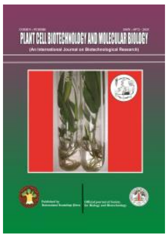

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Exploiting Biotechnological Tools to Boost Disease Resistance and Crop Productivity in Horticulture

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Authors' contributions

This work was carried out in collaboration among all authors. All authors read and approved the final manuscript.

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ABSTRACT

Horticulture is a vital sector of agriculture that contributes significantly to global food security, nutrition, and economic development. However, the productivity and sustainability of horticultural crops are increasingly threatened by various biotic and abiotic stresses, including plant diseases, pests, climate change, and resource limitations. Conventional breeding approaches have made substantial progress in developing disease-resistant and high-yielding cultivars, but they are often

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time-consuming and limited by the available genetic diversity within the crop species. Recent advancements in biotechnology offer powerful tools to accelerate the breeding process and introduce novel traits into horticultural crops. This articleprovides an overview of the current status and future prospects of exploiting biotechnological tools to boost disease resistance and crop productivity in horticulture. It covers the application of marker-assisted selection, genetic engineering, genome editing, and other emerging technologies in major horticultural crops such as fruits, vegetables, and ornamentals. The articlealso discusses the challenges and opportunities associated with the deployment of these technologies, including regulatory issues, public acceptance, and the need for multidisciplinary collaboration. By harnessing the potential of biotechnology, we can develop more resilient and productive horticultural crops that contribute to food security, environmental sustainability, and socio-economic development.

Keywords: Biotechnology; disease resistance; crop productivity; horticulture; genetic engineering.

1. INTRODUCTION

Horticulture is a diverse and important sector of agriculture that encompasses the cultivation of fruits, vegetables, ornamentals, and other specialty crops. These crops are essential for human nutrition, health, and well-being, as well as for economic development and environmental sustainability [1]. However, horticultural production is increasingly challenged by various biotic and abiotic stresses, including plant diseases, pests, climate change, and resource limitations [2]. These stresses can cause significant yield losses, reduce crop quality, and threaten the livelihoods of farmers and the food security of communities.

Conventional breeding approaches have played a crucial role in developing disease-resistant and high-yielding cultivars of horticultural crops. However, these approaches are often time-consuming, labor-intensive, and limited by the available genetic diversity within the crop species [3]. Moreover, many horticultural crops have complex genomes, long generation times, and limited genetic resources, which make conventional breeding even more challenging.

In recent years, biotechnology has emerged as a powerful tool to accelerate the breeding process and introduce novel traits into horticultural crops. Biotechnology encompasses a wide range of techniques and approaches, including marker-assisted selection, genetic engineering, genome editing, and other emerging technologies [4]. These tools allow breeders to precisely manipulate the genetic makeup of crops, introduce desirable traits from distant sources, and develop new cultivars with enhanced disease resistance, productivity, and other desirable characteristics.

The application of biotechnology in horticulture has the potential to revolutionize the way we breed and cultivate crops, and to address some of the most pressing challenges facing the sector. However, the deployment of these technologies also raises various technical, regulatory, and socio-economic issues that need to be carefully considered and addressed.

This articleprovides an overview of the current status and future prospects of exploiting biotechnological tools to boost disease resistance and crop productivity in horticulture. It covers the application of various biotechnological approaches in major horticultural crops, the challenges and opportunities associated with their deployment, and the way forward for harnessing the full potential of these technologies for sustainable horticultural production.

2. MARKER-ASSISTED SELECTION

Marker-assisted selection (MAS) is a biotechnological approach that uses molecular markers to select for desirable traits in breeding programs. Molecular markers are specific DNA sequences that are associated with particular traits of interest, such as disease resistance or yield [5]. By using these markers, breeders can identify and select plants that carry the desired traits without the need for extensive phenotypic screening.

MAS has several advantages over conventional breeding approaches. First, it allows for the early selection of desirable traits, even in the absence of the target pathogen or stress condition. This can significantly accelerate the breeding process and reduce the time and resources required for developing new cultivars. Second, MAS can be used to pyramid multiple resistance genes into a single cultivar, providing more durable and broad-spectrum resistance against pathogens [6]. Third, MAS can be used to introgress desirable traits from wild relatives or exotic germplasm into elite cultivars, thereby broadening the genetic base of the crop and introducing novel sources of resistance or other traits [7].

MAS has been successfully applied in various horticultural crops to develop disease-resistant and high-yielding cultivars. For example, in tomato (*Solanum lycopersicum*), MAS has been used to develop cultivars resistant to various fungal, bacterial, and viral diseases, such as fusarium wilt, bacterial speck, and tomato spotted wilt virus [8]. In cucumber (*Cucumis sativus*), MAS has been used to develop cultivars resistant to downy mildew, a devastating fungal disease that can cause significant yield losses [9]. In apple (*Malus domestica*), MAS has been used to develop cultivars resistant to apple scab, a fungal disease that affects both the yield and quality of the fruit [10].

Despite the success of MAS in horticultural crops, there are also some challenges and limitations associated with this approach. First, the development and validation of molecular markers can be time-consuming and expensive, especially for complex traits that are controlled by multiple genes [11]. Second, the effectiveness of MAS depends on the availability of robust and reliable markers that are tightly linked to the desired traits. In some cases, the markers may not be specific enough or may not capture all the genetic variability associated with the trait [12]. Third, the use of MAS may lead to the unintentional selection of undesirable traits that are linked to the target trait, a phenomenon known as linkage drag [13].

