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Advancements in Genetic Engineering for Enhanced Traits in Horticulture Crops: A Comprehensive Review

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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

Genetic engineering has emerged as a powerful tool in the field of horticulture to enhance the traits of crops, leading to improved yield, quality, and resistance to biotic and abiotic stresses. This comprehensive review explores the recent advancements in genetic engineering techniques applied to horticulture crops, providing a detailed overview of the innovative strategies employed to manipulate plant genomes. The review begins by discussing the evolution of genetic engineering in horticulture, highlighting key milestones and breakthroughs that have paved the way for current advancements. It covers a range of techniques such as CRISPR-Cas9, RNA interference, and synthetic biology, shedding light on their applications in modifying specific genes responsible for desired traits. The main focus of the review is on the enhancement of key horticultural traits, including disease resistance, pest resistance, abiotic stress tolerance, and post-harvest attributes. Examples from various horticultural crops, such as fruits, vegetables, and ornamental plants, illustrate the success stories and potential applications of genetic engineering in each category. The ethical and regulatory aspects of genetic engineering in horticulture are also explored, addressing concerns related to environmental impact, biodiversity, and consumer acceptance. The review emphasizes the importance of responsible and sustainable practices in the application of genetic engineering to ensure the long-term benefits without adverse consequences. Furthermore, the review delves into emerging trends and future prospects in the field, including the potential of genome editing for precision breeding, the use of omics technologies for targeted trait improvements, and the integration of genetic engineering with other breeding techniques. It also discusses the challenges and opportunities associated with the global adoption of genetically modified horticulture crops.

Keywords: genetic; synthetic; biodiversity; engineering.

1. INTRODUCTION

Genetic engineering, also known as genetic modification or gene editing, is a powerful and sophisticated set of biotechnological techniques used to manipulate the genetic material of organisms [1]. This field of science allows researchers to selectively modify the DNA or RNA of an organism, enabling the introduction, removal, or alteration of specific genetic elements. The primary goal of genetic engineering is to bring about desired changes in the traits or characteristics of an organism, whether it be a plant, animal, or microbe [2]. Techniques such as CRISPR-Cas9, RNA interference, and synthetic biology are commonly employed to precisely target and modify genes, facilitating the enhancement of desirable traits such as increased yield, improved nutritional content, resistance to diseases, and tolerance to environmental stresses [3]. Genetic engineering has widespread applications in agriculture, medicine, and various industries, offering innovative solutions to address challenges in food security, healthcare, and the production of valuable bio products. Despite its immense potential, the field of genetic engineering is accompanied by ethical, environmental, and regulatory considerations that necessitate responsible and transparent practices in its application [4].

A powerful biotechnological tool with revolutionary potential for horticulture crops, genome editing enables the exact change of plant DNA to enhance desired traits, increase crop output, and confer resistance to pests, diseases, and environmental challenges. Genome editing has the potential to transform horticultural crops [5]. CRISPR-Cas9, TALENs, and ZFNs are examples of molecular tools that carry out the function of molecular scissors. These tools make it possible to modify specific DNA sequences with an accuracy that has never been seen before. With the help of this technology, scientists are able to introduce or increase desirable features in crops, such as resistance to disease, improved nutrition, and tolerance to drought [6].

Because it encompasses the cultivation and management of plants for the purposes of food, beauty, and medical uses, horticulture is an essential component of both the global food production and the well-being of humans [7]. The production and quality of horticultural crops, on the other hand, are subject to considerable limits as a result of problems such as biotic and abiotic stressors, limited genetic variation, and

