
8	Josephine	9.30	100.107	0.01	n.s.
Control	Average of the experience	9.29	100.00	-	-

LSD5% =2.25 LSD 1% = 3.16 LSD 0.1% = 4.46

The length of the floral stem

The longest length of the flower stem is displayed by the cultivar 'Colour Spectable', with an average of 84.13 cm, followed by the cultivar 'Veritable', with 78.49 cm. The smallest flower stalk average was presented by the 'Princess', with 33.63 cm (Table 3).

Table 3. The length of the floral stem

No. var.	Cultivar	Average stem length (cm)	Relative value (%)	± d (cm)	Significance of differences
1	Record	42.45	69.84	-18.33	oo
2	Tsuki Yori No	66.62	109.61	5.84	n.s.
3	Procyon	64.08	105.43	3.30	n.s.
4	Veritable	78.49	129.14	17.71	**
5	Purple Gem	52.30	86.05	-8.48	o
6	Colour Spectable	84.13	138.42	23.35	***
7	Princess	33.63	55.33	-27.15	ooo
8	Josephine	64.54	106.19	3.76	n.s.
Control	Average of the experience	60.78	100.00	-	-

LSD 5% = 7.62 LSD 1% = 12.71 LSD 0.1% = 20.01

Compared to the control of this character, the average of the experience (60.78 cm), very significant positive differences are observed in the 'Colour Spectable' cultivar and distinctly significant positive differences in the 'Veritable'. Three of the eight cultivars analyzed ('Tsuki Yori No', 'Procyon' and 'Josephine') do not show significant differences compared to the average of the experience.

At the opposite pole, very significantly negative differences of this character are registered in the 'Princess' cultivar, while the 'Purple Gem' cultivar registers significantly negative differences.

As a general opinion, we can say that the varieties with the highest average flower stem length are representative and recommended for use as cut flowers, and those with the lowest average are recommended for use in green spaces.

The number of flowers /plant

One of the most important characteristics that recommend the cultivation of flowers is the number of inflorescences that appear throughout the vegetation period. In Table 4, it can be seen that the cultivar 'Colour Spectable' has the highest average number of flowers per plant (53.2), followed by 'Procyon' with 44.9 flowers per plant, while the cultivars 'Princess' (34.6), and 'Veritable' (35.7) have the lowest number of flowers.

Table 4. The number of flowers /plant

No. var.	Cultivar	Average flowers/plant no.	Relative value (%)	± d (no.)	Significance of differences
1	Record	40.3	100.34	-0.9	n.s.
2	Tsuki Yori No	42.8	103.88	1.6	n.s.
3	Procyon	44.9	108.98	3.7	*
4	Veritable	35.7	86.65	-5.5	oo
5	Purple Gem	39.0	94.66	-2.2	n.s.
6	Colour Spectable	53.2	129.13	12.0	***
7	Princess	34.6	83.98	-6.6	ooo
8	Josephine	38.9	94.42	-2.3	n.s.
Control	Average of the experience	41.2	100.00	-	-

LSD 5% = 3.5 LSD1% = 4.6 LSD 0.1% = 6.1

The average of the experience for the analyzed characteristic among the eight analyzed cultivars had the value of 41.2 flowers/plant.

Following the analysis of the results, it is observed that compared to the average of the experiment considered as a control, the 'Colour Spectable' show a real and very significant positive difference, respectively positively significant ('Procyon') regarding the number of flowers per plant, while at the opposite pole the cultivar 'Princess' shows a very significant negative difference, and 'Veritable' shows a distinctly significant negative difference, compared to the average experience of the studied character.

The comparison made allows us to formulate a general conclusion, the 'Colour Spectable' and 'Procyon' cultivars had the best behavior in the experimental conditions regarding the number of flowers and there is a chance of achieving some successful crops, at the opposite pole finding the cultivars 'Princess' and 'Veritable'.

The diameter of the flowers

The diameter of the inflorescences is of great importance from a decorative point of view, being a character that influences the choice of flowers for use as cut flowers or in landscaping projects. According to this experience we can observe (Table 5) the largest diameter in 'Colour Spectable' (24.9 cm), 'Tsuki Yori No' (18.8 cm) and 'Veritable' (17.2 cm), and the smallest in the cultivars 'Princess' (9.4 cm), 'Record' (10.5 cm), 'Procyon' (10.9 cm) and 'Purple Gem' (11.6 cm).

Table 5. The diameter of the flowers

No. var.	Cultivar	Average diameter flower (cm)	Relative value (%)	± d (cm)	Significance of differences
1	Record	10.5	71.57	-4.17	ooo
2	Tsuki Yori No	18.8	128.15	4.13	***
3	Procyon	10.9	74.30	-3.77	ooo
4	Veritable	17.2	117.25	2.53	***
5	Purple Gem	11.6	79.07	-3.07	ooo
6	Colour Spectable	24.9	169.73	10.23	***
7	Princess	9.40	64.08	-5.27	ooo
8	Josephine	14.1	96.11	-0.57	oo
Control	Average of the experience	14.67	100.00	-	-

LSD 5% = 0.4 LSD 1% = 0.5 LSD 0.1% = 0.7

The average of the experience for the analyzed characteristic among the eight analyzed cultivars had the value of 14.67 cm. The diameter of the dahlia flowers taken in the study is presented in two groups following the data analysis, thus in the cultivars with diameter higher than experience average ('Tsuki Yori No', 'Veritable', and 'Colour Spectable'), and the cultivars with diameter lower than experience average ('Record', 'Procyon', 'Purple Gem' and 'Princess').

The number of ligules/flower

Analyzing the Table 6, with the exception of the 'Tsuki Yori No' cv, which has an average of 150.2 ligules per flower, being in the position of higher, and the 'Purple Gem' cultivar, was in the lowest position, with an average of 93.7 ligules per flower.

Table 6. The number of ligules/flower

No. var.	Cultivar	Average ligules no.	Relative value (%)	± d (no.)	Significance of differences
1	Record	115.6	97.43	-3.05	n.s.
2	Tsuki Yori No	150.2	126.59	31.5	***
3	Procyon	110.3	92.96	-8.35	n.s.
4	Veritable	124.5	104.93	5.85	n.s.
5	Purple Gem	93.7	78.97	-24.95	oo
6	Colour Spectable	115.1	97.01	-3.55	n.s.
7	Princess	139.0	117.15	20.35	*
8	Josephine	100.8	85.00	-17.85	n.s.
Control	Average of the experience	118.65	100.00	-	-

LSD 5% = 19.2 LSD 1% = 23.1 LSD 0.1% = 29.7

According to the results of the statistical analysis, most cultivars do not show a significant difference compared to the average of the experience taken as a control, these cultivars being: 'Record', 'Procyon', 'Veritable', 'Colour Spectable' and 'Josephine'. A very significant positive difference regarding the analyzed character is highlighted in 'Tsuki Yori No', and significant negative differences are presented by the cultivar 'Purple Gem' where the

number of ligules presents distinctly significant negative differences compared to the average of the experience (control). Consequently, we can say that all cultivars can be recommended from the point of view of the number of ligules, except for the 'Purple Gem'. The evaluation of morho-decorative characteristics was also carried out by other researchers in other genera, such as: *Hemerocallis* (Bahrim *et al.*, 2019), *Gladiolus* (Horț *et al.*, 2014), *Gerbera* (Drăghici *et al.*, 2020), *Pelargonium* (Moldovan *et al.*, 2021), which confirm a good adaptability at the crops conditions, fact that supports the recommendation of their extension in the culture.

Studies on the content of soluble dry matter and chlorophyll at *Dahlia*

The content of soluble dry matter in tuberous roots

The study of the present experiment focused on the determination of the content of soluble dry matter in the tuberous roots of *Dahlia* at harvest.

Table 7. The average content of soluble dry matter (%) in the tuberous roots of *Dahlia*

No. var.	Planting material	Cultivar	Average soluble dry content (%)*
V ₁	Tuberous roots	Record	24.81AB
V ₂		Tsuki Yori No	24.37 AB
V ₃		Procyon	15.13 DE
V ₄		Veritable	19.53 ABCD
V ₅		Purple Gem	15.82 CDE
V ₆		Colour Spectable	20.23 ABC
V ₇		Princess	11.63 E
V ₈		Josephine	22.57ABC
			SD 5% = 6.57-7.74

**The difference between any two values followed by at least one common letter is insignificant*

Analyzing Table 7, it can be seen in the case of cultivars 'Princess', 'Procyon' and 'Purple Gem' that the average value of soluble dry matter in the tuberous roots was lower. Significant higher differences are recorded between the V₁ (24.81 %), V₂ (24.37 %) and the variants V₇ (11.63 %), V₃ (15.13 %) variants which are different in value. The variants V₇ - 'Princess' (11.63 %), V₃ - 'Procyon' (15.13) and V₄ - 'Purple Gem' (15.82 %) differ significantly less than the other variants. The results obtained are similar to those obtained by Ciobanu *et al.* (2016) which identifying a greater amount of dry matter in dahlia variants whose tuberous roots were unforced before planting 'Kennemerland' (24.27%) and 'Tsuki Yori No` (24.80 %). Baldini *et al.* (2000) found a wide variability in the genotypes of *Helianthus tuberosus* studied, obtaining an average value of 22.9 % for soluble dry matter.