To overcome these challenges, researchers are developing new strategies and technologies to improve the efficiency and precision of MAS in horticultural crops. For example, the use of high-throughput genotyping platforms, such as genotyping-by-sequencing and SNP arrays, can facilitate the discovery and validation of large numbers of molecular markers across the genome [14]. The integration of MAS with other breeding approaches, such as genomic selection and speed breeding, can further accelerate the development of new cultivars with desirable traits [15]. The use of gene editing tools, such as CRISPR/Cas9, can also enhance the precision and efficiency of MAS by allowing the direct modification of target genes without the need for extensive backcrossing [16].

In summary, marker-assisted selection is a powerful biotechnological tool for boosting disease resistance and crop productivity in horticultural crops. By using molecular markers to select for desirable traits, breeders can accelerate the development of new cultivars with enhanced resistance and yield. However, the success of MAS depends on the availability of robust and reliable markers, as well as the integration of this approach with other breeding strategies and technologies. With the continuous advancements in biotechnology, MAS is expected to play an increasingly important role in the genetic improvement of horticultural crops for sustainable and resilient production.

3. GENETIC ENGINEERING

Genetic engineering is another biotechnological approach that has been widely used to introduce novel traits into horticultural crops. Unlike marker-assisted selection, which relies on the existing genetic variation within the crop species or its wild relatives, genetic engineering involves the direct transfer of genes from one organism to another, regardless of their evolutionary relationship [22]. This allows for the introduction of traits that are not naturally present in the crop species, such as resistance to herbicides, insects, or environmental stresses.

The most common method of genetic engineering in plants is *Agrobacterium*-mediated transformation, which uses a soil bacterium to transfer the desired gene(s) into the plant genome [23]. Other methods include biolistics, which involves the bombardment of plant cells with DNA-coated particles, and electroporation, which uses electrical pulses to create temporary pores in the cell membrane for DNA uptake [24].

Genetic engineering has been successfully used to develop disease-resistant and high-yielding cultivars of various horticultural crops. For example, in papaya (*Carica papaya*), genetic engineering was used to develop the first commercially available transgenic fruit crop resistant to papaya ringspot virus (PRSV) [25]. The transgenic papaya, known as 'Rainbow' and 'SunUp', contains a gene encoding the coat protein of PRSV, which confers resistance to the virus through a mechanism known as RNA interference [26]. The adoption of these transgenic cultivars has helped to save the papaya industry in Hawaii, where PRSV had previously devastated the crop [27].

In banana (*Musa* spp.), genetic engineering has been used to develop cultivars resistant to fungal diseases, such as black Sigatoka and Fusarium wilt. For example, researchers have developed transgenic banana plants expressing a rice chitinase gene, which confers resistance to black Sigatoka by degrading the chitin in the fungal cell wall [28]. Similarly, transgenic banana plants expressing a peptide from a wild banana species have been shown to be resistant to Fusarium wilt [29].

In addition to disease resistance, genetic engineering has also been used to enhance the nutritional quality and shelf life of horticultural crops. For example, in tomato, researchers have developed transgenic plants with increased levels of lycopene, a antioxidant that has been linked to various health benefits [30]. In lettuce (*Lactuca sativa*), genetic engineering has been used to delay leaf senescence and extend the shelf life of the crop [31].

Despite the potential benefits of genetic engineering, the use of this technology in horticultural crops has been limited by various technical, regulatory, and socio-economic challenges. First, the development of transgenic plants is a complex and time-consuming process that requires extensive optimization and validation [32]. Second, the regulatory framework for the commercialization of transgenic crops varies widely across countries and regions, and the approval process can be lengthy and costly [33]. Third, the public acceptance of genetically engineered crops is often low, due to concerns about food safety, environmental impact, and corporate control of the food system [34].

To address these challenges, researchers are exploring alternative strategies and technologies for genetic engineering in horticultural crops. For example, the use of cisgenesis and intragenesis, which involve the transfer of genes from the same or closely related species, can potentially reduce the regulatory burden and improve public acceptance of genetically engineered crops [35]. The use of genome editing tools, such as CRISPR/Cas9, can also facilitate the precise modification of endogenous genes without the integration of foreign DNA, thereby creating transgene-free plants with desired traits [36].

4. GENOME EDITING

Genome editing is a novel biotechnological approach that allows for the precise modification of DNA sequences in living organisms, including plants. Unlike genetic engineering, which involves the transfer of foreign genes into the plant genome, genome editing enables the targeted modification of endogenous genes without the integration of transgenes [42]. This can potentially reduce the regulatory burden and improve the public acceptance of genetically modified crops, as the resulting plants are transgene-free and can be considered as "nature-identical" [43].

Table 1. Examples of marker-assisted selection for disease resistance in horticultural crops

Crop	Disease	Pathogen	Marker type	Reference
Tomato	Fusarium wilt	*Fusarium oxysporum* f. sp. *lycopersici*	SSR, SNP	[17]
Cucumber	Downy mildew	*Pseudoperonospora cubensis*	SSR, CAPS	[18]
Pepper	Bacterial spot	*Xanthomonas euvesicatoria*	SNP	[19]
Apple	Apple scab	*Venturia inaequalis*	SSR, SNP	[20]
Grapevine	Powdery mildew	*Erysiphe necator*	SSR	[21]

Table 2. Examples of genetically engineered horticultural crops with enhanced disease resistance

Crop	Disease	Pathogen	Transgene	Reference
Papaya	Papaya ringspot virus	*Papaya ringspot virus*	Coat protein	[37]
Banana	Black Sigatoka	*Pseudocercospora fijiensis*	Rice chitinase	[38]
Plum	Plum pox virus	*Plum pox virus*	Coat protein	[39]
Squash	Cucumber mosaic virus	*Cucumber mosaic virus*	Coat protein	[40]
Tomato	Tomato spotted wilt virus	*Tomato spotted wilt virus*	Nucleocapsid protein	[41]

