Review

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Integrating Hybrid and Molecular Breeding as Approaches in Vegetable Breeding Strategies

Janko Červenski [1,*], Srđan Zec [1], Gordana Tamindžić [1], Dragana Miljaković [1], Jelena Marinković [1], Boris Adamović [2], Đorđe Vojnović [2] and Aleksandra Ilić [1]

[1] Department of Vegetables and Alternative Crops, Institute of Field and Vegetable Crops, Maksima Gorkog 30, 21000 Novi Sad, Serbia

[2] Faculty of Agriculture, University of Novi Sad, Dositej Obradović Square 8, 21000 Novi Sad, Serbia

* Correspondence: janko.cervenski@ifvcns.ns.ac.rs

Abstract

Considering the daily importance of vegetables in the human diet, breeders are expected to find faster and more accurate methods of creating new varieties of vegetables. To more precisely meet the demands of vegetable producers and consumers, breeders are increasingly combining hybrid and molecular techniques. The integration of hybrid and molecular breeding represents a logical step towards the development of efficient vegetable breeding strategies. For this purpose, the aim of this review is to point out on several representative vegetable species the possibilities, advantages, and disadvantages of the integration of hybrid and molecular methods of breeding. While conventional breeding techniques are based on selective breeding, mass selection, pure line selection, backcrossing, and hybrid breeding that exploit the effects of heterosis, advanced techniques such as phenomics, molecular markers, genome-wide association studies, and next-generation sequencing facilitate the identification and selection of desirable traits and improve nutritional quality. Definitely, molecular techniques represent a faster and more precise part of this mentioned integration. However, classical-hybrid breeding still develops stable, uniform, and marketable varieties without the high costs and significant access of advanced laboratory infrastructure, without the regulatory barriers that accompany genetic engineering.

Keywords: vegetables; hybridization; breeding; molecular marker; CRISPR/Cas9; integration

1. Introduction

Vegetables are grown in nearly 200 countries and represent the largest portion of the human diet in many regions of the world. A global survey has shown that at least 402 types of vegetables from 69 families and 230 genera have been grown and commercialized worldwide [1].

In addition to their widespread production, they provide energy and, in their various forms, ensure an adequate intake of essential vitamins, minerals, dietary fiber, and phytochemicals [2–4]. Many vegetable products meet the daily calorie requirements owing to their carbohydrate content [5], while legumes represent a particularly valuable source of essential amino acids [6,7]. Leafy greens and other vegetables are good sources of vitamins, minerals, and dietary fiber [8–11]. Sharing information about the nutritional value of vegetables and the benefits of changes in human consumption habits can give vegetables an even more important role in the human diet [12–14].

The World Health Organization (WHO) has recognized the importance of vegetable consumption. It promotes and recommends consumption of at least 400 g of vegetables and fruits per day to ensure optimal health and intake of essential nutrients lacking in other food groups [15,16].

Higher living standards have also increased local demands for fresh vegetables, leading to off-season vegetable production [17]. However, the production of high-quality vegetables is challenged by abiotic and biotic factors, including the decrease of arable land, urbanization, and climate change, all of which affect product quality [18–20].

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Biotic stresses are caused by insects, pests, bacteria, fungi, viruses, nematodes, weeds, and other living organisms responsible for a 20–40% reduction in annual crop productivity [21,22]. Abiotic stresses include drought, high salinity, temperature extremes (cold and heat), floods, and heavy metal toxicity. Almost 70% of annual yield loss worldwide is due to abiotic stresses [23,24]. Therefore, plant breeders are required to develop cultivars resistant or tolerant to both types of stress [25].

Conventional plant breeding focuses on the development of new cultivars through traditional methods that depend heavily on natural genetic variations and processes [26]. However, traditional breeding approaches have several limitations; they are time-consuming, often requiring 10–14 years to produce an improved cultivar, as well as being labor-intensive and expensive [27,28]. Advancements in genetics and genomics, along with the introduction of innovative methods and techniques, have significantly improved plant breeding programs. Molecular markers represent one such advancement used in modern breeding strategies to shorten the selection process [29,30]. Molecular markers facilitate the identification and selection of desirable traits [31–33]. Techniques such as marker-assisted selection (MAS), marker-assisted backcrossing (MABC), and marker-assisted recurrent selection (MARS) are widely used to achieve greater precision in plant breeding [34–36].

For the aforementioned reasons, the aim of this review is to emphasize, by using certain vegetable species as examples, the importance of integration of hybrid and molecular techniques in vegetable breeding. In this review, only a few representative vegetable species are highlighted to illustrate the importance and potential of integrating hybrid and molecular techniques in breeding programs.

2. Conventional Plant Breeding

The need for food and fiber turned farmers into the first plant breeders, who unwittingly created locally diverse varieties through several breeding approaches, namely selection, introduction, and domestication. Local genotypes possess diverse traits conferring resistance to insects, pests, and diseases, yet they generally have lower yield potential. Vegetable varieties have a long history, shaped over generations by the efforts of growers and breeders to improve their traits. Over the course of time, scientists found the genetic basis of selection that farmers had already utilized to select plants with desirable traits, giving researchers the motivation to develop cultivars with wider adaptability and higher yield potential under conventional production conditions [37].

Vegetable breeding is challenged to meet the demands of both consumers and producers. Consumers prefer vegetables of good appearance, quality, taste, color, texture, durability, and nutritional content, while producers prioritize cultivars with high yield, uniformity, high market value, resistance to multiple diseases and pests, and tolerance to abiotic stresses [38–40].

Conventional breeding techniques are becoming more expensive, time-consuming, and labor-intensive due to the increasing complexity of traits required in modern vegetable cultivars. In conventional breeding programs, phenotypic selection is a complex and lengthy process that combines desirable traits gradually, step by step. This is particularly true for traits controlled by multiple genes and influenced by environmental variation. Breeding a new and promising cultivar can take 10 or 15 years before it becomes available for commercial production [37,41].

Conventional breeding methods require five to six generations to transfer a trait to the locally adapted, high-yielding cultivars, with a large number of progeny produced in order to select the plants with the desired combination of traits. The improved lines must be subjected to a series of tests at multiple locations before producers can identify a cultivar suitable for production. This process takes at least 7 to 10 years [42].

Conventional breeding typically begins with the selection of parental traits, often from wild relatives or landraces [37], which involves recurrent backcrossing to maintain the crop's genetic basis. Traditional breeding methods are based on strategies such as selective breeding, mass selection, pure line selection, backcrossing, and hybrid breeding, with F1 hybridization and heterosis occurrence that affects productivity and quality [40–43].

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Hybrid breeding is manifested in the superiority of F1 hybrids in relation to parental lines and is based on the exploitation of heterosis, i.e., hybrid vigor. The method is widely used in breeding vegetable crops, such as tomatoes, peppers, cabbages, cucumbers, and onions [43]. The advantage of hybrid breeding is reflected in higher and more stable yields, crop uniformity, improved resistance to diseases and pests, as well as improved technological and nutritional traits [44]. Hybrid breeding requires the development of genetically stable inbred lines, a process of which is long and costly, but the investment is justified by the high performance of the obtained hybrids and their economic value (Figure 1).

One of the quality traits of hybrid vegetables is phenotypic uniformity, which relies on the genetic homozygosity of the inbred parents [43–45].

Screening of different sources of vegetable crops, such as germplasm and breeding lines, resulted in identifying various bioactive compounds. Bioactive compounds such as anthocyanin, isoflavonoids, quercetin, betalins, lycopene, glucosinolates, sulforaphane, carotenoids, chlorophylls, and capsaicin are present in many vegetable crops, and are commonly referred to as nutraceuticals [46]. Conventional breeding strategies, such as selection, backcrossing, and pedigree-followed hybridization, have been used to develop new nutraceutical cultivars of vegetable crops, some of which have been released for commercial cultivation. Genes determining the mentioned quality traits have also been discovered using several molecular approaches, such as marker-assisted selection and transgenic crops [47].

Conventional breeding strategies are heavily dependent on whether the target traits are qualitative or quantitative. Understanding inheritance and genetic associations is critical for quantitative traits, as these traits are influenced by multiple genes and environmental conditions. Many compounds related to the nutritional quality of vegetables are quantitative traits, determined by numerous genes and influenced by environmental conditions, such as the content of pigments in tomatoes [48,49].