Chlorophyll content of leaves

The amount of chlorophyll in the leaves is important in the photosynthesis process of plants, having two essential actions: light absorption and the transformation of light energy into chemical energy. Following the research (Table 8), it can be seen that the cultivar 'Tsuki Yori No' had the highest chlorophyll content, with a value of 524.89 μmol/m², and the lowest value was determined in the 'Josephine', 375.23 μmol/m².

Table 8. Chlorophyll content of *Dahlia* leaves

No. var.	Cultivar	Average chlorophyll ($\mu\text{mol}/\text{m}^2$)	Relative value (%)	$\pm d$ ($\mu\text{mol}/\text{m}^2$)	Significance of differences
1	Record	400.87	87.66	-57.30	o
2	Tsuki Yori No	524.89	114.78	67.59	*
3	Procyon	494.22	108.074	36.92	n.s.
4	Veritable	439.27	96.06	-18.03	n.s.
5	Purple Gem	497.06	108.69	39.76	n.s.
6	Colour Spectable	518.55	113.39	61.25	*
7	Princess	408.31	89.29	-48.99	n.s.
8	Josephine	375.23	82.05	-82.30	oo
Control	Average of the experience	457.30	100.00	-	-

LSD 5% = 56.17 LSD 1% = 72.92 LSD 0.1% = 90.95

The statistical analysis allows us to observe the fact that four of the eight cultivars show non-significant differences compared to the control ($457.30 \mu\text{mol}/\text{m}^2$), and the cultivars 'Tsuki Yori No' and 'Colour Spectable' show significant positive differences compared to the control, while 'Record' shows significant negative differences, and 'Josephine' distinctly significantly negative compared to the control in terms of chlorophyll content.

CONCLUSIONS

Following the observations and determinations made, it is found that the cultivars of *Dahlia* 'Colour Spectable', 'Tsuki Yori No' and 'Veritable' stood out for the large number of flowers and their diameter, great vigor and well branched.

The cultivars 'Record', 'Tsuki Yori No', 'Josephine' and 'Colour Spectable' stood out in terms of the highest amount of soluble dry matter in the tuberous roots and the chlorophyll content showed significant differences in the cultivars 'Tsuki' Yori No' and 'Colour Spectable'.

We conclude that the dahlia is a crop with great potential in Romania floriculture thus, it is evident the need to improve the Romanian assortment with new cultivars which adapted to the ecological conditions, and production demands. Expanding the number of types, cultivars, colors and sizes offers a strong possibility of increasing production, and its quality.

REFERENCES

1. Ardelean M., Sestraș R., Cordea M. (2007). Horticultural experimental technique. Ed. AcademicPres Cluj.
2. Bahrim C., Chelariu E.L., Brînză M., Bernardis R.R. and Draghia L. (2019). Ornamental characterization of some *Hemerocallis* L. cultivars. Scientific papers-series B-Horticulture, vol. 63(1):479-484.
3. Baldini M., Danuso F., Turi M. and Vannozzi G.P. (2000). Variability for characters related to inulin accumulation in topinambur (*Helianthus tuberosus* L.). 15th ISC Toulouse Proceedings.
4. Bye R. and Linares E. (2008). La Dalia, flor nacional de México. CONABIO. Biodiversitas, vol. 76:13-15. (cite by Mariña, 2015).
5. Ciobanu (Țurlea) E.C., Bădulescu L., Badea M.L. and Toma F. (2021). Studies and research on the species and varieties of *Dahlia* in cultivation, Scientific Papers. Series B, Horticulture. Vol. LXV, No. 1:683-688.
6. Ciobanu (Moldovan) I., Cantor M., Buta E., Gocan T. and Crișan I. (2016). Influence of cultivar and planting material on soluble dry matter content of *Dahlia* tuberous roots. Bulletin USAMV Cluj Horticulture 73(2):135-140.

7. Costa P.A., De Souza D.C., Ossani P.C., Mendes M.H.A., De Souza Silva M.L., Carvalho E.E.N. and Resende L.V. (2022). Nutritional and functional compounds in dahlia flowers and roots. *Braz. J. Food Technol.*, Campinas, v. 25, e2022029, 2022 | <https://doi.org/10.1590/1981-6723.02922>.
8. Drăghici D.E., Petra S., Toma F. and Jerca O.I. (2020). The influence of substrate type on growth and flowering of *Gerbera* plants. *Scientific papers, series B-Horticulture*, LXIV(1):569-577.
9. Fălțiceanu M. and Munteanu N. (2006). Useful plants for your garden. Ed. Tipo Moldova, Iași.
10. Horț D., Cantor M., Buta E. and Zaharia A. (2014). The monitoring and evaluation of *Gladiolus* genitors for the Romanian breeding programme, *Journal of horticulture, Forestry and biotechnology* 18(2):57-60.
11. Lara-Cortés E., Martín-Belloso O., Osorio-Díaz P., Barrera-Necha L.L., Sánchez-López J.A. and Bautista-Baños S. (2014). Antioxidant capacity, nutritional and functional composition of edible *Dahlia* flowers. *Revista Chapingo Serie Horticultura*, 20(1):101-116.
12. Lord T. (2003). *Flora, The Gardener's Bible*, vol. I (A-K). Ed. Cassell, London, UK, p 466.
13. Mariña L.J. (2015). Cultivation of the dahlia-review. *Cultivos Tropicales*, vol. 36, no. 1:103-110.
14. Mejía-Muñoz J.M., De Luna-García I., Jiménez-Ruiz E.F., Sosa-Montes E., Flores-Espinosa C., Treviño-De Castro G. and Reyes-Santiago J. (2020). Research on dahlia, the national flower of Mexico. *Acta Horticulturae*, (1288):103-108. <http://dx.doi.org/10.17660/ActaHortic.2020.1288.15>.
15. Mlcek J. and Rop O. (2011). Fresh edible flowers of ornamental plants: A new source of nutraceutical foods. *Trends in Food Science & Technology*, 22(10):561-569. <http://dx.doi.org/10.1016/j.tifs.2011.04.006>.
16. Moldovan I., Szekeley-Varga Z. and Cantor M. (2017). *Dahlia* an unforgettable flower - a new perspective for therapeutic medicine. *Hop and Medicinal Plants*, Year XXV, No. 1-2, 2017.
17. Moldovan A., Mitre V. and Odagiu A. (2021). Monitoring the qualitative indices in „Tango Salmon” variety of *Pelargonium zonale* (L.) L'her., *ProEnvironment* 14(16):62-65.
18. Sho O., Munetaka H. Misa, K., Yoshikuni K., Atsushi H., Fumi T., Motoaki D. and Susumu Y. (2011). Simultaneous post-transcriptional gene silencing of two different chalcone synthase genes resulting in pure white flowers in the octoploid dahlia. *Planta* 234: 945-958.
19. Toma F. (2009). *Floriculture and floral art*, vol 4. Bucharest, RO, Ed. Invel Publishing House, pp. 93-94.
20. Toma F. and Petra S. (2020). *Floriculture and Floral compositions*. Ed. Total Publishing, pp. 317-321.
21. Treviño G., Mera O.L.M., Bye B.R., Mejía M.J.M. and Laguna C.A. (2007). Historia de la Dalia (*Acocoxóchitl*) la flor nacional de México. México, DF: Servicio Nacional de Inspección y Certificación de Semillas citate by Costa et al., 2022.
22. Țurlea (Ciobanu) E.C., Georgescu M., Badea M.L., Ciobanu M.I., Petra S. and Toma F. (2023). Aspects regarding the anatomy of the stem and lifetime as cut flowers of some *Dahlias* cultivars. *Scientific Papers. Series B, Horticulture*. Vol. LXVII, No. 2:490-496. Print ISSN 2285-5653.
23. ***<https://www.paris.fr/en/pages/50-000-new-dahlias-created-for-the-paris-2024-games-27930>.
24. ***<https://www.dahlia.org/growing/the-dahlia-as-a-cut-flower/>.