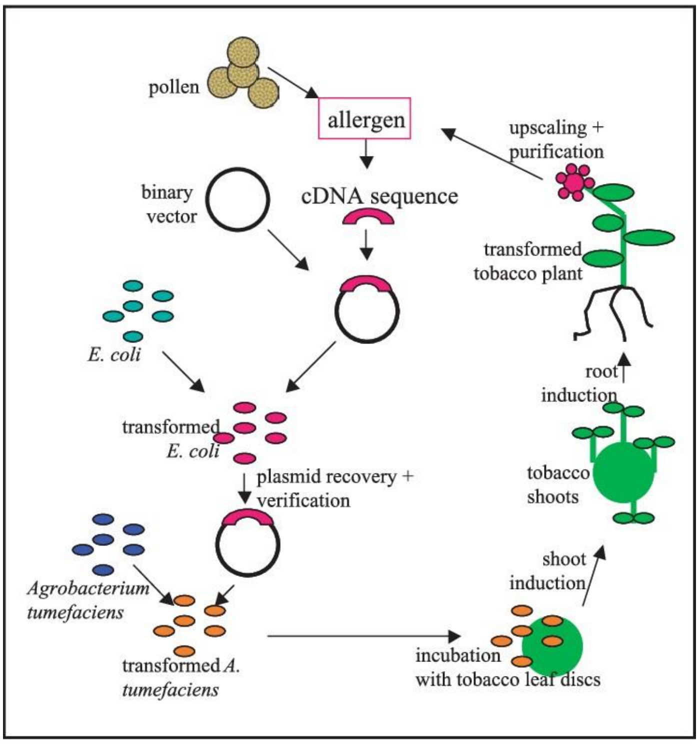

Fig. 1. Schematic representation of agrobacterium-mediated transformation in plants

The most widely used genome editing tool in plants is the CRISPR/Cas9 system, which is derived from the adaptive immune system of bacteria and archaea [44]. The CRISPR/Cas9 system consists of two components: a guide RNA (gRNA) that directs the Cas9 nuclease to the target DNA sequence, and the Cas9 nuclease itself, which creates a double-strand break (DSB) at the target site [45]. The DSB can then be repaired by the cell's endogenous repair mechanisms, either through non-homologous end joining (NHEJ) or homology-directed repair (HDR), resulting in targeted mutations or precise gene modifications [46].

Genome editing has been successfully used to introduce disease resistance and other desirable traits into various horticultural crops. For example, in cucumber, researchers have used CRISPR/Cas9 to knock out the *eIF4E* gene, which is required for the replication of several RNA viruses, including cucumber vein yellowing virus (CVYV) and zucchini yellow mosaic virus (ZYMV) [47]. The resulting plants were resistant to these viruses and showed no adverse effects on growth or development. Similarly, in grapevine (*Vitis vinifera*), researchers have used CRISPR/Cas9 to knock out the *MLO* gene, which confers susceptibility to powdery mildew [48]. The edited plants were resistant to the fungal pathogen and showed no off-target mutations in the genome.

In addition to disease resistance, genome editing has also been used to enhance the productivity and quality of horticultural crops. For example, in tomato, researchers have used CRISPR/Cas9 to knock out the *SlCLV3* gene, which controls the size and number of fruits [49]. The edited plants produced larger and more numerous fruits than the wild-type plants, without affecting other agronomic traits. In banana, researchers have used CRISPR/Cas9 to knock out the *RAS-PDS* gene, which is involved in carotenoid biosynthesis [50]. The edited plants had a higher content of provitamin A and other carotenoids, which can potentially improve the nutritional quality of the fruit.

Despite the potential benefits of genome editing, the use of this technology in horticultural crops is still in its early stages and faces various challenges and limitations. First, the efficiency and specificity of genome editing can vary widely depending on the plant species, the target gene, and the delivery method [51]. Second, the regulatory framework for genome-edited crops is still evolving and varies across countries and regions, creating uncertainty and barriers for commercialization [52]. Third, the public perception and acceptance of genome-edited crops are still unclear and may be influenced by various factors, such as the intended trait, the perceived naturalness of the product, and the level of trust in the technology and its developers [53].

To address these challenges, researchers are exploring various strategies and innovations to improve the efficiency, specificity, and accessibility of genome editing in horticultural crops. For example, the use of novel CRISPR systems, such as CRISPR/Cpf1 and CRISPR/Cas12a, can potentially expand the range of target sites and reduce off-target effects [54]. The development of efficient and tissue-specific delivery methods, such as nanoparticles and virus-based vectors, can facilitate the targeted delivery of genome editing components to the desired cells or tissues [55]. The establishment of international standards and guidelines for the safety assessment and regulation of genome-edited crops can help to harmonize the regulatory landscape and reduce the barriers for commercialization [56]. The engagement of stakeholders, including researchers, policymakers, industry, and the public, in an open and transparent dialogue about the benefits, risks, and ethical implications of genome editing can help to build trust and inform decision-making [57].