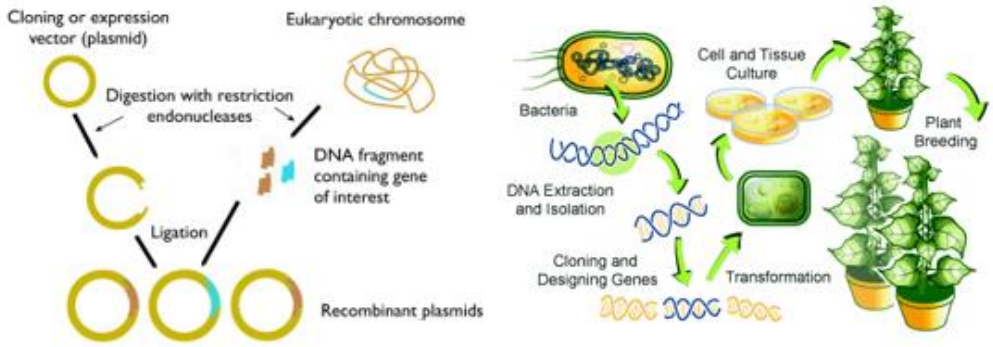

Fig. 1. Genetic engineering

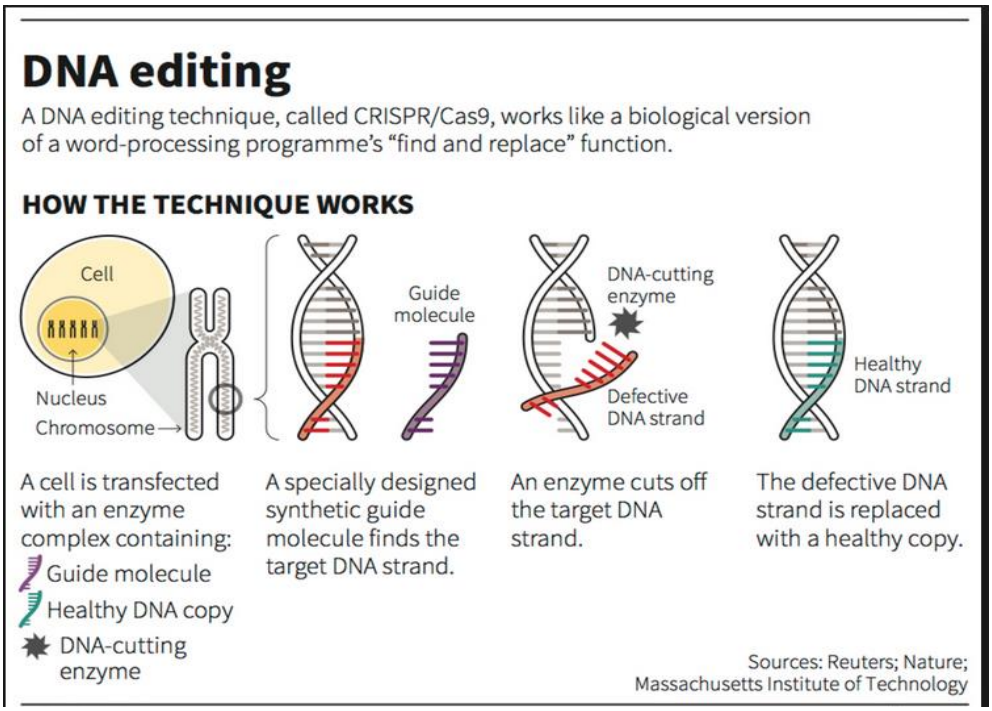

Fig. 2. DNA editing

increasing demands for enhanced features [8]. Traditional techniques of breeding have a number of drawbacks, such as lengthy breeding cycles, restricted genetic variation, and intricate genetic architectures. A revolutionary technology that has the potential to change crop development, especially horticultural crops, genome editing has emerged as a breakthrough in the field of agricultural improvement [9].

As a method for editing genomes, the CRISPR-Cas9 system is widely utilized. This system makes use of guide RNA to control the Cas9 enzyme, which allows for precise DNA cleavage and other alterations [10]. Researchers in the field of horticulture crop research also make use of TALENs and ZFNs, which are alternative genome editing methods. By focusing on particular genes that are related with desirable characteristics, these technologies make it possible to modify plant genomes with pinpoint accuracy [11]. CRISPR-Cas9 has demonstrated its efficacy in enhancing essential characteristics in a wide range of horticulture crops by the application of targeted changes. Researchers have the ability to instruct the Cas9 enzyme to target genes associated with qualities of interest, including as disease resistance, abiotic stress tolerance, nutritional content, and yield-related attributes. This is accomplished by the design of specific gRNAs [12]. The precise precision of CRISPR-Cas9 makes it possible to introduce advantageous mutations or targeted gene knockouts, which allows for the simulation of

natural genetic differences and speeds up the process of breeding [13]. In order to target certain genomic sequences, TALENs and ZFNs make use of designed DNA-binding proteins that can be modified to meet specific requirements. These tools, which are very similar to CRISPR-Cas9, are able to induce targeted DNA cleavage and subsequent alterations at the genomic regions that are required [14]. CRISPR-Cas9 has become increasingly popular because to the fact that it is simple to use, effective, and versatile in terms of its ability to manipulate genetic material across a wide range of organisms, including horticulture crops. The CRISPR-Cas9 system is a potent tool that enables precise genome editing in plants [15]. It originates from the defence mechanism that bacteria use to protect themselves from viral infections. Through the use of this technology, researchers are able to target particular genes that are connected with desirable features and introduce alterations in order to improve agricultural attributes [16].