Pasture with scattered trees

R. M. Tăslăvan^{1,2}, Zs. Szekely-Varga^{1*}, I. Lihăt^{1,3} and E. Kentelky^{1*}

¹Sapientia Hungarian University of Transylvania, Department of Horticulture,
Faculty of Technical and Human Sciences, Târgu Mureș, Romania

²Technical University of Cluj-Napoca, Romania

³“Ion Mincu” University of Architecture and Urban Planning Bucharest,
Romania

*Corresponding author email: kentelky@ms.sapientia.ro; zsolt.szekely-varga@ms.sapientia.ro

ABSTRACT

The "pasture with scattered trees or wooded grasslands" system are traditional land management method that preserves valuable trees, such as fruit-bearing species, on agricultural lands. These trees thrive due to direct sunlight and space, leading to broad, symmetrical crowns. Animals benefit from the trees' shade, while their presence helps fertilize the soil. The trees also contribute to biodiversity, enhancing the agricultural landscape's ecological balance. This system has historical significance in regions like Transylvania, where village laws in the 17th and 18th centuries protected fruit trees. Despite its benefits, traditional landscapes like these are increasingly threatened by modern agricultural practices that favor land homogenization and intensive farming, neglecting the environmental and cultural values provided by scattered trees. Efforts to conserve these landscapes involve engaging local communities, promoting sustainable grazing practices, and recognizing the cultural significance of ancient trees. Pastures with scattered trees offer natural, economic, and aesthetic benefits, providing a sustainable model of land management that balances agriculture and environmental protection. However, challenges include changes in land use and policies that discourage tree preservation, threatening the resilience of these landscapes and their socio-ecological systems.

Keywords: biodiversity, ecological balance, land management, pasture, scattered trees.

INTRODUCTION

The "pasture with scattered trees" is a traditional land management system where valuable trees, such as fruit-bearing species (wild apple, wild pear, rowan, cornelian cherry, sweet chestnut, hazel etc.), are preserved on arable lands or pastures, even after deforestation. These trees are maintained for the benefits they provide to both agricultural productivity and biodiversity. Scattered trees have direct access to sunlight, unobstructed by other trees, which promotes the development of broad, symmetrical crowns. The soil around these trees is naturally fertilized by animals seeking shelter in their shade, while the rubbing of trunks by animals helps eliminate harmful insects. Unlike trees in forests, these trees benefit from ample space both vertically and horizontally, allowing for harmonious growth and the creation of a unique rural landscape characterized by

imposing, well-formed trees. Beyond their aesthetic value, pastures with scattered trees support considerable biodiversity, thereby contributing to the protection of local ecosystems and the conservation of biological diversity. Scattered woody vegetation in open landscapes leads to a great increase in overall biodiversity and a diversification of ecosystem services (Erdos *et al.*, 2018).

This model of agro-ecological management achieves a balance between agricultural use and environmental protection, enhancing soil fertility, reducing erosion risks, and ensuring a balanced microclimate. Scattered tree pastures are an example of sustainable practice, providing benefits to both agriculture and the environment. In Transylvania, such trees are commonly found, even in village gardens, intentionally preserved for their economic and ecological value. The tradition, dating back to the 17th and 18th centuries, when Szekler village laws forbade the cutting of fruit trees, protected these essential resources. In the Harghita Mountains, specific customs allow the claiming of fruit-bearing trees by marking them with branches, straw, or piles of stones.

However, according to local traditions, this marking does not prevent others from accessing the tree's fruit. Foreign travellers, migratory shepherds, mineral water vendors, and potters have the right to harvest a portion of the fruit from marked trees without violating customary rules. These informal regulations reflect a longstanding tradition of communal cooperation and sustainable management of natural resources, respected for centuries in this region (www.arcanum.com).

Scattered trees and the marking of property on wild fruit-bearing trees can be considered early forms of organized fruit cultivation, paving the way for artificial selection. The protection of these trees and the transmission of the marking tradition represented a crucial step in the development of agricultural practices, which later evolved into intensive and controlled fruit tree cultivation. The traditional rural landscapes of Eastern Europe, including Romania, serve as reservoirs of diverse native vegetation, traditional ecological knowledge, and local skills. This agricultural heterogeneity, with small plots, diverse crops, and scattered trees on meadows and pastures, provides not only natural and cultural value but also multiple benefits for local communities, such as enhancing biodiversity and ecosystem potential (Fischer and Tibor, 2012; Molnár, 2014).

Despite their multiple values, traditional agricultural landscapes are increasingly threatened by changes in land use. The homogenization of these landscapes, driven by monofunctional management, leads to the loss of local skills and knowledge, as well as the values associated with the multifunctional management of landscapes. This process represents a critical vulnerability factor for the resilience of local socio-ecological systems (Baumgartner, 2007).

Trees in agricultural landscapes are classic examples of vegetative structures that synergistically enhance the multiple values of traditional farmlands, including aesthetic and cultural benefits, provisioning services, and biodiversity. Traditionally, scattered trees have played a crucial role in agricultural landscapes, being utilized for their fruit, leaves (as fodder), or wood, while also providing shade for animals and contributing to the improvement of soil fertility (Plieninger, 2012).

Scattered trees, of various species and ages, are essential elements of the agricultural landscape's identity. However, in modern agriculture, dispersed woody vegetation is often "eliminated" because the values these trees provide—whether aesthetic, ecological, or economic—are not perceived as sufficiently important. Additionally, official agricultural policies discourage the maintenance and regeneration of trees on farmland, as they are not considered vegetative components with high and consistent production yields (Beaufoy, 2015).

Human societies have undergone major changes over the past centuries, weakening the traditional ties between communities and their landscapes, including pastures with

scattered trees. Population growth, industrial development, urbanization, and globalization have led to landscape modifications to meet new social and economic demands. These transformations have also significantly impacted rural communities in Eastern Europe, which, although more isolated, have nonetheless felt the effects of these changes.

Grazing in European forests has been practiced for centuries, influenced by specific socio-economic and ecological conditions. In the past two centuries, these conditions have changed drastically, profoundly affecting traditional grazing practices in forests and pastures with scattered trees throughout most of Europe. Today's pastures with ancient trees in Europe likely represent only a small fraction of the "functional" pastures that existed centuries ago. For instance, in Hungary, wooded pastures were once widespread, but today they have been reduced to just over 5,000 hectares, managed exclusively for nature conservation (Varga and Bölöni, 2009). In Germany, only approximately 5,500 hectares of wooded pastures have been confirmed, although optimistic estimates suggest that the area could reach up to 100,000 hectares (Luick, 2009). In the Netherlands, nearly all forests were cleared in the 17th century, and today there are approximately 31,000 hectares of landscapes with ancient trees, including orchards, most of which are plantations from the 19th and 20th centuries (Oosterbann, 2009). In the Czech Republic, wooded pastures are almost non-existent (Horák, 2012), while in central Romania, ancient wooded pastures still persist.

MATERIALS AND METHODS

The natural forests of the region, primarily composed of *Quercus* - *Carpinus* and *Fagus* - *Carpinus* associations, are still present, although significantly altered from their original state. In the past, these forests covered slopes and valleys at higher altitudes, dominated by *Carpinus betulus*, a species heavily exploited by local communities. Oak (*Quercus* spp.), Sycamore (*Acer pseudoplatanus*), and Linden (*Tilia cordata*) completed this forest community, typical of Central Europe. In steeper areas, *Fagus* was dominant, adapted to harsher conditions. These forests reflect a balance between ecological influences and human interventions, resulting in mixed forest landscapes, with some relatively intact areas and others modified by traditional management.

Vegetation is an essential component of the natural environment (Iliescu, 2003). This vegetational composition reflects the adaptation of species to well-drained soils capable of retaining moisture, while the diversity of herbaceous species highlights the close ecological connections between these forest habitats and the Dacian landscapes. The *Fagus sylvatica* and *Carpinus betulus* forests on the slopes of the Carpathians form a robust ecosystem, essential for maintaining biodiversity and the ecological balance of the region.

Crataegus, *Prunus spinosa*, and Black Locust (*Robinia pseudacacia*) invade pastures, particularly where cattle graze instead of sheep, and could form extensive thickets if grazing levels decline. Farmers control the invasion of *P. spinosa* through regular burning. An analysis of these thickets reveals associations from the Prunetalia order, such as *Pruno spinosae* - *Crataegetum* and *Ligustro* - *Prunetum*. These spiny thickets are common in the Pannonian Basin and adjacent regions, such as the Banat Hills, the Transylvanian Plateau, and the Southern Carpathians (Sanda and Popescu, 1980; Mountford and Akeroyd, 2005). The wetland vegetation in this landscape indicates ecological diversity adapted to local hydrological conditions. In areas where calcium-rich water has produced springs, a specialized flora has developed. Along the banks of watercourses, *Salix* shrubs dominated, and the floodplain was covered by *Carex* and *Phragmites*. The less flood-prone edges of the floodplain were influenced by mowing and grazing, promoting the development of tall

riparian meadows. The pastures with scattered trees exhibited high floristic diversity, including species adapted to nutrient-rich soils.