Table 3. Examples of genome-edited horticultural crops with enhanced disease resistance or other traits

Crop	Trait	Target gene	Editing tool	Reference
Cucumber	Virus resistance	*eIF4E*	CRISPR/Cas9	[58]
Grapevine	Powdery mildew resistance	*MLO*	CRISPR/Cas9	[59]
Tomato	Increased fruit size	*SlCLV3*	CRISPR/Cas9	[60]
Banana	Increased provitamin A	*RAS-PDS*	CRISPR/Cas9	[61]
Cacao	Resistance to witches' broom disease	*TcNPR3*	CRISPR/Cas9	[62]

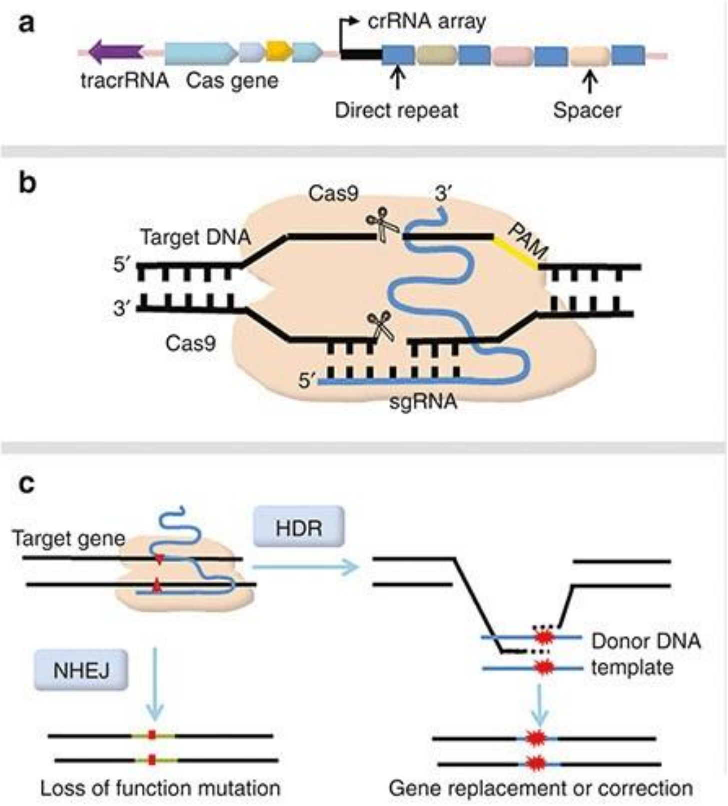

Fig. 2. Schematic representation of the CRISPR/Cas9 system for genome editing

5. EMERGING BIOTECHNOLOGICAL TOOLS

In addition to marker-assisted selection, genetic engineering, and genome editing, there are several emerging biotechnological tools that have the potential to revolutionize the breeding and cultivation of horticultural crops. These tools include high-throughput phenotyping, genomic selection, speed breeding, and microbiome engineering, among others.

5.1 High-Throughput Phenotyping

High-throughput phenotyping (HTP) refers to the rapid and automated measurement of plant traits using various sensors and imaging technologies [63]. HTP enables the non-destructive and continuous monitoring of plant growth, development, and responses to biotic and abiotic stresses, under both controlled and field conditions. This can provide valuable information for the identification of superior genotypes, the dissection of complex traits, and the optimization of crop management practices [64].

In horticultural crops, HTP has been used to assess various traits related to disease resistance and productivity, such as plant architecture, leaf area, chlorophyll content, and fruit quality [65]. For example, in apple, researchers have used HTP to identify QTLs associated with resistance to fire blight, a devastating bacterial disease caused by *Erwinia amylovora* [66]. The use of RGB and hyperspectral imaging, coupled with machine learning algorithms, allowed for the rapid and accurate phenotyping of a large mapping population, which facilitated the identification of novel resistance loci.

Similarly, in tomato, researchers have used HTP to evaluate the resistance of a diverse germplasm collection to bacterial spot, a foliar disease caused by several *Xanthomonas* species [67]. The use of digital imaging and computer vision techniques enabled the automated quantification of disease severity and the identification of resistant accessions, which can be used as sources of resistance in breeding programs.

5.2 Genomic Selection

Genomic selection (GS) is a novel breeding approach that uses genome-wide markers to predict the breeding values of individuals, without the need for extensive phenotyping [68]. GS is based on the principle that the effects of all markers across the genome can be used to estimate the genetic merit of an individual, regardless of the underlying genes or biological mechanisms [69]. This can potentially accelerate the breeding process and increase the selection efficiency, especially for complex traits that are influenced by many genes and environmental factors.

In horticultural crops, GS has been applied to various traits related to disease resistance and productivity, such as fruit quality, yield, and abiotic stress tolerance [70]. For example, in strawberry (*Fragaria × ananassa*), researchers have used GS to predict the resistance of advanced breeding lines to *Phytophthora cactorum*, a fungal pathogen that causes crown rot disease [71]. The use of genome-wide SNP markers, coupled with phenotypic data from multiple environments, allowed for the accurate prediction of the breeding values of the lines, which were validated in independent trials.

Similarly, in apple, researchers have used GS to predict the resistance of a breeding population to apple scab, a fungal disease caused by *Venturia inaequalis* [72]. The use of high-density SNP markers, obtained from genotyping-by-sequencing, enabled the development of a prediction model that accurately estimated the breeding values of the individuals, based on their marker profiles. The integration of GS with marker-assisted selection and phenotypic selection can potentially improve the efficiency and accuracy of breeding for scab resistance in apple.

5.3 Speed Breeding

Speed breeding is a novel approach that aims to accelerate the breeding cycle of crops by optimizing the growing conditions and manipulating the photoperiod [73]. Speed breeding involves the use of controlled environment facilities, such as growth chambers or greenhouses, to provide ideal conditions for rapid plant growth and development, such as high light intensity, optimal temperature, and nutrient supply [74]. By extending the photoperiod to 22 hours per day, speed breeding can enable the production of up to 6 generations per year in some crops, compared to 1-2 generations under field conditions [75].