The selection and development of cultivars adaptable to specific conditions is the first step in breeding, taken to ensure stable, high-quality vegetables with superior nutritional value. Great pressure is put on breeders to develop stable cultivars that can adapt to different environmental conditions [40,48,49] and achieve a high yield [37,50].

If conventional breeding represented one side of the coin, biotechnology would surely be found on the other [42].

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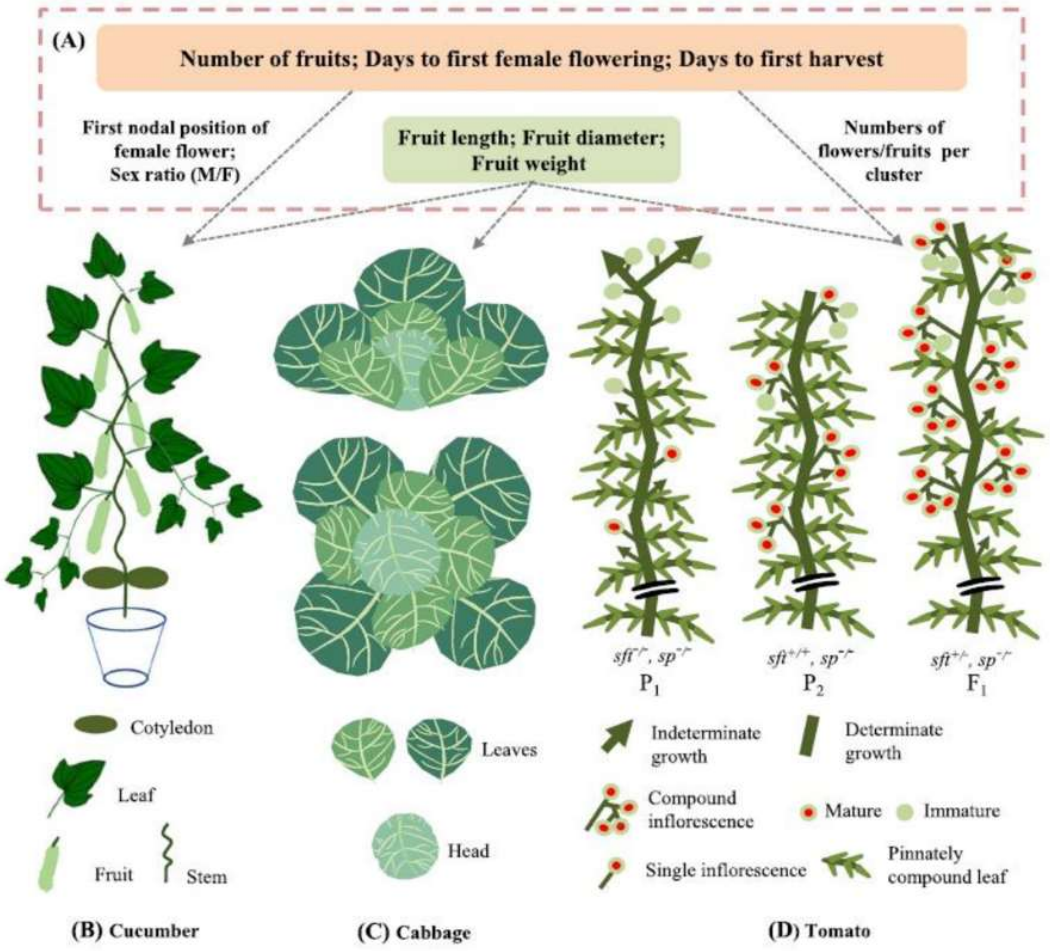

Figure 1. Morphological and phenological traits that contribute to yield heterosis in cucumber, cabbage, and tomato. (**A**) Key traits associated with yield heterosis. (**B**) A gynoecious cucumber line developed for commercial production, characterized by a small number of branches. (**C**) A model of cabbage showing both the aerial view and a cross-section, including the leaves and the head. (**D**) A cross between a tomato plant with single inflorescences and indeterminate growth and one with compound inflorescences and determinate growth yields an F1 hybrid with earlier fruit production, a higher number of compound inflorescences, and determinate growth [51].

Several morphological and phenological traits contribute to yield heterosis in cucumber, cabbage, and tomato (Figure 1). In cucumber, key traits include number of fruits, days to first female flowering, days to first harvest, first nodal position of the female flower, sex ratio (M/F), fruit length, fruit diameter, and fruit weight (Figure 1A, 1B). In cabbage, yield is primarily associated with fruit length, fruit diameter, and fruit weight (Figure 1A, 1C). In tomato, important traits include number of fruits, days to first female flowering, days to first harvest, number of flowers or fruits per cluster, fruit length, fruit diameter, and fruit weight (Figure 1A, 1D) [51].

3. Genetic Diversity Assessment

One of the main prerequisites for successful breeding is the existence of wide genetic variability in the starting material. On the other hand, the successful use of the existing genetic variability requires its reliable assessment. Thus, for example, the effect of hybrid heterosis is determined by the genetic diversity of the parents. The more genetically different the parents are, the better the chance of using heterosis. Therefore, the assessment of genetic diversity has a key role in the development of hybrids and the characterization of germplasm collected from different regions [52].

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Molecular markers are a tool that can be used to precisely determine and analyze the connections between samples based on the assessment of genetic similarity. Therefore, characterizing genotypes at both the genetic and phenotypic levels is the first step towards efficiently conserving, maintaining, and utilizing the existing genetic diversity of plant germplasm [53]. Moreover, knowledge about the existence of genetic links between samples within the germplasm can be a reliable indicator when selecting parents for the development of cultivars and hybrids [54,55].

The study of genetic diversity using molecular markers is a widely practiced approach. Several researchers have evaluated genetic diversity in various vegetable crop species using molecular markers [30,56,57]. Vázquez et al. [58] determined the existence of a very narrow genetic base with more than 50% of deoxyribonucleic acid (DNA) fragments common to 11 pepper breeding lines using random amplified polymorphic DNA (RAPD) markers. Dijkhuizen et al. [59] used restriction fragment length polymorphism (RFLP) to investigate the genetic relationships in two collections of cucumber (*Cucumis sativus* L.) germplasm. Genetic relationships determined from RFLP markers were consistent with predictions based on fruit type and provenance data. During the assessment of world tomato collections, Villand et al. [60] found that samples from South America exhibited greater genetic diversity compared to European samples. Levi et al. [61] determined the existence of 80.2-97.8% genetic similarity among traditional watermelon cultivars using inter-simple sequence repeats (ISSR) and amplified fragment length polymorphism (AFLP) markers and concluded that these markers are very effective in determining genetic diversity among watermelon cultivars. Muminoric et al. [62] used 12 AFLP and 10 ISSR primers in a genetic diversity assessment of 68 radish cultivars. Blanca et al. [63] performed genotyping by sequencing using single nucleotide polymorphism (SNP) markers for 1,254 tomato samples that included European traditional and modern cultivars, early domesticated cultivars, and wild relatives. Very low genetic diversity was determined in the analyzed samples, with only 298 polymorphic loci (at the 95% threshold) out of a total of 64,943 variants.

Over the last few decades, simple sequence repeat (SSR) markers, also known as microsatellites, have gained popularity due to their high reproducibility, multiallelic nature, codominant inheritance, and broad genome coverage. SSR markers have been successfully used to analyze genetic diversity in different plant species. SSR and markers based on sequence-related amplified polymorphism (SRAP) were used to study the genetic variability of certain traditional tomato cultivars in Spain [52]. Singh et al. [53] evaluated the genetic diversity of 47 pea genotypes using 34 SSR markers specific to this plant species.

4. Marker-Assisted Selection (MAS)

Conventional plant breeding is based primarily on the phenotypic selection of superior individuals in segregating populations resulting from hybridization [64]. However, the development of improved cultivars in this way is limited because of its duration. Given the numerous stages of crossing, selection and testing involved in the conventional breeding process, it takes one or two decades to create a new cultivar [65]. This traditional approach is slow, labor-intensive, and expensive. Advances in genetics and genomics rely on new tools, techniques, and approaches that can be applied to improve breeding programs. Molecular markers, genetic linkage maps, marker assays, and whole genome sequencing have been developed in many plant species, including vegetable species. Methods such as marker-assisted selection, marker-assisted backcrossing, marker-assisted recurrent selection, and genomic selection, all applicable to precision breeding, are currently at various stages of development in certain vegetable species. Their use depends on available resources and the complexity of the genetics and breeding of the particular species [66] (Figure 2).