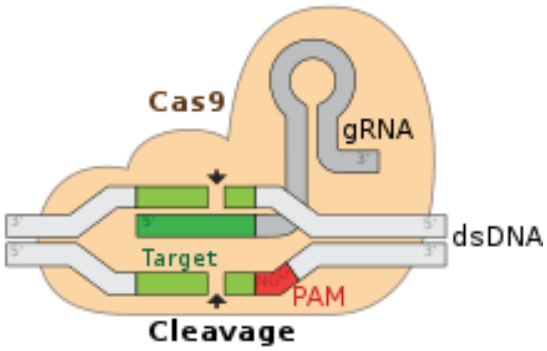

Fig. 3. Cas9 nuclease and the guide RNA

The Cas9 nuclease and the guide RNA (gRNA) are the two components that are necessary for the CRISPR-Cas9 system to function properly. Together, they make it possible to change the DNA of horticultural plants with pinpoint accuracy. A recognition domain is a component of the Cas9 nuclease [17]. This domain is endonuclease-active, meaning that it cleaves DNA at the specific location where it is targeted. The Cas9 nuclease is directed to the target DNA sequence by the guide RNA (gRNA), which in turn activates the processes that are responsible for cellular DNA repair [18]. It has been demonstrated that the CRISPR-Cas9 system is capable of successfully enhancing disease resistance in crops such as tomato, hence offering additional protection against pathogens such as powdery mildew and bacterial spot. Grapevines were subjected to CRISPR-Cas9, which allowed the researchers to target and modify the MLO (Mildew Resistance Locus O) gene [19]. This led to the production of powdery-mildew-resistant grape types. Tomato crops were also targeted and modified using the CRISPR-Cas9 technology in order to increase their resistance against the Tomato Mosaic Virus (ToMV). This was accomplished by targeting and changing the eIF4E gene, which is an essential component in the ToMV infection process. Citrus canker is a devastating bacterial disease that affects citrus crops [20]. The CRISPR-Cas9 technology has been utilized to develop resistance against citrus canker. The researchers were able to successfully develop citrus plants that had greater resistance to citrus canker infection by precisely targeting and altering the susceptibility gene CsLOB1 [21]. The results of this study reveal the transformative potential of CRISPR-Cas9 as a tool for the development of disease-resistant citrus cultivars, which contributes to the progress of horticultural crop protection techniques. By generating site-specific DNA double-strand breaks (DSBs), the CRISPR-Cas9 system makes it possible to precisely damage or knock out target genes. This is accomplished by beginning error-prone DNA repair pathways [22]. Through the use of gene knockouts, researchers are able to get significant insights into the functions that particular genes play in the development of horticulture crops, including issues pertaining to metabolism and responses to biotic and abiotic stimuli.

In a study, the CRISPR-Cas9 method was utilized to carry out a gene knockout of CHS (chalcone synthase) in petunia plants. CHS is an essential enzyme that plays a role in the manufacture of flavonoids [23]. Significant changes in pigment production were brought about as a result of the disruption of CHS, which provided crucial insights into the functional significance of flavonoids in controlling the colouring of petunia flowers. The results of this work demonstrate that CRISPR-Cas9 has the potential to be an effective instrument for examining particular gene activities that are associated with horticultural crop characteristics [24].

The CRISPR-Cas9 system allows for the precise modification of gene expression by directing its action to gene promoters or regulatory regions. This allows for the precise control of gene expression. This skill enables the selective activation or suppression of particular genes, which in turn makes it possible to achieve the