RESULTS AND DISCUSSIONS

The analyzed scattered tree pasture is located in the (ro: Becheciul de Jos/hu: Bekecsalja) area, between the villages of Drojdii and Bereni, near the right bank of the Nirajul Mic stream in Mureş County. This region covers an area of approximately 1.5 square kilometres, situated on the northern slopes of the hills, in a transitional zone between pasture and forest ecosystems. To the north and west, the pasture is bordered by mixed deciduous forests, characterized by a diversity of species typical of this climatic zone. To the south, the area is bordered by a forest strip following the course of the Nirajul Mic stream, providing an ecological transition between the streambed and the pasture. On the eastern side, the pasture is framed by a continuous association of trees and shrubs.

The pasture consists of several terraced areas, due to the relief that varies as one move from the stream to the north. These terraces are characterized by alternating steep slopes and relatively flat surfaces, creating a diverse topographical landscape. On the first terrace, located at the base of the area, there is a sheepfold (the seasonal shelter of shepherds) and the night pen for sheep. This location is strategically chosen due to its accessibility, being easily reachable from County Road 135, an important element for pastoral activities.

Near this sheep pen, numerous wells and water troughs can be observed, installed to provide a constant water source for animals. Around these water sources, due to the increased humidity, hygrophilous vegetation associations have appeared, meaning plants that prefer wet habitats. The presence of these plants is a valuable ecological indicator, historically used by shepherds to locate water sources. During grazing, shepherds would follow these vegetative signs to guide sheep to the best spots for feeding and hydration. Even at this level, there are old trees, some reaching 60, 80, or even 100 years. Surprisingly, these trees are in remarkable health with relatively intact crowns. Their utility and importance for scattered tree pastures are best highlighted by aerial captures made with a drone at the right time (Figure 1–2). During the heat of the day, sheep briefly shelter in the shade of the trees while continuing to graze, underscoring the vital role these trees play in maintaining a suitable environment for grazing.



Figure 1. Aerial captures made with a drone.

Regarding the exact age of the trees, we can only rely on available sources (Figure 2). The fact that similar pastures are shown on maps made by the Hungarian Military Plan in 1941 (Figure3 4) supports the hypothesis that some existing trees are approaching 100 years or more. Furthermore, cartridges from the Austro-Hungarian troops during World War I were discovered under some trees. Since these cartridges were found only near the trees and not in the open areas of the pasture, it is plausible that soldiers took shelter and fired from the shade of these trees.



Figure 2. The present situation of the scattered trees (<https://earth.google.com/>).



Figure 3. The Hungarian Military Plan from 1941 (maps.arcanum.com).

Similar discoveries were made with other mature and older trees, highlighting their historical significance in the local landscape (Figure 4).



Figure 4. Shooting Master Plan (<http://igrek.amzp.pl>).

The map indicates a well with a drawbar, and a similar well still exists in the same area today, emphasizing the continuity of the traditional function of the site. Although it cannot be confirmed that it is the same structure from 80 years ago, similar drawbar wells are increasingly rare in the Nirajul Mic Valley. These structures maintain a practical and cultural link to the past.

The well is situated on a natural rise, offering impressive views of the landscape. From this point, the medieval church tower in Bâra and Beheci Peak (ro: Vârful Beheci/hu: Bekecs tető), a significant geographical landmark for the region, can be seen.

The long-term challenge for local communities will be recognizing the importance of these pastures and implementing concrete measures for their conservation. Protecting these pastoral landscapes is essential not only for biodiversity but also for preserving the agricultural traditions passed down through centuries. Sustainable resource management and raising public awareness will be crucial for maintaining these valuable ecosystems. Our suggestions are in accordance with the results obtained by Tolgyesi *et al.*, 2023 which states that existing the free-standing trees should be preserved, with their centuries-old, massive carbon stock and the many microhabitats and additional ecosystem services they provide.

This can be achieved through strategies to delineate grazing areas where grazing is prohibited or temporarily reduced, and/or by manually planting spiny shrubs to protect seedlings, allowing grazing around them. The effectiveness of these methods can be tested and compared with control sites. However, the success of these initiatives depends on local social support and adaptation to community needs. Testing the effect of different types of farm animals on vegetation structure and biodiversity is another approach. In this case, traditionally cattle-grazed wooded pastures can be compared with those grazed by sheep and abandoned wooded pastures (Figure5).

Assessing the openness of local communities to value and protect old trees (including dead ones) in wooded pastures.

Identifying ways to promote the social value of wooded pastures for local communities (Hartel, 2014).



Figure 5. Traditional grazed wooded pastures.

CONCLUSIONS

The present research underscores the essential role of "pastures with scattered trees" as a traditional land management system that promotes both agricultural productivity and biodiversity. By preserving valuable tree species, particularly those bearing fruit, these pastures play a significant role in enhancing ecosystem health, improving soil fertility, and fostering a balanced microclimate. However, despite their ecological and economic advantages, these landscapes are increasingly threatened by modern agricultural practices and land-use changes that favor monoculture over biodiversity.

The study reveals that traditional customs, which historically played a crucial role in the protection of these trees, are deteriorating in the face of population growth and industrialization. The existence of ancient wooded pastures in regions such as central Romania highlights the necessity of incorporating sustainable practices into contemporary agricultural methods to safeguard these vital ecosystems. The findings indicate a pressing need for community engagement and awareness-raising initiatives to facilitate the conservation of these unique landscapes, accentuating the importance of effective management strategies that harmonize agricultural requirements with ecological preservation.

Future initiatives should aim to elevate the social value of scattered tree pastures and acknowledge their contributions to local culture, biodiversity, and agricultural sustainability. By implementing strategies designed to protect these ecosystems, it is possible to preserve biodiversity while simultaneously supporting the agricultural traditions that have endured for centuries.

REFERENCES

1. Baumgartner S. (2007). The insurance value of biodiversity in the provision of ecosystem services. *Natural Resource Modeling*.
2. Beaufoy G. (2015). Europe's wood-pastures: condemned to a slow death by the CAP? A test case for EU agriculture and biodiversity policy. Booklet produced for the wood-pasture policy seminar in the European Parliament, Brussels, 17th November, 2015.
3. Erdős L., Kröel-Dulay G., Bátori Z., Kovács B., Németh Cs., Kiss P.J. and Tölgyesi C. (2018). Habitat heterogeneity as a key to high conservation value in forest-grassland mosaics. *Biological Conservation* Vol. 226:72–80.
4. Fischer J. and Hartel T. (2012). Conservation policy in traditional farming landscapes. *Conservation Letters*.
5. Google Earth. <https://earth.google.com/>. (accessed on 2024.10.04).
6. Hartel T. (2014). Wood pastures in Southern Transylvania, Romania, Cluj-Napoca.
7. Horák J. (2012). The species richness of click beetles in ancient pasture woodland benefits from a high level of sun exposure. *Peterborough*.
8. Iliescu A.F. (2003). Landscape architecture. Ed. Ceres Bucharest, 189–190.
9. Luick R. (2009). Wood pastures in Germany. Springer.
10. Mapster. <http://igrek.amzp.pl>. (accessed on 2024.10.02).
11. Molnár Zs. (2014). The role of traditional ecological knowledge in managing wood-pastures. Abingdon (UK).
12. Mountford O. and Akeroyd R. (2005). A biodiversity assesment of the saxon villages region of Transylvania (Romania). *Transylv. Rev. Syst. Ecol. Res.* 2, "The Târnava River Basin".
13. Oosterbann A. (2009). Agroforestry in the Netherlands. Springer.
14. Plieninger T. (2012). Resilience and the cultural landscape: understanding and managing change in human shaped environments. Cambridge (UK), Cambridge University Press.
15. Sanda V. and Popescu A. (1980). Cenotaxonomy and chorology of plant groups from Romania. *Studies and communications (Cenotaxonomia și corologia grupurilor vegetale din România. Studii și comunicări)*, Muzeul Brukenthal 24.
16. Scattered trees. (Magyar Néprajzi Lexikon) <https://www.arcanum.com/hu/online-kiadvanyok/Lexikonok-magyar-neprajzi-lexikon-71DCC/h-7297A/hagyasfa-7298E/>. (accessed on 2024.10.05).
17. Tölgyesi Cs., Kelemen A., Batori Z., Kiss R., Habenczyus A.A., Havadtoi K., Varga A., Erdos L., Frei K., Toth B. and Torok P. (2023). Maintaining scattered trees to boost carbon stock in temperate pastures does not compromise overall pasture quality for the livestock . *Agriculture, Ecosystems and Environment* 351 (2023) 108477.
18. Varga A., Bölöni J. (2009). The landscape history of forest grazing, treed pastures and wood-pastures, (Természetvédelmi Közlemények). *HortScience*. 56:506-510. <https://doi.org/10.21273/HORTSCI15683-20>