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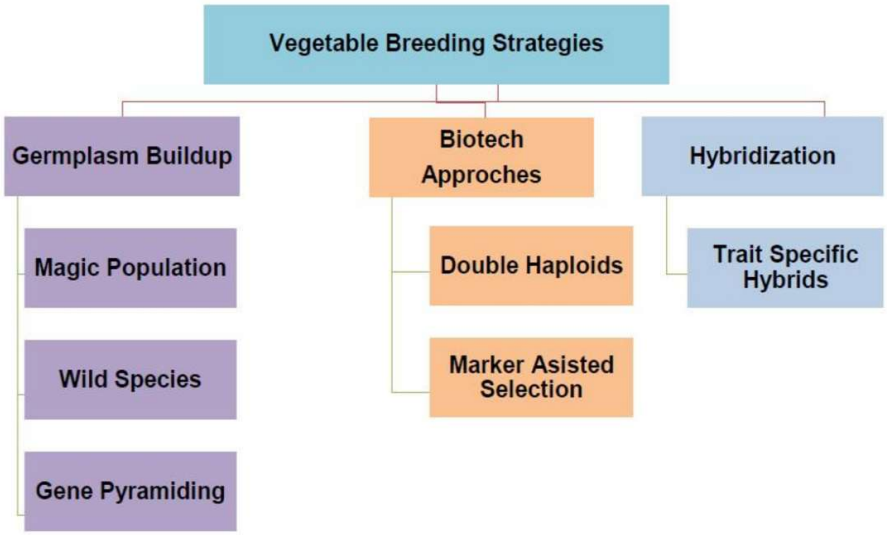

Figure 2. Vegetable breeding strategies [42].

Marker-assisted selection involves the use of molecular markers to select plants carrying genes responsible for traits of interest [32]. With the development and availability of molecular markers and detailed molecular genetic maps, marker-assisted selection has become widely applied in plant breeding for both qualitative and quantitative traits [67]. Marker-assisted selection is a practical application of molecular markers in plant breeding. The process consists of several stages, including identification of sources of desirable alleles, detection of polymorphic markers through genome analysis, establishment of associations between marker allelic variation and target traits, estimation of the effects of candidate genes and loci controlling quantitative traits (QTL), and evaluation of their stability across different environments and genetic backgrounds [68]. On completion of the last MAS phase, it is possible to single out the markers useful for implementation of the so-called molecular breeding [54].

The fundamental principle of marker-assisted selection relies on linkage disequilibrium (LD) between the marker and the gene/QTL of interest. LD refers to the non-random association of alleles that tend to be inherited together due to genetic linkage. With sufficiently strong LD, selection can be carried out directly through markers, with the aim of indirectly increasing the frequency of certain alleles. In this way, marker-assisted selection would enable simpler, faster, cheaper, and more efficient selection [69].

In marker-assisted selection, DNA markers can be used for two primary purposes:

1. Tracking favorable alleles (dominant or recessive) through generations;
2. Identification of most suitable individuals among the offspring in the separation populations, based on the allelic representation in a part of the genome or the entire genome [64].

Most commonly used breeding strategies in MAS are the selection of desirable traits or QTL from breeding lines or populations, introduction of genes from breeding lines or wild relatives, marker-assisted backcrossing, recurrent marker-assisted selection, and gene pyramiding [67]. A schematic representation of conventional, advanced, and modern strategies for vegetable breeding is shown in Figure 3.

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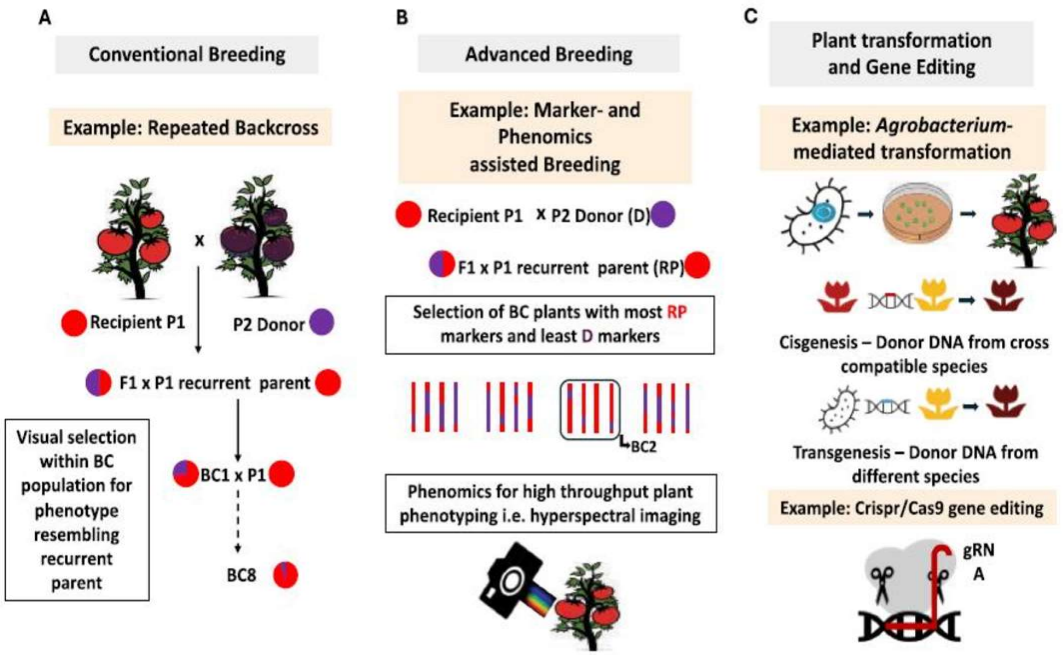

Figure 3. Crop improvement methods categorized as conventional breeding, advanced breeding, and plant transformation and gene editing. (**A**) A conventional strategy involving successive backcrossing and visual phenotypic evaluation of (BC) populations. (**B**) Advanced approaches for screening BC populations using molecular markers and phenomics. (**C**) Genetic engineering approaches such as *Agrobacterium*-mediated plant transformation, cis- and transgenesis, and CRISPR/Cas9-mediated genome editing [40].

4.1. Introgression and Selection of Traits and QTL from Breeding Lines and Wild Relatives

One of the most practical applications of DNA-based markers in breeding programs is the ability to select phenotypic traits using markers tightly linked to the genes controlling the trait [35,70]. The ability to select plants based on genotype rather than phenotype is highly attractive to plant breeders, as it avoids issues associated with phenotypic selection. Interactions with other genetic and environmental factors limit the effectiveness of phenotypic evaluations [70]. On the other hand, the probability of identifying a gene through a marker is inversely proportional to the distance between the gene and the marker. In addition, a large proportion of individuals in segregating generations exhibit high heterozygosity, which complicates visual selection [71]. Selection based on genetic composition helps overcome this limitation [64].

Marker-assisted selection is based on the concept that it is possible to determine the presence of a gene from the presence of a marker if there is a close relationship between them. This technique is used in breeding programs for improvement and rapid selection in early generations [72]. Although markers can be used at any stage of the breeding process, marker-assisted selection is a major advantage in early generations, as plants with undesirable genes and combinations of genes can be eliminated [35]. This allows breeders to focus on a smaller number of priority lines in subsequent generations. An important prerequisite for successful selection in early generations using markers is working with large populations and low heritability of the traits of interest because, in individual selection, the relative efficiency of marker-assisted selection is greatest for traits with low heritability. Unfortunately, the biggest disadvantage of marker-assisted selection in early generations is the cost of genotyping a large number of plants [67].

Marker-assisted selection is an effective approach in which traditional plant breeding is improved by the use of molecular markers, thereby increasing the precision and reliability of selection [25]. Through marker-assisted selection, plant breeders can detect and select appropriate dominant and recessive alleles across generations of a breeding population [73]. Within MAS, QTL

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associated with significant agronomic traits and valuable resistance genes are used, together with their specific DNA markers that are tightly linked to the trait of interest [74].

Many fruit traits are controlled by a relatively large number of QTL, each contributing positively or negatively to the phenotype. DNA markers are particularly useful in the selection of such quantitative traits that are difficult to select for based on phenotypic assessment alone. QTL regions controlling such traits have been identified in several cultivated plant species, such as tomato [64].