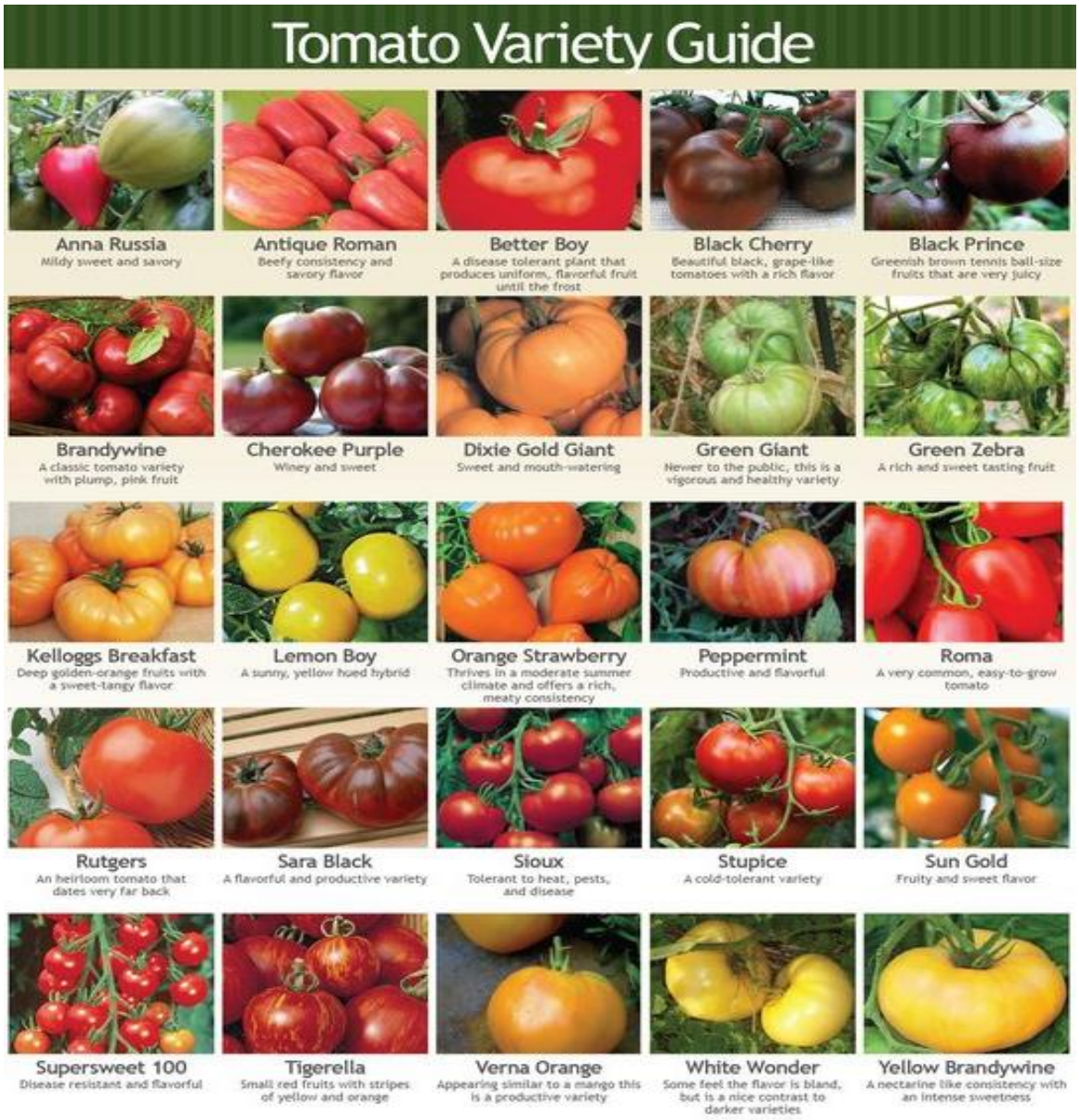

Fig. 4. Tomato varieties

necessary changes in crop phenotypes through the manipulation of key genes connected with a variety of biological processes [25]. This approach has the potential to be used for precise gene regulation in horticultural crops, as demonstrated by the fact that researchers have successfully exploited CRISPR-Cas9 to increase lettuce production by activating essential genes related to growth. The researchers Nitarska et al. used CRISPR-Cas9 to activate endogenous F3'H gene expression in poinsettia plants. This resulted in increased F3'H gene expression as well as a change in the colour of the bracts, which went from vibrant red to vivid reddish orange [26]. The researchers Huang et al. employed CRISPR-Cas9 to inhibit the expression of the SlEIN2 gene in tomatoes. They did this by inducing tiny deletions in the promoter region of the SlEIN2 gene. This led to a decrease in the production of SlEIN2 and a delay in the ripening of the fruit [27].

CRISPR-Cas9 was utilized by Huynh in order to activate the expression of the ZmDREB2A gene in maize plants. This resulted in an increase in the expression of ZmDREB2A, which is an essential gene for effectively responding to drought stress. The maize plants that were altered showed greater drought tolerance, as seen by higher survival rates and enhanced

growth in settings when there was a lack of water [28]. The exploitation of CRISPR-Cas9 presents a valuable tool for the process of agricultural domestication. This method makes it possible to rapidly modify plant species that are either wild or underutilized with the intention of changing them into potential horticulture crops. This novel technique gives researchers the ability to introduce specific genetic modifications that are associated with desired agronomic properties [29]. These traits include a reduction in bitterness, an improvement in nutritional composition, and the possibility for higher yield. Notably, the application of CRISPR/Cas9 technology has been examined in crops such as watermelon. The results of this investigation showed that targeted mutation of the ClBG1 gene by CRISPR/Cas9 led to a reduction in seed size and an increase in seed germination [30].