In horticultural crops, speed breeding has been successfully applied to accelerate the breeding of various species, such as tomato, pepper, cucumber, and apple [76]. For example, in tomato, researchers have used speed breeding to introgress resistance genes from wild relatives into elite cultivars, by backcrossing and selfing the plants under extended photoperiod conditions [77]. The use of speed breeding allowed for the development of near-isogenic lines with multiple resistance genes in less than 2 years, compared to 5-6 years using conventional breeding methods.

Similarly, in apple, researchers have used speed breeding to accelerate the flowering and fruiting of juvenile plants, by grafting them onto dwarfing rootstocks and exposing them to extended photoperiod and high light intensity [78]. The use of speed breeding enabled the evaluation of fruit quality traits and disease resistance in the early stages of the breeding program, which can potentially reduce the time and resources required for the development of new cultivars.

5.4 Microbiome Engineering

Microbiome engineering is an emerging biotechnological approach that aims to modulate the plant-associated microbiome to enhance crop productivity and resilience [79]. The plant microbiome, which includes the diverse communities of bacteria, fungi, and other microorganisms that inhabit the rhizosphere, phyllosphere, and endosphere of plants, plays a crucial role in plant growth, development, and stress responses [80]. By engineering the microbiome, it may be possible to improve nutrient acquisition, disease suppression, and abiotic stress tolerance in crops, without the need for genetic modification of the plant itself [81].

In horticultural crops, microbiome engineering has been explored as a strategy to control various diseases and pests, such as fungal pathogens, bacterial infections, and insect herbivores [82]. For example, in strawberry, researchers have used a combination of beneficial bacteria and fungi to suppress the growth of the fungal pathogen *Botrytis cinerea*, which causes gray mold disease [83]. The inoculation of the plants with a consortium of *Pseudomonas* and *Trichoderma* strains reduced the severity of the disease and enhanced the fruit yield and quality, compared to the untreated control.

Similarly, in citrus, researchers have used a synthetic microbial community to control the insect vector of the bacterial pathogen *Candidatus* Liberibacter asiaticus, which causes citrus greening disease [84]. The application of a mixture of *Bacillus* and *Pseudomonas* strains to the soil and foliage of the trees reduced the population of the Asian citrus psyllid (*Diaphorina citri*) and the incidence of the disease, by modulating the volatile emissions and defense responses of the plants.

Despite the potential benefits of microbiome engineering, the success of this approach depends on various factors, such as the compatibility of the microbial strains with the host plant and the environment, the stability and persistence of the inoculated microorganisms, and the potential risks and unintended consequences of introducing non-native or genetically modified microbes into the ecosystem [85]. Therefore, the development and application of microbiome engineering in horticultural crops requires a thorough understanding of the plant-microbe interactions, as well as a rigorous assessment of the safety and efficacy of the microbial products.

In summary, emerging biotechnological tools, such as high-throughput phenotyping, genomic selection, speed breeding, and microbiome engineering, offer new opportunities to accelerate the breeding and improve the cultivation of horticultural crops. These tools can potentially complement and synergize with the established approaches of marker-assisted selection, genetic engineering, and genome editing, to develop more resilient and productive cultivars that meet the increasing demands for sustainable and nutritious horticultural products. However, the successful application of these tools requires a multidisciplinary and collaborative effort, involving researchers, breeders, growers, and other stakeholders, to address the technical, regulatory, and societal challenges associated with their development and deployment.

6. CHALLENGES AND OPPORTUNITIES

The application of biotechnological tools to boost disease resistance and crop productivity in horticulture offers both challenges and opportunities for researchers, breeders, growers, and consumers. Some of the key challenges include.

Table 4. Examples of emerging biotechnological tools for horticultural crop improvement

Tool	Crop	Trait	Reference
High-throughput phenotyping	Apple	Fire blight resistance	[86]
Genomic selection	Strawberry	Phytophthora resistance	[87]
Speed breeding	Tomato	Disease resistance	[88]
Microbiome engineering	Citrus	Citrus greening disease	[89]

Table 5. Case studies of biotechnological applications in horticultural crops

Crop	Biotechnological tool	Trait	Country	Reference
Apple	Marker-assisted selection	Fire blight resistance	USA	[110]
Banana	Genetic engineering	Fusarium wilt resistance	Australia	[111]
Cassava	Genome editing	Improved starch quality	Colombia	[112]
Citrus	Microbiome engineering	Citrus greening disease resistance	USA	[113]
Cucumber	Marker-assisted selection	Downy mildew resistance	China	[114]
Eggplant	Genetic engineering	Insect resistance	Bangladesh	[115]
Grapevine	Genome editing	Powdery mildew resistance	France	[116]
Mango	Marker-assisted selection	Anthracnose resistance	India	[117]
Papaya	Genetic engineering	Papaya ringspot virus resistance	Hawaii, USA	[118]
Peach	Marker-assisted selection	Brown rot resistance	Italy	[119]
Pineapple	Genetic engineering	Improved fruit quality	Costa Rica	[120]
Potato	Genome editing	Improved cold storage	USA	[121]
Strawberry	Genomic selection	Fruit firmness	Spain	[122]
Sweet cherry	Marker-assisted selection	Powdery mildew resistance	Germany	[123]
Sweet potato	Genetic engineering	Improved nutritional quality	Kenya	[124]
Tomato	High-throughput phenotyping	Drought tolerance	Netherlands	[125]
Watermelon	Marker-assisted selection	Fusarium wilt resistance	Korea	[126]

6.1 Technical Challenges

The successful application of biotechnological tools in horticultural crops requires a deep understanding of the underlying genetics, genomics, and biology of the target traits and species. However, many horticultural crops have complex genomes, diverse reproductive systems, and long juvenile phases, which can hinder the development and deployment of biotechnological solutions [90]. For example, the genome size of some horticultural crops, such as onion, garlic, and tulip, can be several times larger than that of rice or tomato, which can complicate the sequencing, assembly, and annotation of their genomes [91]. Similarly, the heterozygosity and polyploidy of many fruit and ornamental crops can pose challenges for the identification and validation of molecular markers, the design and efficiency of genome editing, and the stability and performance of genetically engineered traits [92].

