

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

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ABSTRACT

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

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

INTRODUCTION

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

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

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

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

Vegetable Genetic Resources, Domestication, and Breeding

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

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

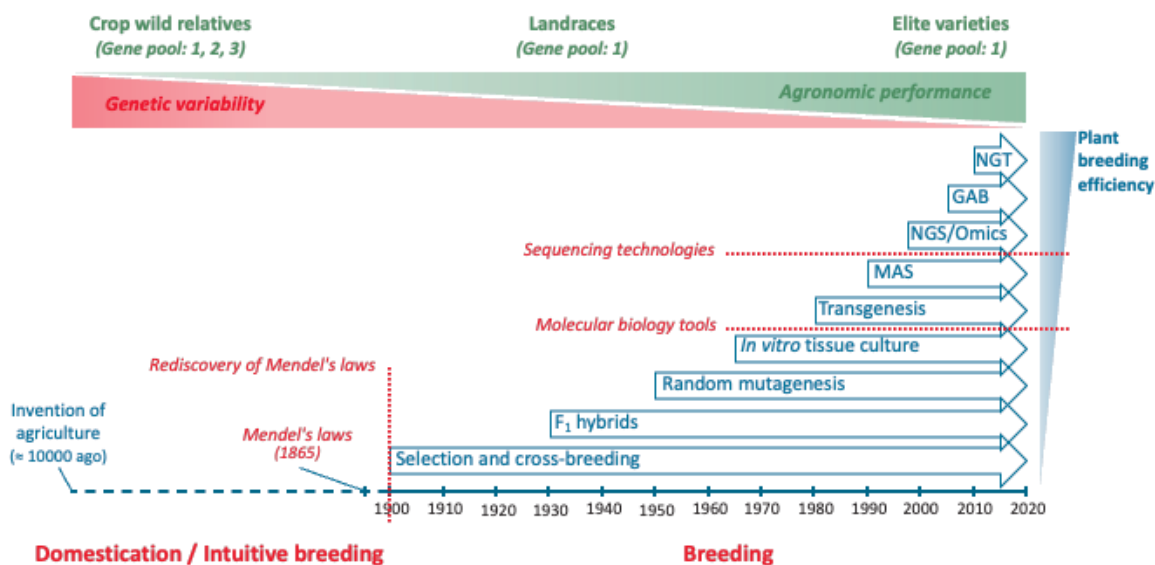


Figure 1. Domestication and breeding of crop plants. MAS, Marker Assisted Selection; NGS/Omics, Next Generation Sequencing/Omics sciences; GAB, Genomics Assisted Breeding; NGT, New Genomic Techniques (modified from Fricano *et al.*, 2016).

‘Omics’ and Biotechnological Tools

‘Omics’ technologies

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

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

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

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

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

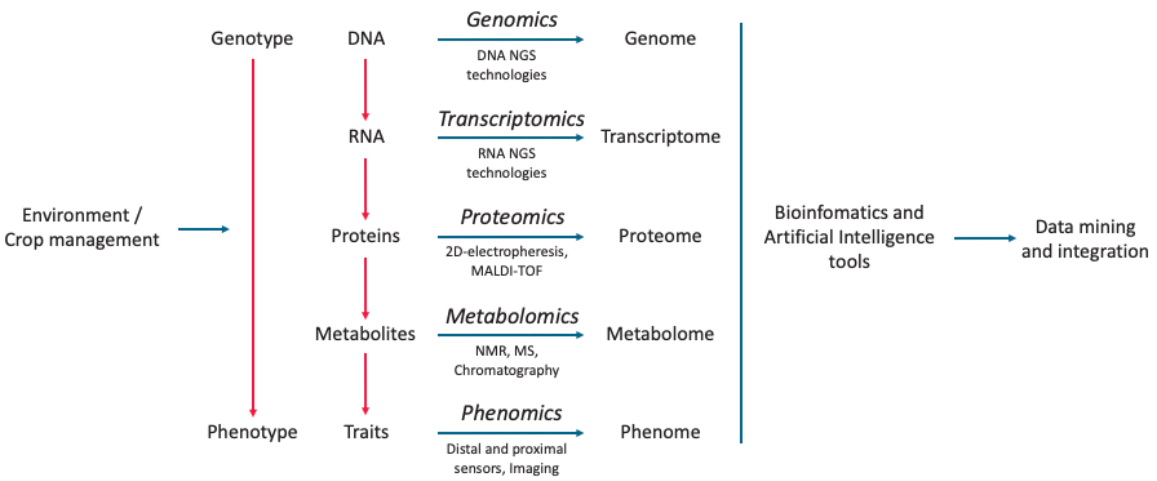


Figure 2. Omics technologies for the genome-scale analysis of different molecules and traits to find out genotype-to-phenotype relationships. Examples of some analytical techniques used are also indicated for each ‘omics’ science.