Seed dormancy, an intrinsic mechanism that inhibits germination under unfavourable conditions, has been subjected to negative selection in the context of agricultural domestication. This mechanism has proven to be harmful to crop production. The CRISPR-Cas9 method was applied by researchers in order to explore the regulation of seed dormancy in tomatoes. More specifically, the researchers focused on Lycopene and modified the DELAY OF GERMINATION 1 (DOG1) gene when conducting their investigation [31]. Through the introduction of particular mutations in the SH4 gene, scientists were able to successfully diminish the amount of seed shattering and improve the ease with which harvesting could be accomplished. CRISPR-Cas9 has the ability to improve the quality characteristics of horticultural crops by modifying genes such as flavour, nutritional content, texture, scent, and colour. This might be accomplished by the modification of genes [32]. Using this method, researchers have been able to successfully target genes related with anthocyanin production. This has led to the generation of unique hues in flowers and fruits, which has improved both their sensory and nutritional aspects.

2. HISTORICAL PERSPECTIVE

Over the course of thousands of years, biological processes have been modifying genomes. Natural selection has made it possible for plants that possess particular genetic variants to endure. Over the course of more than 10,000 years, humans have utilized artificial selection in order to domesticate crops, resulting in the production of modern corn from its wild predecessor, teosinte. Alterations or variations in genetic material are essential to the development of agricultural yields; nevertheless, our predecessors had to make do with mutations that occurred spontaneously [33]. In the twentieth century, researchers created and tested reagents, including as radiation and chemical mutagens, to generate DNA mutations and analyse the phenotypic changes that resulted from these mutations. The notion of mutant breeding was first introduced in the 1940s, and it has since produced a number of noteworthy achievements. One example is the wheat varieties that have greatly better yields, which were essential to the Green Revolution that took place in the 1970s [34].

The discovery that *Agrobacterium tumefaciens* (Agrobacterium), the bacterium that causes crown gall disease, is a natural genetic engineer that introduces a piece of its own DNA into the genome of a plant that it infects, in addition to possibly carrying along a DNA sequence that was provided by a researcher, was a significant step forward in the field of genetic modification [35]. The development of "binary vectors" developed from Ti-plasmids that are capable of replicating in Escherichia coli and Agrobacterium while also integrating into plant genomes served as the foundation for plant biotechnology [36]. It is conceivable to combine genes from creatures that are not closely related to one another through a process known as transgenesis or cisgenesis, which both make use of these tools. There are, however, a number of downsides associated with this method. These include the random nature of the gene insertion, the likelihood of disrupting functioning genes, public concerns around genetically modified organisms (GMOs), and the failure to make use of the plant's original genetic repertoire [37]. In the 1980s, Mario Capecchi was the first person to develop gene-targeting technology. He also pioneered the idea of using double-strand breaks (DSBs) as a tool for editing the genome. Later on, the capability of modifying genomes by the production of site-specific double-strand breaks (DSBs) was created. Following the generation of double-strand breaks (DSBs), the cell's own repair machinery can be harvested to determine the genetic outcome [38]. This can be accomplished through either the inaccurate repair process of non-homologous end joining (NHEJ) or the precise repair process of homology-directed repair (HDR).

2.1 Importance

- Food insecurity, a global problem that threatens millions of people with starvation, is getting worse as a result of the expanding population of the world as well as new risks such as climate change, desertification, salinization, human use, and developing diseases [39]. To ensure that future generations will have access to sufficient food, the current rate of agricultural production must be increased by a factor of two. The problem of food insecurity has been addressed by plant breeders through the use of natural and artificial mutations, as well as breeding for hybrid vigour [40].
- Increasing the amount of food produced per unit of land cultivated and preventing crop failures are two of the current ways that are being put into practice in order to improve agricultural yield. In order to improve the size of each grain, the number of plants that can be cultivated in a given area, and the number of grains that can be produced by each plant, breeders have focused on genes that increase these characteristics. These characteristics entail the manipulation of plant architecture by achieving a balance between the activity of meristems and the production of hormones [41].
- Breeders have developed specific features that assist crops in withstanding stressors in order to reduce the likelihood of crop failures and to improve production stability. For the purpose of abiotic stress, researchers have focused on tolerance to a variety of stimuli, including heat, cold, high light, high salt, heavy metals, and others. Researchers have identified alleles that give resistance to a variety of viral, bacterial, and fungal diseases, as well as loci that impact interactions with animal and plant pathogens. This is in relation to biotic stressors [42].
- In order to improve the nutritional value of crops, the current methods are geared toward the provision of varied and well-balanced diets that contain sufficient quantities of vitamins and minerals that are beneficial to human health. Recent advancements in crop biotechnology have made it possible to manipulate important enzymes in metabolic pathways [43]. This has the potential to increase the amount of essential nutrients, such as vitamins and iron, while simultaneously decreasing the amount of chemicals that are not desirable. In an effort to address the issue of nutritional deficits, bio fortified crops such as rice, maize, and wheat have been developed. Golden Rice, which has been genetically modified to generate a substantial quantity of β-carotene, is an example of a crop that has been created to provide assistance to individuals who are at danger of experiencing a deficiency in vitamin A consumption [44].