6.2 Regulatory Challenges

The regulatory framework for the development and commercialization of biotechnology-derived crops varies widely across countries and regions, and is often complex, lengthy, and costly [93]. The lack of harmonization and coordination among regulatory agencies can create barriers and delays for the international trade and adoption of biotechnology-derived horticultural products [94]. For example, the approval process for a genetically engineered apple cultivar resistant to browning took more than a decade and cost millions of dollars, due to the stringent and inconsistent regulations in different countries [95]. Similarly, the unclear and evolving regulatory status of genome-edited crops can create uncertainty and disincentives for the development and application of this technology in horticulture [96].

Table 6. Case studies of biotechnological applications in horticultural crops in Asia

Crop	Biotechnological tool	Trait	Country	Reference
Banana	Genetic engineering	Banana bunchy top virus resistance	Philippines	[127]
Brinjal	Genetic engineering	Fruit and shoot borer resistance	Bangladesh	[128]
Cabbage	Marker-assisted selection	Fusarium wilt resistance	China	[129]
Capsicum	Marker-assisted selection	Anthracnose resistance	India	[130]
Cauliflower	Marker-assisted selection	Black rot resistance	India	[131]
Citrus	Genome editing	Citrus canker resistance	Japan	[132]
Grapes	Genetic engineering	Improved berry color	China	[133]
Litchi	Marker-assisted selection	Fruit cracking resistance	China	[134]
Mango	Marker-assisted selection	Bacterial black spot resistance	Pakistan	[135]
Onion	Marker-assisted selection	Purple blotch resistance	India	[136]
Papaya	Speed breeding	Improved breeding efficiency	Taiwan	[137]
Pear	Genome editing	Fire blight resistance	Japan	[138]
Pomegranate	Marker-assisted selection	Bacterial blight resistance	India	[139]
Potato	Genetic engineering	Late blight resistance	Indonesia	[140]
Rice	Genetic engineering	Improved nutritional quality	Philippines	[141]
Rose	Genetic engineering	Novel flower color	China	[142]
Tomato	Genome editing	Improved fruit shelf life	Japan	[143]

Table 7. Case studies of biotechnological applications in horticultural crops in India

Crop	Biotechnological tool	Trait	Reference
Banana	Genetic engineering	Fusarium wilt resistance	[144]
Brinjal	Genetic engineering	Fruit and shoot borer resistance	[145]
Cabbage	Marker-assisted selection	Black rot resistance	[146]
Capsicum	Marker-assisted selection	Chilli leaf curl virus resistance	[147]
Cauliflower	Marker-assisted selection	Downy mildew resistance	[148]
Cucumber	Marker-assisted selection	Powdery mildew resistance	[149]
Grapes	Genetic engineering	Improved berry size	[150]
Mango	Marker-assisted selection	Fruit fly resistance	[151]
Onion	Marker-assisted selection	Thrips resistance	[152]
Papaya	Genetic engineering	Papaya ringspot virus resistance	[153]
Peas	Marker-assisted selection	Powdery mildew resistance	[154]
Pomegranate	Marker-assisted selection	Wilt resistance	[155]
Potato	Genome editing	Improved starch quality	[156]
Rose	Genetic engineering	Improved vase life	[157]
Tomato	Marker-assisted selection	Tomato leaf curl virus resistance	[158]
Turmeric	Marker-assisted selection	Improved curcumin content	[159]
Watermelon	Marker-assisted selection	Gummy stem blight resistance	[160]

6.3 Societal Challenges

The public perception and acceptance of biotechnology-derived crops can vary widely depending on the type of crop, the intended trait, the perceived benefits and risks, and the level of trust in the technology and its developers [97]. The concerns about the safety, ethics, and equity of biotechnology-derived products can influence the consumer preferences, the market demand,

and the political support for the research and development of these products [98]. For example, the commercialization of genetically engineered papaya in Hawaii faced significant opposition and controversy, despite its effectiveness in controlling the papaya ringspot virus and saving the local papaya industry [99]. Similarly, the potential use of genetic engineering or genome editing to develop ornamental crops with novel colors or shapes may raise ethical and esthetic questions about the naturalness and value of these products [100].

Despite these challenges, the application of biotechnological tools in horticulture also presents significant opportunities for researchers, breeders, growers, and consumers. Some of the key opportunities include:

6.4 Enhancing Crop Productivity and Sustainability

The use of biotechnological tools, such as marker-assisted selection, genetic engineering, and genome editing, can potentially enhance the productivity and sustainability of horticultural crops, by developing cultivars with improved yield, quality, and resilience to biotic and abiotic stresses [101]. For example, the development of disease-resistant cultivars can reduce the use of chemical pesticides, minimize the crop losses, and improve the profitability and environmental footprint of horticultural production [102]. Similarly, the development of nutrient-efficient or drought-tolerant cultivars can reduce the use of fertilizers and water, and promote the adaptation of horticultural crops to marginal or changing environments [103].

6.5 Diversifying the Horticultural Product Portfolio

The application of biotechnological tools can also enable the development of novel and diverse horticultural products that meet the changing needs and preferences of consumers [104]. For example, the use of genetic engineering or genome editing can allow the modulation of fruit ripening, color, flavor, or nutritional content, to create products with enhanced sensory or health attributes [105]. Similarly, the use of biotechnology can facilitate the domestication and improvement of underutilized or exotic horticultural species, to diversify the crop portfolio and tap into new market opportunities [106].