Wild relatives of cultivated plants are important genetic resources, as potential sources of desirable alleles or genes lost through breeding over the years. In this regard, tomato provides a well-known example of the importance of wild relatives in improving agronomic traits, as they have been extensively used in tomato breeding programs. *Lycopersicon pimpinellifolium* has been an attractive source of germplasm for breeders due to its closeness to cultivated tomatoes and the simplicity of backcrossing. Genes for tolerance to Fusarium wilt and bacterial spot caused by *Fusarium oxysporum* f. sp. *lycopersici* and *Pseudomonas syringae* pv. *tomato*, respectively, have thus been transferred from wild relatives to cultivated tomato. *Lycopersicon parviflorum* and *Lycopersicon chmielewskii* are interesting because of the high sugar and vitamin C content in their fruits [75,76]. Furthermore, a series of backcrosses between *L. chmielewskii* (10-11% dry matter) and the Californian variety VF-145 (about 5% dry matter) resulted in lines with increased sugar content (about 7.5% dry matter) and acceptable fruit size and color [77]. Also, it has been noted that *Lycopersicon hirsutum*, in its natural habitat, is characterized by a high degree of resistance to insects, which was also confirmed by laboratory tests. For instance, during a mite attack, mites are trapped in the sticky exudate of the glandular scales that cover the *L. hirsutum* plant, which confirms a significant negative correlation between glandular malleat density and mite infestation. Moreover, *L. hirsutum* contains the natural insecticide 2-tridecanone in a 72-fold higher concentration compared to the tomato varieties cultivated for commercial purposes. As a result, *L. hirsutum* is resistant to a large number of insects and therefore is a valuable source of germplasm, enabling breeders to increase tolerance in commercially grown tomato cultivars. In addition to its well-documented insect resistance, *L. hirsutum* also contains genes for resistance to other pathogens. For example, certain specimens of *Lycopersicon hirsutum* f. *glabratum* are resistant to the bacterial spot and to the root rot caused by nematodes (*Meloidogyne* spp.). Furthermore, it serves as an important source of resistance to fungal diseases such as *Septoria lycopersici* and viral diseases such as the tobacco mosaic virus . Consequently, due to its preference for higher altitudes, *L. hirsutum* was investigated as a possible source of genes for tolerance to low temperatures [77,78].

Additionally, markers can also be used in the introgression of desirable traits from wild germplasm into cultivated varieties. In particular, markers associated with genes from the wild donor parent, together with markers distributed across the genome of the improved cultivar (recurrent parent), are used to select progeny derived from such crosses. Thus, markers can be used to track the favorable donor alleles and will also help reduce the genetic background of the donor in the offspring [64].

4.2. Marker-Assisted Backcrossing

In traditional backcrossing, the reconstruction of the recurrent parent genotype takes more than six generations, whereas marker-assisted backcrossing helps complete this process in three generations or less. Marker-assisted backcrossing is particularly effective for introducing individual alleles into a different genetic framework, for example, when improving an existing variety by introducing a specific trait [67].

Backcrossing is used in plant breeding to transfer favorable traits from a donor plant (donor parent) to an elite genotype (recurrent parent). During repeated crosses, the first original cross (F1) is crossed again with the recurrent parent until most of the genes from the donor plant have been eliminated. The purpose of backcrossing is to keep all the desirable traits already present in the recurrent parent and to "take" only the desired allele from the parent representing the donor of the gene of interest [79]. However, segments of the donor plant linked to the target allele may remain

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relatively large even after many generations of backcrossing, a limitation that can be addressed by using molecular markers [67].

In this context, markers can be used in backcrossing to control the target gene (foreground selection), accelerate reconstruction of the genotype of the recurrent parent (background selection), or select offspring with the target gene and the occurred recombinations (recombinant selection). Moreover, the transfer of recessive genes through conventional breeding techniques requires generations of inbreeding after each backcross, a procedure that is unacceptably slow for commercial breeding programs [64].

Marker-assisted backcrossing is likely to become a popular approach, largely for the same reasons that encouraged traditional backcrossing [80].

4.3. Marker-Assisted Recurrent Selection (MARS)

Recurrent selection is a key breeding strategy aimed at gradually increasing the frequency of desirable alleles in a population through repeated cycles of selection and recombination. In contrast, MARS applies molecular marker information to detect and select multiple genomic regions controlling complex traits, enabling the accumulation of superior alleles into high-performing genotypes within one or more related populations [81].

Marker-assisted recurrent selection is a breeding approach that aims to accumulate favorable alleles for a relatively large number of QTL in a given population, using a set of markers significantly associated with the traits of interest [82]. It is an important population improvement method that focuses on cyclical selection and enriching favorable alleles from biparental or multiparental introgression at several loci [83]. Marker-assisted recurrent selection begins with a heterogeneous base population and thereafter uses superior recombinants during each cycle to produce an improved population, inbred line, or hybrid. Recognizing its potential, breeding programs in both public and private sectors have successfully applied this approach to cultivated crops [25,83,84].

4.4. Cumulative Gene Pyramiding

The study of genetic resistance against disease in vegetable crops has seen tremendous progress over the past 3 decades. Research of qualitative resistance has had a head start compared with the more complex quantitative resistance, which still has many unanswered questions [85]. Crop plants often experience more than one biotic and abiotic stress. Stress tolerance is genetically complex and results from interplay of multiple genes [86].

Gene stacking, also referred to as pyramiding, represents an effective strategy for incorporating multiple target genes or QTL derived from distinct parental lines into a single genotype within a relatively short timeframe (approximately two to three generations). In contrast, traditional breeding approaches typically require at least six generations recovering about 99.2% of the recurrent parent genome [87,88]. One of the main limitations of conventional crop improvement is that, alongside the target traits, undesirable genes may persist even after multiple backcross generations, and identifying these residual genes can be challenging. In contrast, developments in molecular technologies have enabled more accurate, efficient, and accelerated breeding approaches through the use of molecular markers [89,90]. Marker-assisted selection represents an indirect breeding approach in which molecular markers closely associated with target genes are used to identify and select desirable traits. This strategy is particularly valuable for agronomic characteristics that are difficult to evaluate phenotypically, such as resistance to pathogens, diseases, and abiotic stresses, thereby contributing to the prevention of yield reduction and deterioration of quality traits [91].

To pyramid disease or pest resistance genes containing similar phenotypic effects, often without suitable breeds available, marker-assisted selection may be the only practical method – especially in cases where one gene masks the presence of others. The fact that pyramiding must be repeated after each cross must not be overlooked when applying these strategies in practical breeding, because the pyramided resistance genes segregate in the progeny [67].

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The integration of gene pyramiding strategies, particularly those supported by MAS and related methodologies, has significantly expedited the production of durable, resistant or tolerant lines, enabling precise selection and substantially reducing the time required for their development [92]. A good example of gene pyramiding for biotic stress tolerance could be seen in the tomato trait, where resistance toleaf curl and spotted virus has been pyramided by genes *Ty-1*, *Ty-3*, and *Sw-5* [93]. Another example of gene pyramiding is observed in pepper, where the genes (the *Me1* and*Me2* have been used to confer resistance to root-knot nematode [94]. It is also a known fact that the probability of detecting a marker associated with a gene responsible for tolerance to a certain disease is inversely proportional to the genetic distance between the marker and the gene [95].

For a better estimate, the genetic distance between the markers and genes should be calculated using a large population or, even better, from multiple crosses. Associations between markers and monogenic resistance genes are typically identified through genetic mapping. Although RFLP and RAPD markers were frequently used in this strategy [72], kompetitive allele specific PCR **(**KASP) markers [96] and SNP markers [97] are now increasingly applied.

5. Examples of Marker-Assisted Selection in Vegetable Crops

5.1. Tomato

Tomato (*Solanum lycopersicum* L.), a member of the *Solanaceae* family, ranks as the second most important vegetable crop worldwide, following potato (*Solanum tuberosum* L.) [98]. Most of the existing tomato cultivars were developed by conventional breeding techniques. However, numerous problems of the breeding process have led to the development and application of new technologies in vegetable crop breeding, including tomato breeding [69,99].