The effect of alternating temperature treatment on seed germination of *Pulsatilla tongkangensis*

H.M. Kim¹, M. S. Roh², G. Burchi³ and Y.H. Joung^{1,*}

¹ School of Biological Sciences and Technology, Chonnam National University, Korea

² 4210 Westbrook Dr. 4210, Ann Arbor, USA

³ CREA Research Centre for Vegetable and Ornamental Crops, Pescia, Italy

*Corresponding author email: yhjyoung@chonnam.ac.kr

ABSTRACT

The effect of alternating temperature on the germination of *Pulsatilla tongkangensis* seeds was investigated. Open pollinated seeds were wrapped in a moist paper towel for alternating temperature treatments. Seeds were sown in cells filled with Miracle-Gro Potting Mix and then topped with 0.5 cm of Miracle-Gro Seed Starting Potting Mix. The number of germinated seeds was counted daily until no further germination was observed for 3 to 4 days. The maximum germination rate (49%) was achieved in 18 days when seeds were treated at 22°C for 7 days followed by 5 °C for 7 days, and germinated at 20°C. The low germination rate could result from the inclusion of empty seeds. Mature *P. tongkangensis* seeds may not have a shallow physiological dormancy since germination was not improved with 14 days of 5°C.

Keywords: dormancy, full seeds, germination rate, Pasque flower, X-ray imaging.

INTRODUCTION

The genus *Pulsatilla* Mill. contains approximately 33 species, which grow in a wide area including Asia, North America, and Europe (Wang and Bartholomew, 2001; USDA, Agricultural Research Service, National Plant Germplasm System, 2024). *Pulsatilla tongkangensis* Y. N. Lee et T. C. Lee (<https://www.ipni.org/n/20005221-1>; accessed 22 August 2023) is growing limited regions in Korea, specifically near calcified rock cavity cliffs and in well-drained areas (Lee *et al.*, 2010; Lee and Lee, 2014; Oh *et al.*, 2014). This species is cultivated as ornamentals because of bell-shaped showy flowers, and white seed head. However, this species is considered a hybrid population in both natural habitats analyzed with molecular markers (Lee *et al.*, 2010).

The germination rate of *P. tongkangensis* seeds is highest (73.35%) when harvested 28 days after artificial fertilization (An *et al.*, 2010). Seeds of *P. koreana* germinate in 14 days at 25°C (Sang *et al.*, 1993, 1996). The germination rates (<38%) is lower than the 72% reported previously by Yuan *et al.* (2019) and might result from the inclusion of non-viable seeds (Yuan *et al.*, 2021).

The optimum temperatures for *P. koreana* seed germination are reported as 20°C (Yuan *et al.*, 2021), 25°C (Sang *et al.*, 1993), and 30°C (Yoo *et al.*, 2020). Additionally, germination rates were higher at 25/15°C and 30/20°C compared to 20/10°C and 35/25°C (Yoo *et al.*, 2020). However, the effects of alternating temperature treatments, such as high-low-high cycles, have not been studied in *P. tongkangensis*. The objective of this research was

therefore to investigate the effect of alternating temperature treatments on seed germination of *P. tongkangensis*.

MATERIALS AND METHODS

Open-pollinated seeds of *P. tongkangensis* seeds were collected from Jungsun, Kangwon-do Province, Korea, and were tested in this experiment. *Pulsatilla tongkangensis* seeds, received on 21 May 2023, after removing filaments were wrapped in a moist paper towel for alternating temperature treatments. The seeds (Frame A, Figure 1) were kept at 22°C or 5°C and then potted after 22°C treatment, as indicated by S (Table 1). Seeds were then sown into conical-shaped hexagon cells (3.8 cm top diameter × 2.5 cm bottom diameter × 6.5 cm depth, Ferry-Morse Home Gardening, Norton, MA, USA) (Frame B, Figure 1). The number of germinated seeds was counted daily until no further germination was observed for 3 to 4 days. Each treatment was replicated twice, with 50 seeds per replication. Data were analyzed separately for each species using Microsoft Excel (Microsoft Corporation, 2019). Means were compared using Tukey's honestly significant difference (HSD) test to determine significant differences.

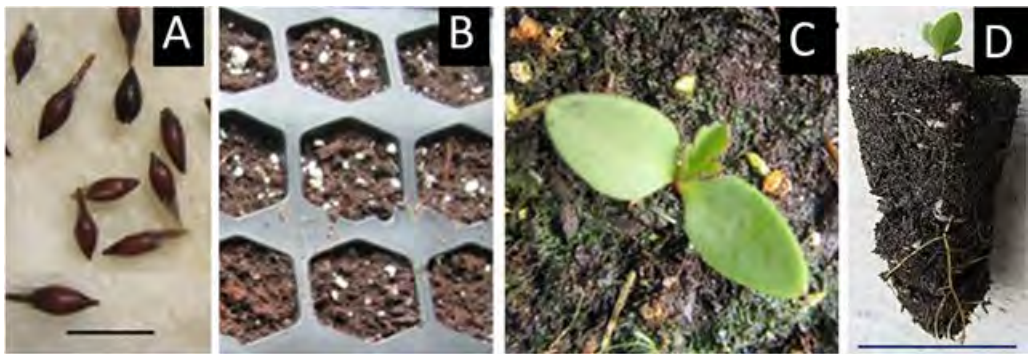


Figure 1. Preparation and germination of *Pulsatilla tongkangensis* seeds: Filaments were removed from seeds (frame A) and sown into conical-shaped hexagon cells (B), and germinated seeds were counted when cotyledon emerged from the sowing medium. Seeds that developed the first true leaves (C) and developed roots (D) were transplanted when seedlings formed 3 – 4 true leaves for subsequent experiments

RESULTS AND DISCUSSIONS

Pulsatilla tongkangensis seeds treated with moist treatment at 22°C for 28 days (Treatment 1; T1) exhibited an initial germination rate of 36.2% in 14 days, and plateaued in 21 days after sowing, which was significantly longer compared to the other treatments (Table 1). The highest final germination rate of 49.2% was observed when seeds were initially treated at 22°C for 7 days, followed by 5°C for 7 days, and 22°C (Treatment 2; T2). In accordance with other studies, the germination rate in this result was lower than the *in vitro* germination rate of 65% (An *et al.*, 2010) and the 72% germination rate in *P. koreana* (Yuan *et al.*, 2021). However, it is higher than the 38% germination rate observed after a 2-week at 5°C treatment reported by Yuan *et al.* (2019). The relatively low germination rate observed in this study could be partially attributed to the germination temperature of 22°C, which is lower than the optimal 25°C reported in *P. tongkangensis* (An *et al.*, 2010). A higher germination rate of 55.3% was also reported in *P. koreana* at a 30°C temperature (Sang *et al.*, 1993; Yoo *et al.*, 2020). Additionally, Wang *et al.* (2013) reported that the optimal temperature range for *P. turczaninovii* is between 25

and 30°C. The presence of empty seeds might have also contributed to the lower germination rates.

Table 1. *Pulsatilla tongkangensis* seeds received moist storage treatment at 22°C and 5°C at 7-day intervals

Treat- ment (T)	No. of days of temperature treatment (22 or 5°C) and sowing (S ^a)				No. of days to germination ^b		From A to B	Final germi- nation rates (%)
	0–7	8–14	15–21	22–28	First seed (A)	Reaching plateau ^c (B)		
T1	22 (S)	22	22	22	14	21	7	36.2
T2	22	5	22 (S)	22	8	18	10	49.2
T3	22	5	5	22 (S)	10	19	9	30.1
T4	5	22 (S)	22	22	7	19	12	26.2
T5	5	5	22 (S)	22	6	13	7	27.0
HSD 1% ^d					3.4	3.9	2.8	5.72

^a Seeds were sown (S) at the conclusion of the indicated 22°C treatment.
^b The number of days to germination was counted from the sowing dates and seeds germinated were recorded daily.
^c Recording of germinated seeds was terminated after reaching the plateau when there were no more seeds germinated for 3 to 4 days after reaching the plateau.
^d Tukey’s honestly significant difference (HSD) test ($P \leq 0.01$).