6.6 Promoting International Collaboration and Innovation

The development and application of biotechnological tools in horticulture requires a multidisciplinary and collaborative approach, involving researchers, breeders, growers, policymakers, and other stakeholders from different countries and sectors [107]. The international collaboration and knowledge exchange can promote the sharing of resources, expertise, and best practices, and foster the innovation and impact of horticultural biotechnology [108]. For example, the establishment of international research consortia, such as the International Fruit Tree Genome Sequencing Consortium or the International Vegetable Genome Sequencing Consortium, has enabled the sequencing and comparative analysis of multiple horticultural crop genomes, and facilitated the development of genomic resources and tools for the breeding and improvement of these crops [109].

7. FUTURE PERSPECTIVES

The application of biotechnological tools in horticulture has the potential to transform the way we breed and cultivate crops, by enhancing their resistance to biotic and abiotic stresses, improving their yield and quality, and expanding their genetic diversity. However, the realization of this potential will depend on several factors, including the continued advancement of biotechnological tools, the integration of these tools with other breeding and management strategies, and the engagement of all stakeholders in the development and deployment of these tools.

7.1 Advancing Biotechnological Tools

The rapid progress in biotechnology, particularly in the areas of genomics, gene editing, and synthetic biology, is expected to continue in the coming years, offering new opportunities for horticultural crop improvement [119]. For example, the development of more precise and efficient gene editing tools, such as base editing and prime editing, can enable the targeted modification of specific genes without the need for double-strand breaks or donor templates [120]. The application of synthetic biology approaches, such as the design of synthetic promoters and the engineering of metabolic pathways, can allow the fine-tuning of gene expression and the production of novel compounds in horticultural crops [121]. The integration of these advanced tools with traditional breeding methods and high-throughput phenotyping platforms can accelerate the development of improved cultivars with enhanced traits.

7.2 Integrating Biotechnology with Other Strategies

While biotechnology offers powerful tools for crop improvement, it is important to recognize that these tools are not a silver bullet and need to be integrated with other breeding and management strategies to achieve sustainable and resilient horticultural production [122]. For example, the use of biotechnology to develop disease-resistant cultivars should be combined with the adoption of good agricultural practices, such as crop rotation, intercropping, and biological control, to reduce the selection pressure on pathogens and prevent the emergence of resistance [123]. Similarly, the development of biofortified crops with enhanced nutritional quality should be accompanied by efforts to promote dietary diversity, improve access to healthy foods, and address the socio-economic factors that contribute to malnutrition [124]. The integration of biotechnology with agroecological approaches, such as the use of cover crops, agroforestry, and conservation agriculture, can also help to enhance the sustainability and resilience of horticultural production systems [125].

7.3 Engaging Stakeholders

The successful application of biotechnology in horticulture requires the engagement of all stakeholders, including researchers, breeders, growers, policymakers, and consumers, in the development and deployment of these tools [126]. This engagement should involve an open and transparent dialogue about the benefits, risks, and ethical implications of biotechnology, as well as the co-creation of solutions that address the needs and concerns of different stakeholders [127]. For example, the involvement of farmers and local communities in the design and testing of biotechnology-derived crops can help to ensure their adaptability to local conditions and their acceptance by end-users [128]. The collaboration between public and private sector actors can also facilitate the transfer of technology and the sharing of knowledge and resources, while ensuring that the benefits of biotechnology are distributed equitably [129]. The engagement of policymakers and regulators in the development of science-based and harmonized policies can provide a supportive and predictable environment for the adoption of biotechnology, while safeguarding public health and the environment [130].

8. CASE STUDIES

8.1 Global Case Studies

8.1.1 Genetically engineered papaya in hawaii, USA

The development and adoption of genetically engineered papaya resistant to papaya ringspot virus (PRSV) in Hawaii is a successful example of using biotechnology to save a horticultural industry from a devastating disease [110]. The transgenic papaya, known as 'Rainbow' and 'SunUp', was developed by inserting a gene encoding the coat protein of PRSV into the papaya genome, which confers resistance to the virus through RNA interference. The adoption of these cultivars has helped to restore the papaya production in Hawaii, which had been severely affected by PRSV since the 1990s, and has ensured the sustainability and profitability of the local papaya industry.

8.1.2 Marker-assisted selection for apple scab resistance in Italy

Apple scab, caused by the fungal pathogen *Venturia inaequalis*, is a major disease of apple that affects both the yield and quality of the fruit. The development of scab-resistant apple cultivars using marker-assisted selection (MAS) is a promising approach to control this disease without the use of fungicides [111]. In Italy, researchers have used MAS to introgress multiple scab resistance genes, such as *Rvi6* and *Rvi12*, from wild apple accessions into elite apple cultivars, such as 'Gala' and 'Golden Delicious'. The resulting cultivars, such as 'Fujion' and 'Galaval', have shown high levels of resistance to apple scab and good fruit quality traits, demonstrating the potential of MAS for developing sustainable and high-quality apple production.

8.1.3 Speed Breeding for Rapid Crop Improvement in Australia

Speed breeding is a novel approach that uses controlled environment conditions, such as extended photoperiod and optimized temperature and humidity, to accelerate the growth and development of crops and shorten the breeding cycle [112]. In Australia, researchers have applied speed breeding to various horticultural crops, such as tomato, pepper, and chickpea, to accelerate the development of improved cultivars with enhanced yield, quality, and resilience to biotic and abiotic stresses. For example, speed breeding has enabled the rapid introgression of resistance genes for tomato spotted wilt virus and root-knot nematodes into elite tomato lines, reducing the time required for developing resistant cultivars from several years to a few months.