Although tomato was one of the first cultivated plant species for which genetic markers and maps were used in breeding programs, until the early 1980s, almost all tomato breeding programs relied mainly on phenotypic selection. With the discovery of more suitable genetic markers for breeders, including polymerase chain reaction (PCR)-based markers and SNP, there has been an increased interest in the use of markers to facilitate the tomato breeding process. Marker-assisted selection is often utilized in tomato breeding programs to introduce and combine genes, especially when breeding for tolerance to multiple diseases. For instance, sequence characterized amplified region **(**SCAR), cleaved amplified polymorphic sequences **(**CAPS), and other PCR-based markers are used in most tomato breeding programs to select for resistance traits to major diseases controlled by a single gene [66]. Six genes (*Ty1, Ty2, Ty3, Ty4, Ty5, Ty6*) conferring resistance to tomato leaf curl virus, a major cause of crop losses, have been identified using molecular markers [52]. Markers are also routinely used for many other purposes, including testing hybrid purity and screening breeding populations for fruit quality traits. On the other hand, they are not typically used in breeding for certain complex traits, such as polygenic traits for disease resistance, abiotic stress tolerance, yield, and various fruit quality traits [66].

5.2. Pepper

Pepper (*Capsicum annuum* L.) plays a significant role in agriculture, yet its production remains highly susceptible to abiotic and biotic factors such as drought conditions, infectious diseases, and temperature fluctuations. Although the variety development process takes many years, advances in biotechnology over the past 25–30 years have resulted in a reduction in breeding time. The use of advanced DNA-based methods in breeding allows breeders to easily overcome the challenges of the traditional breeding process and has reduced the time required to release new varieties [100]. Genetic diversity studies using molecular methods allow breeders to rapidly characterize the genetic material of interest [101–103], develop markers linked to desired traits [104,105], make selections using developed markers [106], and map species genomes [107]. RFLP [108], Some of the molecular marker systems used in the diversity of *Capsicum* germplasms are RAPD [109], AFLP [110,111], microsatellite markers [112,113], SNP [114,115], and SRAP [116–118].

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Several markers linked to specific traits have been developed for use in pepper breeding programs, including allele-specific CAPS markers for the *pvr1*, *pvr11*, *pvr12*, and *pvr2* genes, which confer resistance to various strains of the tobacco mosaic virus. Also, several closely related markers have been developed for tolerance to important pepper diseases, including those caused by *Phytophthora capsici*, pepper mottle virus, tomato spotted wilt virus, and anthracnose [66].

Using derived cleaved amplified polymorphic sequences (dCAPS) and SCAR markers, two genes that controlled capsinoid biosynthesis (*p-AMT* and *Pun1*) were incorporated into a new pepper cultivar called Maru Salad, resulting in a higher concentration of capsinoids in this pepper cultivar compared to the control [106]. Capsaicinoids are a specific class of compounds found in hot peppers, responsible for their pungency [119].

By applying marker-assisted backcrossing and pyramiding with the recessive allele *pun*[1,120] succeeded in the introgression of the male sterility gene *ms10* from the hot pepper (*Capsicum annuum* var. *annuum*) genotype into the genotype of bell pepper (*C. annuum* var. *grossum*). The selection of plants containing the gene of interest was aided by the use of the SSR marker linked to the *ms10* gene located on chromosome I and the SCAR marker, indicating the presence of the no-heat gene *pun1*.

In short conclusion, by enabling precise tracking of desirable alleles for traits such as disease resistance, fruit quality, and abiotic stress tolerance, MAS accelerates breeding cycles and improves selection efficiency compared with traditional phenotypic approaches. Overall, MAS will remain a key component of pepper breeding strategies, helping to meet global demands for resilient, high-yielding, and high-quality pepper varieties.

5.3. Cabbage

Some of the most important objectives in cabbage breeding are to achieve crop uniformity, high yield, desirable appearance, and the appropriate size, shape, and color of economically important plant organs [39]. Today, most of the currently grown cabbage cultivars are F1 hybrids, characterized by high yields and early maturity as a result of heterosis [121]. Conventional breeding of head cabbage begins with crosses between parent lines carrying desirable traits, followed by successive generations of self-pollination and selection aimed at creating improved cultivars [122].

However, as with other Brassica species, the development of modern head cabbage (*Brassica oleracea* var. *capitata* L.) cultivars increasingly depends on molecular markers implemented via MAS. Further markers are use in hybrid breeding. Markers for Male Sterility Maintenance: PCR [123,124], CAPS [125], SSR [125,126], insertion-deletion (InDel) [127–129], RAPD [130], extended random primer amplified region (ERPAR), SCAR [126,131,132], AFLP [132], KASP [133].

Following markers for evaluation of combining ability, population genetic diversity and phylogenetic relationships: RAPD [134–136], SSR [136–140], ISSR [141], AFLP [142,143], expressed sequence tags–single sequence repeats (EST-SSR) [144], SNP [145], KASP [146].

Here are also markers for genetic purity testing of F1 hybrid seeds: RAPD markers [147], Combination of RAPD, ISSR and SSR markers [148], Combination of SSR, SRAP and ISSR markers [149], SSR [150].

Last in this group are fingerprinting markers sets: set of RAPD markers [151], set of KASP markers [152] and sets of SNP markers [153].

Next group are markers for morphological traits and it contains markers for petal color: InDel markers [154,155], cabbage waxiness: SSR markers [156–159], InDel markers [158,160], PCR markers [161], KASP markers [162], and leaf color: InDel markers [163,164].

Group of markers for resistance to abiotic factors is defined by markers for resistance to head splitting: SSR markers [165], InDel makers [166]. Next are bolting resistance and flowering time markers: SSR [167] and InDel markers [1,168]. Last are markers for tolerance to prolonged high and low temperatures: PCR markers [169,170], InDel markers [164] and SNP markers [171].

Last group contain markers for disease resistance. In this group are markers for Fusarium wilt resistance: PCR-RFLP markers [150], SCAR markers [172], SSR markers [173–175], SNP/InDel markers [173], InDel markers [176,177], CAPS markers [177], and dCAPS markers [177]. Further are

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Black rot resistance markers: SSR markers [178,179] and InDel markers [180]. Clubroot resistance markers are: SCAR, CAPS markers [181], SSR markers [182,183], and SCAR, SSR, CAPS markers [184]. Last in the group are Downy Mildew resistance markers: InDel [185] and KASP markers [186].

Therefore, although current markers already play a significant role in speeding up the breeding process, future research should prioritize a deeper genetic analysis of quantitative traits, the development of more advanced and efficient markers—such as KASP and SNP markers derived from whole-genome sequencing—and the expansion of available marker systems to encompass a wider range of valuable traits [187].

5.4. Lettuce

Lettuce (*Lactuca sativa* L.) is a widely cultivated vegetable of significant economic importance [188]. The species includes seven principal genotype groups grown in numerous regions, across varying altitudes, and represented by many traditional cultivars [189].

The development of new lettuce cultivars relies on both traditional and modern breeding strategies to fulfill the specific demands of producers and consumers. However, the generation of F1 hybrids has proven unsuccessful because the pollen of *L. sativa* is heavy, viscous, and poorly transferable.

The main aim of traditional lettuce breeding is to develop varieties that perform well in a wide range of environments. Given current global challenges, breeders must also account for changing agricultural land, biotic and abiotic stresses, and the need for better quality and higher yields [190,191].

Several drawbacks are associated with traditional breeding techniques, including difficulty in ensuring the genetic purity of progeny, slow breeding cycles, limited offspring production, and the introduction of undesirable traits that can only be removed through prolonged backcrossing.

Breeding programs for *L. sativa* mainly aim to improve plant quality and strengthen resistance to early wilting, pests, and diseases. These objectives are achieved through biotechnology and genetic engineering methods, such as gene transfer. Modern technologies have enabled the preservation of diverse genotypes, supported healthier growth environments, and reduced the time required for breeding compared with traditional *in vivo* approaches [192].

Different molecular markers techniques are use for various genetic purposes of *L. sativa* species. Usage of RFLP markers we found in investigations of Landry et al.[193], Kesseli et al. [194], Vermeulen et al.[195], Kesseli et al. [196] and El-Esawi [197]. Further, RAPD markers described Kesseli et al.[196], Yamamoto et al. [198], Tardin et al. [199] and El-Esawi [197]. Utilization of AFLP markers we could find in works from Hill et al. [200], Jeuken et al. [201], Koopman et al. [202], Van Treuren and Van Hintum [203] and El-Esawi [197]. Microsatellite makers in lettuce breeding were described by Witsenboer et al. [204], Van de Wiel et al. [205], Van de Wiel et al. [206], Van Treuren and Van Hintum [203], Rauscher and Simko [207], El-Esawi [197] and Rui et al. [208]. Next type of EST-SSR markerswere demonstrated by Simko [209] and Riar et al. [210]. SNP type of markers were listed in works of MorenoVa'zquez et al. [211], Simko et al. [212], El-Esawi [197] and Walley et al. [213]. Last type of markers, or SRAP and Sequence-specific Amplified Polymorphism (SSAP) were investigated [203,214].