To improve the germination rate of *P. tongkangensis* to over 50%, alternating temperature treatments of 30/20°C, as reported in *P. koreana* (Yoo *et al.*, 2020), applying aaaaaaaa temperature of 30–35°C during the first 7 days followed by 5°C for 7 days could be beneficial (An *et al.*, 2010). Since germination was not improved with 14 days of 5°C treatment before or after 14 days at 22°C, mature *P. tongkangensis* seeds may not have a shallow physiological dormancy (Baskin and Baskin, 2004).

CONCLUSIONS

The maximum germination rate of *P. tongkangensis* (49.2%) was achieved within 18 days by treating seeds first at 22°C for 7 days, followed by 5°C for 7 days. The relatively low germination rate observed in this study could be attributed to the mixture of both full and empty seeds and the suboptimal germination temperature of 22°C. Mature *P. tongkangensis* seeds may not have a shallow physiological dormancy since germination was not improved with 14 days of 5°C

ACKNOWLEDGEMENT

This research was supported by the research grant awarded to Y.H. Jounɡ from the New Breeding Technologies Development Program (Project No. RS-2024-00322366) through the Rural Development Administration (RDA), Korea.
 Germplasm used in this project was provided by J.W. Kang, D.W. Suh, Y.K. Yoo, and M.C. Chung, H.M. Kim and Y.H. Jounɡ: was involved in the preparation of draft, review and editing, and received the English editing service, and finalize the manuscript for submission. G. Burchi: reviewed and edited the manuscript preparation. M.S. Roh: conceived the idea, performed the experiment, and finalize the manuscript for submission.

REFERENCES

1. An C.H., Lee H.S., Park Y.I., and Lee J.S. (2010). Habitat survey and seed germination test of *Pulsatilla tongkangensis* in Du-ta mountain, Sam-cheok City. Proceedings of the Korean Society of Environment and Ecology Conference. 20 (1): 59-61.
2. Baskin J.M., and Baskin C.C. (2004). A classification system for seed dormancy. *Seed Science Research*. 14, 1-16. DOI: <https://doi.org/10.1079/SSR2003150>
3. Lee A.K., Yuan T., Suh J.K., Choi D.S., Choi I.Y., Roh M.S., Joung Y.H., Ko J.Y., and Lee J.S. (2010). *Pulsatilla tongkangensis*, a natural hybrid swarm population hybridized with *P. koreana* based on RAPD and SNPs of chloroplast DNA. *Horticulture Environment and Biotechnology*. 51: 409-421. <http://www.horticulture.or.kr/english/>
4. Microsoft Corporation. (2019). Microsoft Excel, Available at: <https://office.microsoft.com/excel>.
5. Oh H.K., Lee K., Lee Y., and Choung Y. (2014). Geographical distribution and ecology in microhabitats of the rare species, *Pulsatilla tongkangensis* YN Lee et TC Lee. *Korean Journal of Ecology and Environment*. 47(spc), 74-82. (In Korean with English abstract). [http://dx.doi.org/10.11614/KSL.2014.47\(5\).074](http://dx.doi.org/10.11614/KSL.2014.47(5).074)
6. Sang C.K., Kim E.H., and Kim H.Y. (1993). Germination and life span of *Pulsatilla cernua* var. *koreana* seeds. *Journal of Korean Society for Horticultural Science*. 34:207-212 (In Korean with English Summary).
7. Sang C-K., Choi B-J., and Koh, J-C. (1996). Effect of storage humidity and temperature on seed germination of Pasque flower (*Pulsatilla cernua* var. *koreana*). *Journal of Korean Society for Horticultural Science*. 37: 47-450 (In Korean with English summary).
8. USDA Agricultural Research Service National Plant Germplasm System (2024). Germplasm Resources Information Network (GRIN-Taxonomy). National Germplasm Resources Laboratory, Beltsville, MD. Search Taxonomy GRIN-Global (ars-grin.gov). 19 Jan. 2024.
9. Wang W., and Bartholomew B. (2001). 27. *Pulsatilla* Miller, Gard. Dict. Abr., ed. 4, (1136). 1754. In *Flora of China* 6. 329-333.
10. Wang F., Zhang L-M, Zhang D., Yu X-m, and Song, H. (2013). Effect of different treatments on seed germination of *Pulsatilla turczaninovii* and *Pulsatilla dahurica*. *Northern Horticulture*. 2: 65-67 (In Chinese with English abstract).
11. Yoo Y.K., Roh Y.S., Yuan T., and Roh M.S. (2020). Germination of *Pulsatilla cernua* var. *koreana* seeds influenced by temperature, light, and GA₃ treatments. *Acta Horticulturae* . 129(1): 83-190. DOI:10.17660/ActaHortic.2020.1291.23
12. Yuan T., Wei Q., and Bauchan G. (2019). Germination of *Pulsatilla* seeds as influenced by seed morphology, moist 5° C and gibberellin (GA3) treatment, and detection of nickel in seeds. *HortScience*. 54: 2015-2023. <https://doi.org/10.21273/HORTSCI14056-19>
13. Yuan T., Wei Q., Jourdan P., and Yoo Y.K. (2021). Germination of *Pulsatilla cernua* var. *koreana* seeds as influenced by harvest dates and development of seeds analyzed by X-ray imaging.

Integrating Romanian traditional motifs with sustainable succulent landscapes

A.P. Cotoz, V.S. Dan and M. Cantor*

Department of Horticulture and Landscape Design, University of Agricultural Sciences and Veterinary Medicine of Cluj-Napoca, Romania

*Corresponding author email: maria.cantor@usamvcluj.ro

ABSTRACT

This study explores sustainable landscape design solutions within the Romanian cultural context by examining two distinct landscape concepts that incorporate succulent and perennial plants, mainly from the *Sedum* genus. Both designs integrate floral mosaics inspired by traditional Romanian motifs, employing innovative planting practices. Design 1 features a modern suspended floral mosaic, while Design 2 presents a larger, historically themed landscape integrated within an ethnographic museum setting. Each design utilized cultivars of *Sedum* species (*Sedum acre*, *Sedum spurium* and *Sedum spathulifolium*), showcasing the aesthetic, cultural, and practical implications of using succulent-based mosaics in landscape architecture. The study highlights their durability and minimal maintenance requirements, positioning them as viable options for sustainable urban landscaping.

Keywords: sedum plants, mosaics, traditional motifs, sustainable design

INTRODUCTION

The driving motivation behind this study is the growing demand for urban green spaces that contribute to both environmental sustainability and cultural expression (Semeraro *et al.*, 2021). In urban and historical contexts, green spaces serve a dual purpose: they improve environmental conditions, such as air quality and biodiversity (Kruize *et al.*, 2019), while also acting as public art installations that reflect local cultural traditions and aesthetics (Blessings, 2024; Vishal and Vuilliomen, 2021). By integrating cultural elements into green spaces, public art installations contribute to both the aesthetic appeal and the cultural vibrancy of urban environments, thereby enriching the experience of visitors and residents alike. These artistic interventions often evoke a connection to local cultural traditions and serve as platforms for storytelling and collective memory, as noted in the article (Blessings, 2024). Romanian traditional motifs, with their intricate patterns and strong ties to national identity, are especially well-suited for sustainable landscape design. This approach aims to blend ecological resilience with cultural heritage. A similar cultural fusion can be seen in public art displays like the renowned Flower Carpet event in Brussels. This event features elaborate floral designs crafted by artists using thousands of begonias, drawing inspiration from traditional motifs and highlighting the intersection of nature and art in celebrating cultural diversity (<https://flowercarpet.brussels/en>). Landscape design presents a unique opportunity to integrate cultural heritage with ecological sustainability, offering innovative solutions to modern environmental challenges. In Romania, traditional motifs found in textiles, ceramics, and other forms of folk art provide a rich source of inspiration for contemporary landscape architecture (Radu and Cantor, 2014). This study explores the use of succulent plants, particularly

species from the *Sedum* genus, to create floral mosaics that are not only visually striking but also environmentally sustainable.

By leveraging the durability and low-maintenance needs of succulents, these designs offer an aesthetic that merges traditional artistry with modern ecological concerns, demonstrating how cultural heritage can coexist with sustainable practices.

Historically, cultural exchange has played a pivotal role in the socio-economic development of societies and has had a lasting impact on professional fields such as landscape design. The evolution of garden design is a testament to this exchange of ideas. Ancient Egyptian geometric gardens influenced Greek landscaping, which in turn shaped Roman garden traditions. This chain of influence continued through to the development of French gardens, which subsequently inspired British landscape aesthetics, leading to a global reform in garden design (<https://www.britannica.com/art/garden-and-landscape-design/Historical-development>). One of the most prominent examples is the geometric gardens of Villandry in France, which display a blend of traditional French design elements with clear influences from Persian geometric motifs (Figure 1).