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

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

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

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

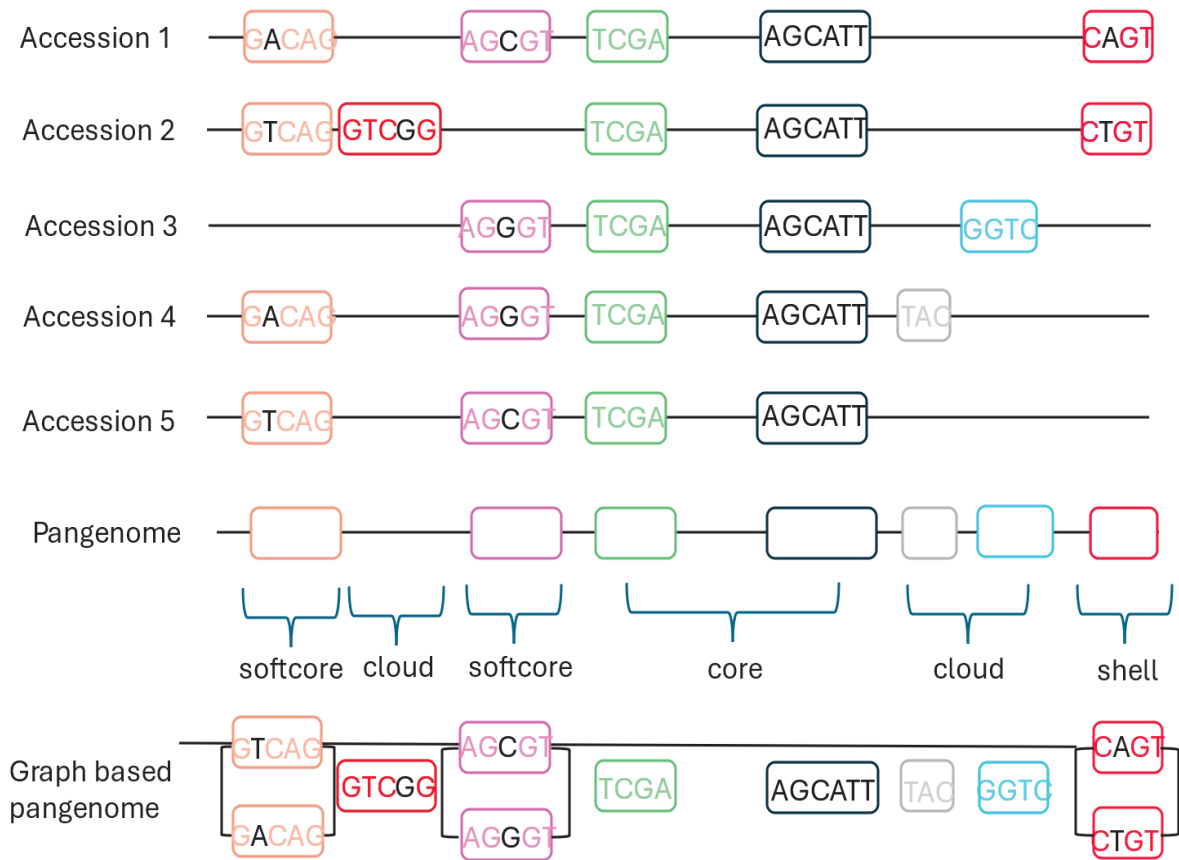


Figure 3. Schematic representation of a pangenome. The core includes genes that are present in all genomes analyzed, softcore in the large majority, shell in a few, and cloud in only one accession.

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

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

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

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

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

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

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

Biotechnologies

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

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

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

Management and Enhancement of Vegetable Genetic Resources

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

Collections management

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

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

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

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

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

Gene discovery and pre-breeding

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

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

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

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

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

Table 2. Examples of pangenomes constructed in vegetable species.

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

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

^b SV, Structural variants

^c Based on gene loci (pan-loci)

^d Based on whole genome sequences

^e Not specified

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

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

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

Breeding

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

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

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

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

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

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

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

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

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

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

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

^b SV, Structural variants

^c Based on gene loci (pan-loci)

^d Based on whole genome sequences

^e Not specified

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

Challenges and Perspectives

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

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

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

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

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

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

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

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

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

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

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