Conventional breeding remains a primary strategy for the incorporation of novel alleles by crossing genotypes derived from diverse plant genetic resources to improve multiple agronomic traits. For example, modern varieties are hybridized with locally adapted genotypes to achieve early maturity, increased yield, improved quality, and enhanced resistance to abiotic and biotic stresses. Moreover, modern biotechnological approaches, including advanced DNA sequencing and molecular tools, are essential for the development of lettuce cultivars possessing desirable characteristics [215].

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6. Genomic Selection as a Bridge Between Molecular and Hybrid Breeding

Genomic selection (GS) represents a major advancement that enables the integration of molecular and hybrid breeding techniques in vegetables. Unlike MAS, which targets markers closely linked to genes controlling traits of interest or major QTL, GS utilizes genome-wide marker information combined with phenotypic data to predict the breeding value of individuals based on genomic estimated breeding values (GEBVs) [216,217]. Breeders can use this method to capture the cumulative effects of numerous small-effect loci controlling complex quantitative traits, such as yield, fruit quality, and abiotic stress tolerance [218].

In hybrid vegetable breeding, GS provides substantial benefits by enabling the early prediction of parental line performance and potential hybrid combinations before extensive phenotypic evaluation. Genomic prediction typically involves establishing a relatively small training population alongside a larger virtual test population. High-density genotypic and phenotypic data are collected from the training population to develop genomic prediction models, which are then applied to candidate populations to estimate the effects of individual genetic markers on target traits or to determine the breeding values of prospective hybrids [219]. Kondo et al. [220] demonstrated that genomic prediction integrating SNP genotypic data and parental phenotypic traits enabled highly reliable prediction of F1 hybrid phenotypes in chili peppers. Genomic prediction models also allow for more precise estimation of general combining ability (GCA) and, increasingly, specific combining ability (SCA), facilitating the definition of heterotic groups based on genomic relationships rather than solely on pedigree information. This approach enhances parental line selection and improves hybrid performance prediction [221]. For example, Yue et al. [222] found that genomic distance between parental lines, as determined by SNP markers, improved the prediction of heterosis and F1 hybrid performance in Chinese cabbage (*Brassica rapa* L. ssp. *pekinensis*). GS has the potential to accelerate the improvement of complex traits in line and hybrid development, particularly for traits with low heritability and pronounced genotype × environment interactions, where conventional phenotypic selection is less effective. This reduces the need for extensive field trials, shortens breeding cycles, and lowers operational costs [223].

The integration of GS and other modern vegetable breeding approaches offers additional benefits. Combining GS with doubled haploid (DH) technology enables rapid cycle breeding, as DH lines can simultaneously serve as training and selection populations, increasing prediction accuracy and shortening generation time while improving annual genetic gain [224]. Singh et al. [225] demonstrated in cauliflower that the combination of fully homozygous DH lines and microsatellite markers allows more accurate prediction of heterosis for key agronomic traits, since genetic distance derived from these tools provides a more reliable assessment of hybrid performance than phenotypic methods. Furthermore, combining GS with high-throughput phenotyping (HTPP) platforms [226] and machine learning algorithms [227] further enhances prediction accuracy. With the rapid development of artificial intelligence (AI), machine learning algorithms are increasingly applied to capture the effects of multiple genes, further improving prediction precision [228]. Among the most advanced applications of AI in this field are genomic language models (gLMs), which interpret genomic sequences as structured linguistic data. These models learn contextual relationships between genetic elements, thereby enabling precise gene annotation, regulatory motif identification, and accurate prediction of variant effects. Although the use of gLMs in vegetable crops is still at an early stage, models trained on the tomato genome have already shown notable potential in predicting promoter activity, guiding single guide ribonucleic acid (sgRNA) design for clustered regularly interspaced short palindromic repeats (CRISPR) editing, and identifying non-coding regulatory elements involved in stress responses [229].

With declining sequencing costs one of the main limiting factors, [223], GS is likely to become a key component of modern vegetable breeding programs, complementing MAS and enabling more efficient improvement of complex agronomic traits.

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7. CRISPR/Cas9 Technology in Vegetable Breeding

The innovative breeding method called CRISPR-associated protein 9 (Cas9) enables precise and rapid enhancement of multiple crop traits, including yield, quality, resistance to diseases, tolerance to abiotic stresses, and nutritional value [230]. In the vegetable breeding CRISPR-Cas9 technology has the next improvements: improvement of biotic stress resistance, abiotic stress resistance improvement, herbicide resistance improvement, and quality improvement [230]. Compared with conventional breeding methods, this technology provides substantially greater precision and significantly reduces the time required for trait improvement [231].

7.1. Tomato

Tomato has emerged as an ideal model crop for evaluating CRISPR/Cas9 applications due to its significant economic importance, the feasibility of *Agrobacterium*-mediated transformation, and the successful development of new varieties without introducing foreign genes into its genome [230,232].

CRISPR-Cas9 technology through *Agrobacterium*-mediated transformation in tomato was modified for further traits: wiry phenotype [233], resistance against downy mildew [234,235], Fusarium wilt susceptibility [236], resistance to *Botrytis cinerea* [237], decrease in heat stress tolerance [238], decrease in chilling stress tolerance [239], decrease in drought stress tolerance [240], herbicide resistance [241], resistance against *Phelipanche aegytiaca* [242,243], introduction of desirable traits with morphology, flower number, fruit size and number, and ascorbic acid synthesis [244], fruit size, inflorescence branching, and plant architecture [245], yellow-coloured tomato [246], pink-coloured tomato [247], purple-coloured tomato [248], repression of genes controlling sugar and auxin metabolism [249], increase in gamma-aminobutyric acid content [250], lycopene content [251], MADS-box transcription factor regulating fruit ripening [252], activation and inhibition of fruit ripening [253], pectin degradation control [254], long shelf-life [255], jointless fruit stem [256], parthenocarpic [257–259], early flowering with simplified inflorescence architecture [260], day-length-sensitive flowering [261]. Furthermore, targeted mutation of the polygalacturonase (*SlPG*) gene using CRISPR/Cas9 led to a delay in fruit softening, thus extending postharvest firmness and shelf life [262]. To increase fruit firmness, [263] simultaneously knocked out the *FIS1* and *PL* genes using CRISPR/Cas9, as these genes negatively affect fruit firmness. The obtained plants showed a significantly increased firmness of the fruits and better storage, without any negative impact on the basic traits of the fruit.

The results of increased lycopene content in tomatoes using CRISPR/Cas9 have been demonstrated in studies by Li et al. [251] and Ahmad et al. [264], who used the CRISPR/Cas9 technology to simultaneously mutate multiple genes involved in carotenoid metabolism in order to increase the concentration of lycopene, an antioxidant with important nutritional properties that affects the color of tomato fruit.

Traditional breeding techniques for major agronomic crops like tomato are unlikely to keep pace with emerging challenges, making the development of advanced, disease-resistant crop varieties essential for ensuring global food security. In this regard, advanced genome-editing tools such as CRISPR/Cas9 have become increasingly significant, as they have been applied in tomato to improve yield, enhance nutritional quality, and increase resistance to both biotic and abiotic stresses [265].

7.2. Pepper

To achieve long-term sustainability in production, it is essential to incorporate cutting-edge genetic and molecular strategies into pepper breeding and improvement programs. Innovations in genomic technologies—including CRISPR-Cas9 editing and integrated multi-omics approaches—are enabling the creation of varieties resilient to environmental stress [266].

Research conducted by [267] aimed to enhance pepper resistance to anthracnose diseases caused by *Colletotrichum* spp. through *CaERF28* gene mutation. The resulting lines showed significantly improved resistance to infection compared to the wild type, and the T-DNA and marker-free mutants

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retained the edited phenotype in subsequent generations. Functional validation of CRISPR/Cas9 editing in pepper was performed using the phytoene desaturase (*CaPDS*) gene as a visual marker. Approximately 62.5% of the regenerated plants displayed albino or mosaic phenotypes due to loss of PDS protein function, confirming successful genome editing in *Capsicum* [268]. Parthenocarpic (seedless) pepper fruits were selectively generated by introducing the CRISPR/Cas9-RNP (ribonucleoprotein) complex into the protoplasts of the leaves of pepper lines, demonstrating potential applications for production under adverse climatic conditions [269].