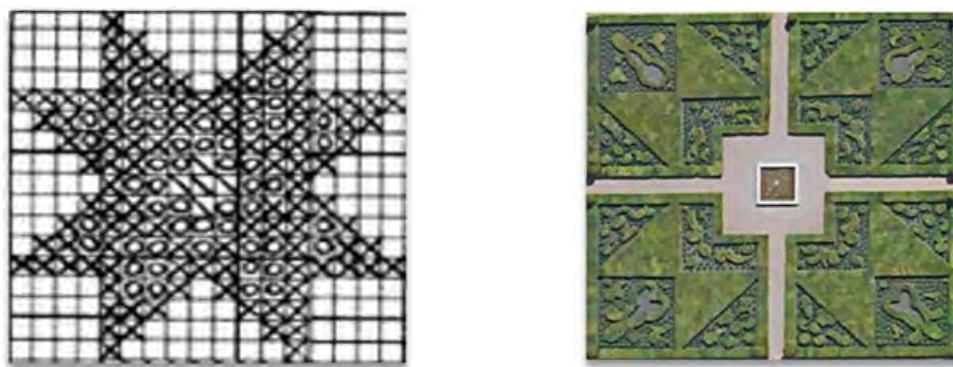


Figure 1. Traditional Persian motif (left) geometric landscape design of Villandry (right)
Source: Norbury, 1973; Google Earth

This study places particular emphasis on the use of *Sedum* species in green infrastructure, drawing on Romania's cultural heritage as a key design element. Cotoz (2024) states that *Sedums* species are highly versatile and adaptable to various environmental conditions, making them ideal ground cover plants. *Sedum* plants are known for their resilience, thriving with minimal water and maintenance (Durhman *et al.*, 2006; Wolf and Lundholm, 2008; Zaharia *et al.*, 2016), making them ideal candidates for sustainable urban landscaping. By incorporating these plants into floral mosaics that reflect Romania's traditional geometric motifs, this approach aims to create urban green spaces that are not only ecologically sound but also culturally meaningful.

MATERIALS AND METHODS

Two landscape design concepts were developed to explore the integration of tradition and sustainability, utilizing *Sedum* plants to create floral mosaics. The first design was implemented on the 180 m² south-facing terrace of the Advanced Horticultural Research Institute of Transylvania (A.H.R.I.T.) building at the University of Agricultural Sciences and Veterinary Medicine campus, while the second concept was set within a fictional historical context, covering 865 m².

Design 1: Suspended floral mosaic

Design 1 (Figure 2) was implemented on the 180 m² south-facing terrace of the A.H.R.I.T. building on the campus of the University of Agricultural Sciences and Veterinary Medicine of Cluj-Napoca. The primary objective was to create a modern, modular succulent

installation that reflected traditional Romanian patterns. To achieve this, the design employed a suspended garden structure with a geometric layout inspired by folk textile motifs.



Figure 2. Planting scheme within the floral mosaic

- **Plant selection:** Three varieties of *Sedum* were chosen for their distinct colors and growth habits: *Sedum spurium* 'Purpur Winter' (red), *Sedum spathulifolium* 'Cape Blanco' (white), and *Sedum spathulifolium* 'Purpureum' (dark purple). These varieties were selected to mimic the red, white, and black color schemes typical of Romanian embroidery patterns.
- **Implementation:** A suspended garden system was used to support the mosaic, ensuring that the installation remained lightweight yet structurally sound (Cotoz, 2024). The use of a specialized green roof substrate provided excellent drainage and aeration while supporting the growth of the succulents. The substrate was composed of a blend of organic and inorganic materials optimized for succulent health, ensuring long-term sustainability with minimal maintenance.
- **Layout:** The mosaic was designed in a grid pattern, with each square representing a different color block. This arrangement allowed for easy maintenance and replanting if necessary. The evergreen nature of the selected plants ensured year-round visual appeal, with seasonal flowering adding additional interest during certain periods.

Design 2: Historically themed landscape

Design 2 (Figure 3) was conceived as a ground-level installation in a fictive historical context, covering 865 m². The setting was a former noble family mansion, converted into an ethnographic museum, with the floral mosaic serving as the centerpiece of the landscape design (Cotoz, 2024).



Figure 3. Planting scheme within the floral mosaic

- Plant selection:** The second design utilized a broader range of *Sedum* species, including *Sedum acre*, *Sedum spurium* 'Purpur Winter', and *Sedum spurium* 'Voodoo'. These species were chosen for their adaptability to ground-based installations, their capacity for ground coverage, and their ornamental appeal. The color scheme for this design was more muted than in Design 1, reflecting the historical theme of the landscape.
- Layout:** The floral mosaic was arranged in a trapezoidal pattern, echoing the geometric patterns found in Romanian folk art. The mosaic was framed by native trees and shrubs, which enhanced the sense of history and created a visual connection between the mosaic and its surroundings. The use of native plants also contributed to the overall sustainability of the design, reducing water and fertilizer needs.

Site preparation and substrate: A well-draining substrate was essential for both designs, as succulents are prone to root rot if waterlogged. For the historical site, a mix of sand, compost, and pumice was used to provide the necessary drainage while retaining enough moisture for the plants to thrive. The plants were spaced at high densities to ensure full ground coverage and prevent weed growth.

Table 1. Plant inventory for the landscape designs

No. Crt.	Specification	Quantity	Density	Height (cm)
succulent plants – design 1				
1	<i>Sedum spurium</i> 'Purpur Winter'	482	1/pot	10-15
2	<i>Sedum spathulifolium</i> 'Cape Blanco'	983	1/pot	10
3	<i>Sedum spathulifolium</i> 'Purpureum'	581	1/pot	10
succulent plants – design 2				
4	<i>Sedum acre</i>	3,588	13/m ²	10
5	<i>Sedum spurium</i> 'Purpur Winter'	2,223	13/m ²	10-15
6	<i>Sedum spurium</i> 'Voodoo'	2,093	13/m ²	10-15

Table 2.Decorative periods of the selected plants species

No. Crt.	Specification	Months											
		I	II	III	IV	V	VI	VII	VIII	IX	X	XI	XII
succulent plants – design 1													
1	<i>Sedum spurium</i> 'Purpur Winter'				✿			✿	✿	✿		✿	
2	<i>Sedum spathulifolium</i> 'Cape Blanco'				✿			✿	✿			✿	
3	<i>Sedum spathulifolium</i> 'Purpureum'				✿			✿	✿	✿		✿	
succulent plants – design 2													
4	<i>Sedum acre</i>				✿			✿	✿	✿		✿	
5	<i>Sedum spurium</i> 'Purpur Winter'				✿			✿	✿	✿		✿	
6	<i>Sedum spurium</i> 'Voodoo'				✿			✿	✿	✿		✿	

Tools and software

To visualize and plan these designs, the study utilized the 3D modeling software Sketchup for creating virtual environments, while Lumion was used for rendering high-quality visualizations. These tools were instrumental in refining the planting schemes and ensuring the aesthetic alignment with traditional motifs.

Maintenance considerations

Succulents are known for their minimal water needs and ability to survive in challenging environments. However, initial care is essential to ensure successful establishment. During

the first few months after planting, the succulents in both designs require regular watering to establish their root systems. After this period, the plants were largely self-sustaining, with additional watering only necessary during extended periods of drought (Stephenson, 1994).

RESULTS AND DISCUSSIONS

Design 1: Floral mosaic with Sedum varieties

The suspended floral mosaic in Design 1 successfully demonstrated the aesthetic potential of *Sedum* species in replicating traditional Romanian motifs. The use of *Sedum spurium* 'Purpur Winter' for red tones, *Sedum spathulifolium* 'Cape Blanco' for white, and *Sedum spathulifolium* 'Purpureum' for black created a vibrant, year-round display that required minimal upkeep. The design's modular nature allowed for easy maintenance, with the mosaic framed by clear strips to facilitate trimming and preserve the integrity of the patterns.

The mosaic's resilience to varying environmental conditions further reinforced the practicality of using succulents in such designs. The plants thrived in the selected substrates, which were chosen for their ability to maintain adequate moisture levels without waterlogging. The temperature-regulating properties of the substrates also contributed to the health of the plants, as observed in the doctoral study on which the design was based (Cotoz, 2024).