2.2 Principles and Implementation of Genetic Engineering in Horticulture Crops

Over the course of human history, the technique of hybridization breeding has been around since ancient times. This involves the deliberate selection and preservation of naturally hybridized individuals who possess desirable characteristics. After some time had passed, people became aware of the distinctions that existed between the reproductive organs of male and female plants [45]. They also discovered that new progeny with superior characteristics might be produced by artificial mating or cross-pollination. Plant hybridization breeding, a defining characteristic of contemporary agriculture and horticulture, came into being as a result of this. Breeders have the ability to integrate beneficial characteristics from two or more sources into a single plant through the process of purposeful hybridization, which can take place over one or more generations [46]. When hybridization breeding is used, one of the most successful uses is the utilization of heterosis, which is a phenomena in which a hybrid (F1) progeny is often superior with respect to size, growth traits, and yield when compared with either of the parents. The hybridization and selection processes have resulted in the production of a wide variety of fruit and vegetable crops, including the garden strawberry, apple, sweet orange, tomato, and squash [47].

However, there are some constraints associated with crop hybridization breeding that are difficult to overcome. The first thing to note is that hybridization can only be effectively carried out between two plants that are compatible with one another and belong to the same species or genus. Second, when plants are hybridized, many positive characteristics are transferred along with unfavourable characteristics, such as low yield potential or poor quality [48]. This is

because this process is known as hybridization. In the third place, the process of breeding numerous woody horticulture crops like apple and walnut can take as long as twenty to thirty years to produce a single individual that possesses a combination of several desirable characteristics. Even while molecular marker-assisted selection and fast track breeding techniques have the potential to speed up the breeding and selection processes, this still takes a significant amount of labour and land resources of an enormous magnitude [49]. During the course of crop evolution, there were instances in which spontaneous variations with novel features emerged, and these types of variations have been maintained. A significant increase in grain yield has occurred as a result of the utilization of these variants in crop breeding, such as the semi-dwarf variation of cereal crops; this phenomenon is referred to as the "green revolution." When it comes to perennial horticulture crops, new cultivars produced from spontaneous mutations are highly fruitful [50]. Some examples of these cultivars are the new red-skinned Fuji apple, the large-berry tetraploid Kyoho grape, and a variety of ornamentals that have a distinctive appearance. The low frequency of natural mutation can be solved by purposefully subjecting various plant components, including as seeds, cuttings, pollen, or tissue grown calli, to either physical or chemical mutagens. This can provide a solution to the problem of natural mutation. Plant mutation breeding was eventually brought about as a result of this discovery. In spite of the fact that the number of mutations has significantly increased, it is important to note that mutation is a random and non-specific process. Furthermore, the majority of mutations are harmful and chimeric [51].

On the basis of the gene, plant breeding is carried out. Breeders chose new phenotypes that possessed desirable characteristics in the early phases of the breeding process without being aware of the genotype. In the field of modern biotechnological breeding, the introduction of molecular genetics has opened up a wide range of opportunities. Through the use of DNA recombinant technologies, also known as transgenic technology, molecular biologists are able to precisely edit the gene that codes for a trait in order to develop novel phenotypes [52]. This is made possible by the fact that they are aware of the specifics of how desirable and undesirable qualities are inherited and genetically controlled. In 1986, France and the United States of America were the locations where the first public experiments of genetically modified plants were carried out. In 1994, the FlavrSavr tomato was the first transgenic product to be given the green light for commercial sale in the United States [53]. The papaya that is resistant to viral diseases is yet another example of effective transgenic plants in the agricultural industry. A great number of horticultural crop varieties, including tomato and papaya, have been developed through the application of transgenic technology and have been made available to the public [54]. There are several technical hurdles associated with transgenic technology, despite the fact that it has enjoyed a great deal of success in enhancing crop breeding and has a significant amount of commercial value.