Further, testing the delivery capability of the CRISPR/Cas9 system using A*grobacterium* in two pepper cultivars provided the next example of how the system can be established for the future pepper breeding. The goal was to test the *Agrobacterium*-mediated CRISPR/Cas9 system for editing the *CaMLO2* gene, which is associated with susceptibility to diseases [270].

On the other side, employing CRISPR genome editing to improve breeding program chili peppers, next stress responses were obtained: cold, high temperature, NaCl, and mannitol [271–275], drought and salt [276–278], chilling and salt [279], pathogen (*R. solanacearum*] and high temperature [280–283], pathogens (*X. axonopodis*, *TMV* and *P. syringe*) [284], *R. solanacearum* infection [285,286], high temperature [287–289], pathogen stress responses [290,291], heat shock and *R. solanacearum*[292], silencing of *CaWRKY50*-showed enhanced pepper resistance to *C. scovillei* [293], pathogen (*P. capsici*) [294], high temperature, salt and drought [295–297], pathogen (*Pseudomonas syringae* pv. *tomato* DC3000), drought and salt [298], pathogen (*P. syringae* pv. *tomato* DC3000) [299,300], water and salt [301].

With emerging pathogens and environmental challenges posing ongoing risks to crop production, the future of pepper improvement is being driven by the integration of breeding and molecular strategies [266].

7.3. Cabbage

The main goals of cabbage crop improvement include improving crop uniformity for maturity, increasing yield and yield-related traits, and developing climate-resilient varieties and hybrids that are resistant to biotic and abiotic stresses and rich in nutraceutical properties [302]. CRISPR/Cas technology has been widely applied for the modification of physiological and agronomic traits in Brassica species, including growth control, gene methylation and functional trait characterization [168].

The first CRISPR-Cas9 gene editing in cole vegetables was reported by Jansson [303], who used cabbage as a model plant and targeted the *PsbS* gene to create a Brassica deletion mutant.

Zhang et al. [228] used a modified CRISPR/Cas9 system to deactivate the *BoBPM6* and *BoDMR6* genes, resulting in broad-spectrum disease resistance in *Brassica oleracea*. This example illustrates how CRISPR/Cas9 technology can directly contribute to increased disease resistance in commercial cabbage varieties, an important trait for improving yield stability and reducing pesticide use.

In another research, the CRISPR/Cas9 system with an endogenous tRNA system was used to simultaneously target several genes in cabbage, such as: *BoPDS* (phytoene desaturase - related to pigment biosynthesis), *BoSRK* (S-receptor kinase - determinant of the autoincompatibility mechanism), and *BoMS1* (gene related to male sterility) [304]. Multiple, inherited mutants were obtained in a single transformation, including lines that became self-compatible and male-sterile, which can be extremely useful for hybrid cabbage production [305]. This example shows how CRISPR/Cas9 can be used not only for functional analysis of genes but also for direct breeding of agronomic traits (e.g., removal of auto-incompatibility and fertility control).

Further, CRISPR/Cas9 ribonucleoprotein (RNP) complexes were introduced into cabbage protoplasts without the integration of foreign DNA. The approach enabled genome editing without transgenic sequences, which is important for both regulatory and consumer understanding [306]. The target genes were *FRI* and *PDS*, and the mutations were generated directly in the protoplasts. The approach represents an important breeding strategy, as it can result in varieties that are not legally classified as genetically modified organisms (GMOs) [306].

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To illustrate another application, the CRISPR/Cas9 system has been successfully used to mutate the BoPDS gene in cabbage, causing clearly detectable albino phenotypes. The aim of the research was not to directly improve commercial properties but to serve as a technological foundation for later targeting of more important agronomic genes via the CRISPR/Cas9 system [304].

To improve black rot resistance in *B. oleracea* var. *capitata* CRISPR/Cas9 editing was used [307]. Black rot, a major disease of vegetable brassicas such as *B. oleracea* var. *capitata*, is caused by the bacterial pathogen *Xanthomonas campestris* pv. *campestris* (Xcc) [308,309]. Another study employed CRISPR/Cas9 to optimize the modification of a potential host susceptibility gene, *BoSWEET15b* (*SWEET15b* gene in *B. oleracea*), in the cabbage cultivar 'Ohgane' [310]. Recent progress in genomics, combined with CRISPR/Cas technologies, has facilitated faster identification, validation, and deployment of resistance genes for clubroot and black rot.

By applying CRISPR/Cas systems, researchers can better understand Brassica–pathogen interactions and the genetic processes involved in abiotic stress responses in Brassica plants.

7.4. Cucumber

Progress in transgenic cucumber breeding has been constrained by the absence of a reliable genetic transformation system, which restricts the use of tools such as CRISPR/Cas9 [311]. CRISPR/Cas9 is a precise gene editing method that enables targeted changes in the plant genome by introducing double-strand DNA breaks at specific loci, leading to mutations via non-homologous end joining (NHEJ) or homology-directed repair (HDR) pathways. This technology has proven highly useful for improving agronomic traits, such as disease resistance, flowering, or fruit quality traits [312].

Research using CRISPR/Cas9 mainly targets characteristics like resistance to viruses, fruit length, shoot growth, branching, and the production of gynoecious lines for hybrid breeding programs [313,314]. One strategy to optimize CRISPR/Cas9 systems is to utilize a stronger CsU6 promoter and include a GFP tag to aid in transformant detection [313].

Moreover, targeted mutations in *Csa2G264590* led to a ten-fold increase in the number of spines on cucumber fruit compared to the wild type, which is an indication that structural traits can also be modified specifically [315]. Besides, mutating the *MLO*-type genes, or *CsaMLO1*, responsible for powdery mildew resistance, would be another potential strategy in breeding for resistance to mold, powdery mildew, or nematodes through mutation of the CsMS gene [316].

To develop viral resistance, CRISPR/Cas9 was used for the targeted mutation of the *eIF4E* (eukaryotic translation initiation factor 4E) gene in cucumber. This gene is known as the susceptibility (S) gene, which viruses use for infection. Gene editing resulted in plants resistant to several viral diseases, including cucumber vein yellowing virus (*ipomovirus*), zucchini yellow mosaic virus (*potyvirus*) and papaya ringspot virus-W (*potyvirus*). Homozygous progeny showed complete or significant resistance without other visible negative effects on plant development. The result shows how non-transgenic cucumbers with long-term virus resistance can be developed by using CRISPR/Cas9 [317].

Various breeding methods have been employed to achieve genetic enhancements in cucumbers, guided by specific breeding objectives. The primary goal of cucumber breeding programs is to develop varieties that are high-yielding, produce superior-quality fruits, and exhibit resistance to both biotic and abiotic stresses. The growing challenges in cultivation, along with rapid advancements in breeding technologies, have facilitated the development of cucumber varieties with improved resistance to diseases and tolerance to environmental stresses [318].

7.5. Lettuce

CRISPR/Cas9-based gene editing has been successfully demonstrated in lettuce, which is a model vegetable crop with a variety of characteristics. The CRISPR/Cas9 system was optimized to improve editing efficiency and stability by using the endogenous U6 promoter derived from the lettuce genome. The *PHOT2* gene was targeted to check the efficiency of editing, i.e., the setting of

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gene efficiency and system optimization in lettuce [319]. However, the *AtU6-26* promoter has been heterologously applied to drive sgRNAfor the CRISPR/Cas9 system in lettuce [320–322].

Nguyen et al. [322] use CRISPR gene editing for creating plants with novel phenotypes for faster plant production or marketing. The mentioned authors demonstrated the improved efficiency of a modified CRISPR/Cas9 construct through minimizing T-DNA positional effects and simplifying the process of transgene selection. In the study of 322], a CRISPR/Cas9 construct targeting the *LsVAR2* gene, which controls leaf variegation, was used. Homozygous mutations produced an albino phenotype, whereas heterozygous plants displayed variegated or mottled leaves.

Choi et al. [323] developed the late-bolting lettuce varieties using the CRISPR/Cas9 system to introduce a precise single-base modification of the *SOC1* gene, which regulates the onset of flowering in lettuce. Delayed bolting increases vegetative growth and may result in higher leaf yield.