Figure 4. Mosaic composition – “suspended garden”

From a plant care perspective, it is recommended to acquire specimens in pots with a diameter of 9 cm. These plants will need to be transplanted into substrates specifically designed for green roofs, as previous results have shown (Cotoz *et al.*, 2023) that this substrate generally exhibits the most suitable qualities and characteristics. Although it may be inferior in terms of nutrient content (Cotoz *et al.*, 2023), the green-roof substrate still provides a good source of essential nutrients for plant development. Because it does not compact, the green roof substrate allows for good air circulation around the plant roots, facilitating efficient nutrient absorption.

Watering once or twice a week, depending on weather conditions and soil moisture levels, will suffice (VanWoert *et al.*, 2005). Once established, the plants generally require minimal watering, surviving primarily on rainwater. However, during prolonged periods of drought or extreme heat, additional watering may be necessary. The frequency of watering should be adjusted according to seasonal changes (Stephenson, 1994).

Design 2: Floral mosaic in a historical context

Design 2 presented a more complex landscape, where the floral mosaic served as a visual centerpiece within a larger historical narrative. The use of *Sedum acre*, *Sedum spurium* 'Purpur Winter', and *Sedum spurium* 'Voodoo' mirrored the design principles of Design 1 but within a grander setting. The mosaic's geometric and traditional Romanian patterns connected the four symmetrically arranged garden islands, adding both thematic coherence and aesthetic harmony.

The inclusion of native trees and shrubs around the mosaic further enhanced the historical context of the design, providing a sense of continuity with the surrounding environment. The careful selection of plants, based on their growth habits and decorative periods, ensured that the landscape remained dynamic throughout the year, with different elements coming into prominence as the seasons changed.



Figure 5. Mosaic composition -fictitive historical site

The study explored two distinct landscape design concepts that integrated succulent plants and Romanian traditional motifs to achieve both aesthetic and sustainable outcomes. The comparative analysis of these designs, one urban and one historical, reveals several important insights into the balance between modern and traditional elements, the practical advantages of using succulents, and the cultural significance of integrating heritage into contemporary settings.

Aesthetic and cultural integration

Both designs leveraged the symbolic power of traditional Romanian motifs, using floral mosaics to establish a visual connection between the past and present. However, their success in doing so varied due to differences in setting and scale. Design 1, which was located in an urban, rooftop environment, relied on the contrast between modern architecture and traditional floral motifs. This juxtaposition created a visually interesting dynamic, but the potential risk of the design feeling out of place underscores the challenge

of blending cultural elements with contemporary structures. The design's success depended heavily on careful integration with its surroundings to avoid disrupting the modern aesthetic of the A.H.R.I.T. building.

In contrast, Design 2, situated in a historical context, achieved a more harmonious integration by aligning the floral mosaic with the architectural narrative of the site. The use of traditional motifs within a historically themed environment allowed the design to enhance, rather than compete with, its surroundings. The symmetry and geometric patterns of the mosaic reinforced the sense of order and balance often associated with historical landscapes. This thematic cohesion made the mosaic feel like a natural extension of the site's cultural heritage, creating a visually captivating and contextually relevant landscape.

Functionality and Sustainability

The use of succulent plants, particularly *Sedum* species, proved essential to the functionality and durability of both designs. *Sedum*'s low water requirements and ability to thrive in harsh conditions made it an ideal choice for public spaces, where long-term sustainability and minimal maintenance are critical. These plants provided both aesthetic value and environmental benefits, as their resilience reduced the need for irrigation and upkeep, also noted by Durhman *et al.* (2006) and Wolf and Lundholm (2008).

In Design 1, the rooftop setting posed challenges related to weight and drainage. The green roof substrate addressed these concerns by maintaining moisture without becoming waterlogged and by providing a microenvironment with slightly elevated temperatures, which supported plant growth. The substrate's lightweight, well-draining properties were crucial in preventing structural issues on the rooftop, ensuring that the mosaic would remain sustainable over time.

Design 2, which occupied a larger, ground-based site, also benefitted from the use of *Sedum* plants. The trapezoidal mosaic, surrounded by native trees and shrubs, created a focal point within the landscape, emphasizing the cultural and natural elements of the historical setting while the low-maintenance characteristics of succulents allowed the design to retain its visual impact without requiring extensive care, making it an ideal solution for large, historically significant spaces.

Comparative outcomes

Both designs successfully demonstrated the versatility of succulent-based floral mosaics in different contexts. Design 1, with its compact, urban arrangement, highlighted the potential for integrating green infrastructure into densely populated areas. The minimal maintenance required by succulent plants made it an efficient solution for small urban spaces, where resources and space are limited. In this context, the floral mosaic served not only as an ornamental feature but also as a functional element of the green roof, contributing to urban sustainability.

Design 2, on the other hand, showcased the potential for integrating succulents into larger, more complex landscapes. The cultural and historical significance of the setting enhanced the impact of the floral mosaic, making it a central visual and thematic feature of the site. The alignment of traditional motifs with the historical context created a deeper sense of place, enriching the visitor experience by connecting them to Romania's cultural heritage.

Broader implications

The findings from this study suggest that the use of succulent plants in landscape design is a highly effective strategy for achieving both sustainability and cultural relevance. Succulents not only reduce the environmental footprint of landscaping projects but also offer a versatile medium for expressing cultural narratives through design. By integrating

traditional motifs into modern urban and historical landscapes, the study demonstrated how landscape design can bridge the gap between past and present, enhancing both aesthetic appeal and cultural significance.

CONCLUSIONS

This study highlights the potential of integrating plants, specifically *Sedum* species, into landscape designs that draw on Romanian cultural motifs. The use of floral mosaics in both the modern and historical contexts demonstrated how traditional patterns could be effectively incorporated into contemporary landscapes. The durability and low maintenance requirements of succulent plants make them an ideal choice for sustainable urban green spaces.

The findings suggest that floral mosaics inspired by cultural traditions can enhance both the aesthetic and cultural value of public landscapes. By carefully selecting plant species and designing layouts that reflect traditional motifs, landscape architects can create spaces that are not only visually appealing but also resonate with local history and identity. The use of succulents offers a long-lasting, eco-friendly alternative to conventional floral arrangements, making them a viable option for future landscape projects.

Permanent solutions that use succulent and hardy plants are more suitable due to their lower maintenance costs and ease of implementation. These plants require less water, are more resistant to varied environmental conditions, and generally have a longer lifespan compared to conventional plants used in floral mosaics.

REFERENCES

1. Blessings N. (2024). The Impact of Public Art Installations on Urban Recreation Spaces. *International Journal of Arts, Recreation and Sports*. 3. 13-24. 10.47941/ijars.1936.
2. Cotoz A.P., Dan V.S., Gocan T.M., Andreica I., Rózsa S. and Cantor M. (2023). *Sedum* Growth Patterns under Different Pedoclimatic Conditions, *Plants*, 12(14), 2739.
3. Cotoz A.P. (2024). PhD thesis Research regarding *Sedum* plants in mosaic designs and their adaptation to various pedoclimatic conditions. USAMV Cluj-Napoca. Pp. 15, 69-75, 126-130.
4. Durhman A. K., Rowe D. B. and Rugh C. L. (2006). Effect of watering regimen on chlorophyll fluorescence and growth of selected green roof plant taxa. *HortScience*, 41(7), 1623-1628.
5. Norbury J. (1973). *Traditional Knitting Patterns, from Scandinavia: The British Isles, France, Italy, and Other European Countries*. Courier Corporation.
6. Radu D. M. and Cantor M. (2014). A new concept of landscape design with Romanian traditional patterns used for green areas increase and preservation. *ProEnvironment Promediu*, 7(17).
7. Semeraro T., Scarano A., Buccolieri R., Santino A. and Aarvevaara E., (2021). Planning of Urban Green Spaces: An Ecological Perspective on Human Benefits, *Land*. 10(2):105.
8. Stephenson R. (1994). *Sedum: cultivated stonecrops*. Timber Press Inc.
9. Vishal K. and Vuillienet A. (2021). Urban Nature: Does Green Infrastructure Relate to the Cultural and Creative Vitality of European Cities? *Sustainability* 13, no. 14: 8052.
10. VanWoert N.D., Rowe D. B., Andresen, J. A., Rugh, C. L. and Xiao, L. (2005). Watering regime and green roof substrate design affect *Sedum* plant growth. *HortScience*, 40(3), 659-664.
11. Wolf D. and Lundholm J.T. (2008). Water uptake in green roof microcosms: Effects of plant species and water availability. *Ecological Engineering*, 33(2), 179-186.
12. Zaharia A., Jucan D. and Buta E. (2016). Reaction of *Sedum spurium* "Purpureteppich" exposed to thermal and hydric stress.
13. <https://www.britannica.com/art/garden-and-landscape-design/Historical-development>.
14. <https://flowercarpet.brussels/en>.
15. Google earth.



Magazine financially supported by
the Romanian Society of Horticulturist