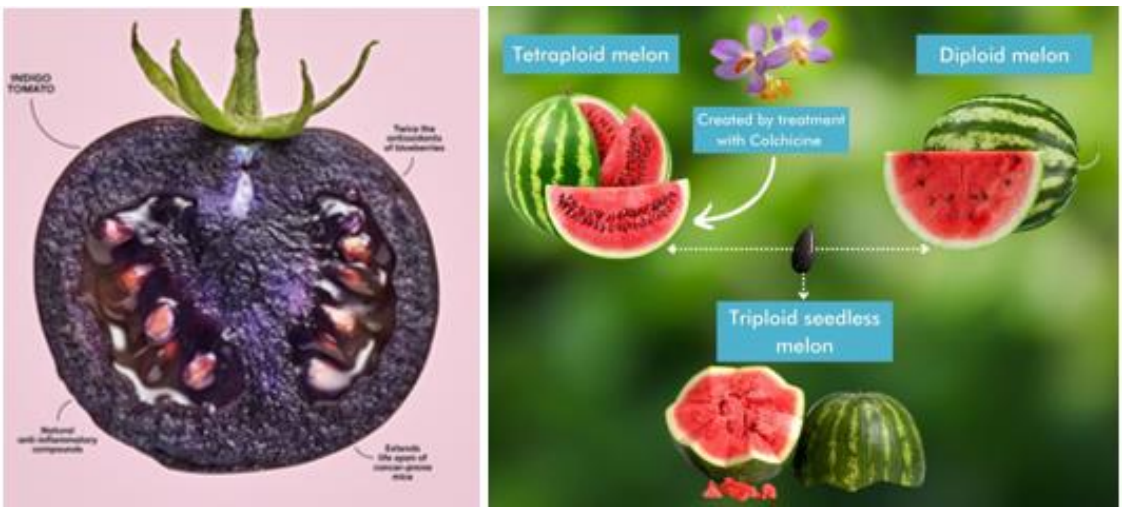

Fig. 5. Food insecurity, a global problem

GM Events for crops have been developed to improve crop quality, resistance to herbicides, and control of pollination [55]. Some of the most notable GM events include the Arctic "Golden Delicious" Apple (GD734), Carnation (*Dianthus caryophyllus*), Moonshadow (11363), and Creeping Bentgrass (*Agrostis stolonifera*) [56].

In 1995, Australia and Norway received several awards for their efforts in developing and implementing GM events. In addition to improved product quality, these countries also developed systems that allow for herbicide tolerance and control of pollination [57]. For example, Bejo Zaden BV developed the Roundup Ready Creeping Bentgrass in 1997, which was used in the United States of America. The company of Hybrid Seeds in Maharashtra developed Melon (*Cucumis melo*) from Bangladesh in 1999, and later made it available in two varieties: Melon A (NA) and Melon B (NA). The United States Department of Agriculture's Agricultural Research Service in the United States of America has also reported on the resistance of diseases in potato crops [58]. Insect resistance levels have been studied by various institutions, including Cornell University and the University of Hawaii in the United States of America, Canada, Japan, South China Agricultural University, and the University of Florida in the United States of America. Monsanto Company and Scotts Seeds Corporation in the United States of America have also made modifications to their products, such as the InnateTM Russet Burbank Potato and the Atlantic NewLeafTM potato [59].

In summary, GM events for crops have been developed to improve crop quality, resistance to herbicides, and control of pollination. Companies owned by Monsanto Company, including those wholly and partially owned by them, have contributed to the development of these products. The success of these GM events in improving crop quality and resistance to diseases is a testament to the ongoing progress in genetic engineering [60].