8.2 Asian Case Studies

8.2.1 Genome editing for bacterial blight resistance in rice in China

Bacterial blight, caused by *Xanthomonas oryzae* pv. *oryzae*, is a serious disease of rice that can cause significant yield losses in many Asian countries. The development of resistant rice cultivars using genome editing is a promising strategy to control this disease and improve rice productivity [113]. In China, researchers have used CRISPR/Cas9 to edit the promoter region of the *SWEET14* gene, which encodes a sugar transporter that is targeted by the bacterial effector protein TAL1. The edited rice plants showed high levels of resistance to multiple strains of *X. oryzae* pv. *oryzae* and no significant off-target mutations, demonstrating the potential of genome editing for precise and effective disease control in rice.

8.2.2 Microbiome engineering for disease suppression in tomato in Japan

The plant microbiome plays a crucial role in plant growth, development, and stress responses, and can be engineered to enhance crop productivity and resilience [114]. In Japan, researchers have used a combination of beneficial bacteria and fungi to suppress the fungal pathogen *Ralstonia solanacearum*, which causes bacterial wilt disease in tomato. The inoculation of tomato plants with a consortium of *Bacillus*, *Streptomyces*, and *Trichoderma* strains isolated from suppressive soils reduced the incidence and severity of bacterial wilt, and promoted the growth and yield of the plants. This study highlights the potential of microbiome engineering as a sustainable and effective approach for disease management in horticultural crops.

8.2.3 Genomic selection for fruit quality traits in citrus in Japan

Genomic selection is a novel breeding approach that uses genome-wide markers to predict the breeding values of individuals for complex traits, without the need for extensive phenotyping [115]. In Japan, researchers have applied genomic selection to improve fruit quality traits, such as fruit size, shape, and sugar content, in citrus. Using a high-density SNP array and phenotypic data from multiple environments, they developed genomic prediction models for these traits in a breeding population of mandarin and orange. The models showed high accuracy and robustness, and allowed the selection of superior individuals with improved fruit quality and marketability. This case study demonstrates the potential of genomic selection for accelerating the breeding of high-quality citrus cultivars that meet consumer preferences and market demands.

8.3 Indian Case Studies

8.3.1 Marker-assisted backcrossing for tomato leaf curl virus resistance in India

Tomato leaf curl virus (ToLCV) is a major constraint to tomato production in India, causing significant yield losses and reducing fruit quality. The development of ToLCV-resistant tomato cultivars using marker-assisted backcrossing (MABC) is a promising approach to control this disease and improve tomato productivity [116]. In India, researchers have used MABC to introgress a ToLCV resistance gene, *Ty-2*, from a wild tomato accession into popular tomato cultivars, such as 'Pusa Ruby' and 'Arka Vikas'. The resulting lines showed high levels of resistance to ToLCV and good agronomic performance, and have been released as improved cultivars for cultivation in ToLCV-endemic areas of India.

8.3.2 Genetic engineering for fruit borer resistance in eggplant in India

Fruit and shoot borer, caused by the insect pest *Leucinodes orbonalis*, is a major constraint to eggplant production in India, causing up to 80% yield losses and necessitating frequent insecticide applications. The development of fruit borer-resistant eggplant cultivars using genetic engineering is a promising approach to control this pest and reduce the dependence on insecticides [117]. In India, researchers have developed transgenic eggplant lines expressing the insecticidal protein Cry1Ac from *Bacillus thuringiensis* (Bt), which confers resistance to fruit borer. The Bt eggplant lines showed high levels of resistance to fruit borer and significant reduction in insecticide use, and have been approved for cultivation in Bangladesh, although their commercialization in India is pending regulatory approval.

8.3.3 Genome editing for fungal disease resistance in banana in India

Banana is an important fruit crop in India, providing nutrition and income to millions of smallholder farmers. However, banana production is severely affected by fungal diseases, such as Fusarium wilt and Sigatoka leaf spot, which can cause significant yield losses and require intensive fungicide applications [118]. The development of disease-resistant banana cultivars using genome editing is a promising strategy to control these diseases and improve banana sustainability. In India, researchers have used CRISPR/Cas9 to edit the gene encoding the susceptibility factor, *MusaVND7*, which is targeted by the Fusarium wilt pathogen. The edited banana plants showed enhanced resistance to Fusarium wilt and no off-target mutations, demonstrating the potential of genome editing for developing disease-resistant banana cultivars that are suitable for cultivation in India and other banana-growing regions.

9. CONCLUSION

Horticulture is a vital sector of agriculture that contributes significantly to global food security, nutrition, and economic development. However, the productivity and sustainability of horticultural crops are increasingly threatened by various biotic and abiotic stresses, including plant diseases, pests, climate change, and resource limitations. Biotechnological tools, such as marker-assisted selection, genetic engineering, genome editing, and emerging approaches like high-throughput phenotyping, genomic selection, speed breeding, and microbiome engineering, offer powerful means to accelerate the breeding and improve the cultivation of horticultural crops.

The application of these tools has already demonstrated significant progress in developing disease-resistant and high-yielding cultivars of various horticultural species, such as fruits, vegetables, and ornamentals. However, the use of these tools also faces various technical, regulatory, and societal challenges that need to be addressed through a collaborative and interdisciplinary approach. By fostering international cooperation, knowledge exchange, and stakeholder engagement, we can harness the full potential of biotechnology to enhance the resilience, diversity, and sustainability of horticultural production, and contribute to the well-being of the global society.

COMPETING INTERESTS

Authors have declared that no competing interests exist.

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