Genetic engineering enables the direct manipulation of lettuce genes. By creating lines with enhanced tolerance to various environmental conditions, it can complement conventional breeding approaches, improving lettuce production efficiency and reducing losses caused by biotic and abiotic stresses [192]. Leveraging advances in molecular biology and genome sequencing, genetic engineering offers tools to increase both the yield and quality of lettuce. This approach has led to highly efficient and reliable transformation systems capable of directing nucleases to specific genetic sequences. In lettuce, transformation methods such as *Agrobacterium tumefaciens*-mediated techniques have shown strong success. These achievements have paved the way for genome editing approaches, including CRISPR/Cas9, which hold significant promise for enhancing performance and boosting yield. Consequently, genetic engineering in lettuce is poised to surpass traditional transformation techniques in effectiveness and efficiency [192].

8. Advantages of Integrating Hybrid and Molecular Breeding of Vegetables

Traditional breeding is a method of selective breeding in which crops are chosen for propagation based on their superior performance. This approach is time-consuming and relies heavily on the observable characteristics (phenotype) of plants. By combining with hybrid breeding, where heterosis (hybrid vigor) is exploited, highly productive and high-quality vegetable hybrids can be developed more quickly [324,325]. However, selection based solely on phenotypic traits is often imprecise. Consequently, breeders began incorporating multiple disciplines of biology into plant breeding, leading to the development of modern breeding techniques [326].

Today, this incorporation or integration of hybrid and molecular breeding approaches in vegetables offers several key advantages, including:

- The possibility of increasing breeding efficiency and speed [327,328].
- Greater precision in the selection of genetically complex traits [20,329],
- Improved stability and stress resistance of varieties [330],
- Optimized genetic resources and diversity [42],
- Reduction of costs and resources through targeted selection [331,332].

This application of molecular markers in hybrid programs enables more precise introgression and pyramiding of beneficial traits, including resistance to diseases and stress conditions [20]. Combining molecular techniques with hybrid programs, it is easier to identify and use genetically diverse parental materials to create superior hybrids. Although initial costs in marker development may be higher, marker-assisted selection reduces the need for large field populations and extensive phenotypic evaluations, ultimately lowering overall costs and accelerating the process [20,51,324,325]. The integration of molecular methods enables more precise mapping and introduction of genes for resistance to biotic and abiotic stressors (diseases, drought, high temperatures), which increases the stability of vegetable yields [51].

Through germplasm assessment, gene discovery, and the development of molecular markers, researchers and plant breeders are collaborating to enhance breeding technologies aimed at producing superior cultivars. As extensive genomic data and advanced tools continue to emerge, deeper partnerships will be essential to advance next-generation strategies such as genome-based

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breeding by design to develop high-yielding, high-quality vegetable cultivars that are environmentally sustainable and well adapted to changing conditions [333].

9. Limitations and Challenges of Integrating Conventional Breeding and Molecular Techniques

Although the integration of hybrid and molecular breeding in vegetable production brings precision, accelerates selection, and potentially increases genetic progress, it also has several significant disadvantages, including:

- High complexity and technical demands [326],
- Challenges in translating theoretical approaches into practical applications, with limited applicability under field conditions [334],
- Genetic uniformity and the risk of reducing genetic variability [335,336],
- High costs and long duration [337],
- Regulatory and societal constraints [20,338],
- Demands for a multidisciplinary approach [28,326].

Successful integration of hybridization and molecular techniques requires a multidisciplinary approach including classical genetics, molecular biology, statistics, and agronomy [28,326]. The lack of multidisciplinary teams can limit the possibilities for developing new cultivars or lines that successfully combine the aforementioned methods. The integration of molecular methods and hybrid breeding requires significantly greater technological and laboratory resources than conventional selection, including sophisticated tools for genotyping, gene analysis, and bioinformatic support, which can be an obstacle for smaller research teams or countries with more limited resources [327,339]. Furthermore, although molecular methods speed up the process, the initial investment is high, primarily in equipment, laboratory infrastructure, and experts for genetic analysis [340]. To achieve optimal profitability of such investments, long-term with a broad base of breeding programs must be developed.

Despite the fact that molecular methods can theoretically accelerate selection and improve accuracy in recognizing useful genes, their effectiveness in practice is often limited: it is difficult to predict how marker-related traits will behave in different climates and agroecosystems, and there is often a lack of clear strategies for transferring laboratory results into cultivated varieties with a realistically increased yield [334]. Marker-assisted selection (MAS) is a more efficient strategy for improving traits that have low heritability or are costly and labor-intensive to measure. However, it is most effective when applied to a limited number of quantitative traits that account for a significant proportion of the variation within a population [341].

Molecular techniques such as marker-assisted selection (MAS) have limitations, especially for complex, polygenically controlled traits (e.g., stress tolerance, nutritional quality). Marker-assisted selection is often inefficient for traits controlled by numerous small-effect genes, and precise mapping of all relevant quantitative traits is demanding and expensive. On the other hand, hybridization and selection with molecular markers can lead to a reduction in genetic variability if they focus only on a few high-potential genotypes. A lack of genetic variability can lead to increased susceptibility to diseases and stress factors in changing growing conditions [20,333,342].

These limitations must be addressed through appropriate funding, education, and multidisciplinary collaboration in order to realize the full potential of these approaches in vegetable production.

10. Conclusions

The integration of new technologies allows breeders to plan and develop inbred lines containing different combinations of desirable traits, based on predefined molecular profiles. Carefully selected parental lines, both genetically and phenotypically, then enable the development of F1 hybrids with optimally complementary traits. Advanced technological approaches thus accelerate the

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development of elite inbred lines and commercially valuable F1 hybrids with complex sets of traits, such as specific combinations of genes or QTL which correspond to specific growing conditions, that a significant reduction in breeding costs and time.

Similar to the marker-assisted selection (MAS), other modern technologies have also encouraged vegetable breeders to approach breeding with greater precision. In the past, breeders generally focused on one or a few traits that they improved over a long period of time. Conventional breeding techniques involved the development of numerous inbred lines and a detailed phenotypic evaluation, as well as testing innumerable experimental F1 hybrids before selecting those with market value potential.

The development of more advanced SNP chips will also enable early-stage genotyping of breeding material for a large number of traits and thus significantly reduce the time required for the identification of elite inbred lines and the development of desirable hybrid combinations.

If the breeder's resources allow the full breadth of integration of conventional and molecular breeding to be fully exploited, genetic progress and the faster development of higher-quality vegetable varieties are proportionally increased compared to either of these approaches alone. The true values of integration are a combination of speed, precision, saving time and resources, greater accuracy of selection, and more efficient use of plant genetic resources.

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Abbreviations

The following abbreviations are used in this manuscript:

WHO	World Health Organization
MAS	Marker-Assisted Selection
MABC	Marker-Assisted Backcrossing
MARS	Marker-Assisted Recurrent Selection
DNA	Deoxyribonucleic Acid
RAPD	Random Amplified Polymorphic DNA
ISSR	Inter-Simple Sequence Repeats
AFLP	Amplified Fragment Length Polymorphism
RFLP	Restriction Fragment Length Polymorphism
SNP	Single Nucleotide Polymorphism
SSR	Simple Sequence Repeat
SRAP	Sequence-Related Amplified Polymorphism
QTL	Quantitative Trait Loci
LD	Linkage Disequilibrium
KASP	Kompetitive Allele Specific PCR

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SCAR	Sequence Characterized Amplified Region
CAPS	Cleaved Amplified Polymorphic Sequences
PCR	Polymerase Chain Reaction
dCAPS	Derived Cleaved Amplified Polymorphic Sequences
InDel	Insertion-Deletion
ERPAR	Extended Random Primer Amplified Region
EST-SSR	Expressed Sequence Tag-Simple Sequence Repeats
SSAP	Sequence-specific Amplified Polymorphism
GS	Genomic Selection
GEBVs	Genomic Estimated Breeding Values
GCA	General Combining Ability
SCA	Specific Combining Ability
DH	Doubled Haploid
HTPP	High-Throughput Phenotyping
AI	Artificial Intelligence
gLMs	Genomic Language Models
sgRNA	Single Guide Ribonucleic Acid
CRISPR	Clustered Regularly Interspaced Short Palindromic Repeats
CRISPR/Cas9	CRISPR- associated Protein 9
RNP	Ribonucleoprotein
GMOs	Genetically Modified Organisms
NHEJ	Non-Homologous End Joining
HDR	Homology-Directed Repair

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