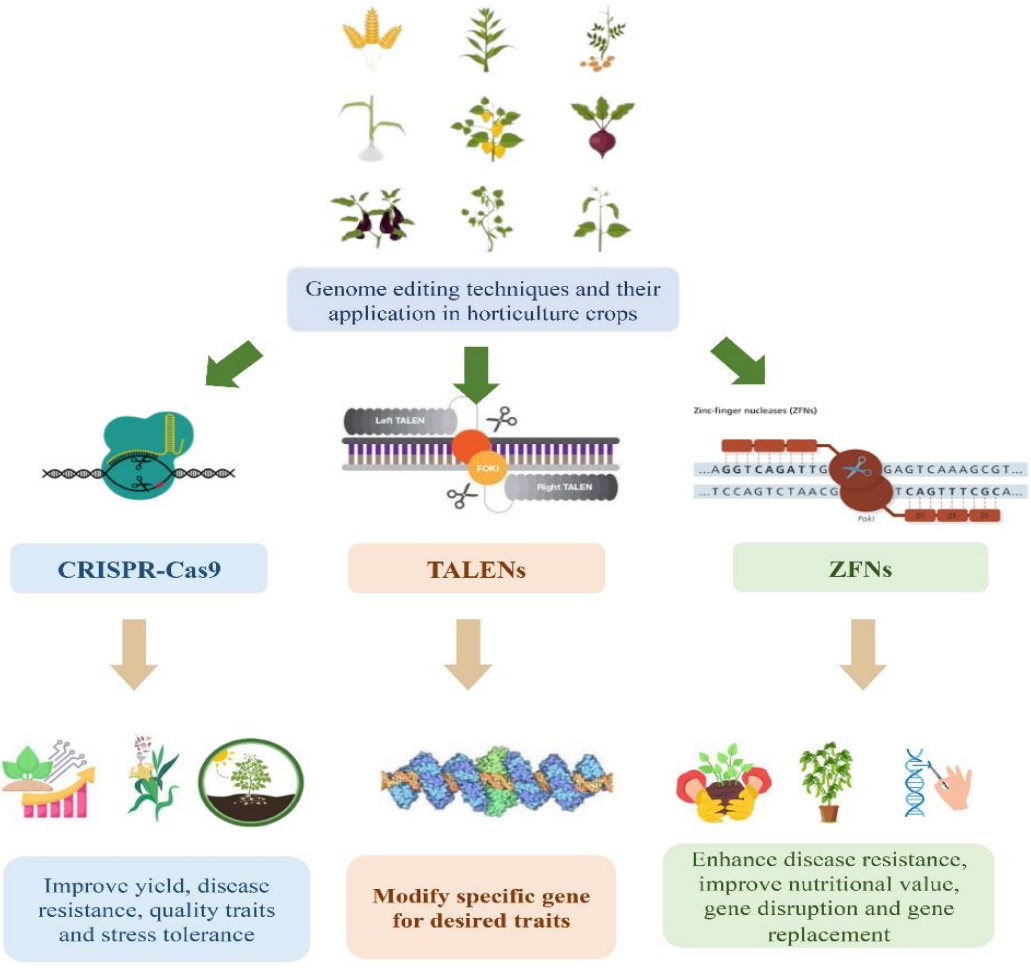

Fig. 6. Genome editing technique

The insect and disease resistance characteristics of various potato varieties, including the New LeafTM Russet Burbank potato, RBMT15-101, SEMT15-02, -07, and -15, and the Superior NewLeafTM potato varieties SPBT02-5 and SPBT02-7. The potato has been cited in various countries, including the United States, Australia, Canada, Japan, Mexico, New Zealand, Philippines, South Korea, and Australia [61]. The New LeafTM Plus Russet Burbank potato model number is RBMT22-082, -186, -238, and -262. It also includes herbicide tolerance in the potato. The Superior NewLeafTM potato varieties SPBT02-5 and SPBT02-7 have an insect resistance level in the United States, Canada, Japan, Mexico, New Zealand, Philippines, South Korea, Australia, Canada, Japan, Mexico, New Zealand, and South Korea [62].

Two WKS82/130-4-1 roses, also known as rose hybrids, have been modified to improve their product quality. These roses were located in Japan, Suntory Limited, the United States of America, Japan, Australia, and Colombia. The tomato (*Lycopersicon esculentum*) from China was also modified to improve its product quality [63]. In 1996, Agritope Inc. of the United States of America modified the product quality. Other modified potato varieties include B (NA), Da (NA), F (NA), Huafan No 1 (NA), Huazhong Agricultural Sciences (China), Da Dong No 9 (NA), and Monsanto Company USA (FLAVR SAVR™). In addition to insect and disease resistance, the potato has also been modified to fight sickness and insects. The quality of the modified product has been improved by various companies, such as Agritope Inc., Agritope Inc., Zeneca Plant Science and Petoseed Company, The Institute of Microbiology, CAS (China), Huazhong Agricultural Sciences (China), Monsanto Company USA (USA), and PK-TM8805R (NA) [64].

2.3 Genetic Engineering Technologies Trends

2.3.1 TALENs (Transcription Activator-like Effector Nucleases)

CRISPR-Cas9 and TALENs are two of the most popular genome editing techniques currently available. They are utilized to make precise gene alterations in horticultural crops. The ability of these designed nucleases to induce double-strand breaks (DSBs) at particular DNA regions makes it possible to alter genes in a targeted manner. The nuclease domain is commonly obtained from the FokI endonuclease, while the DNA-binding domain is derived from transcription activator-like effectors (TALEs) [65]. Both of these domains are customized to meet the specific needs of the target. When the DNA-binding domain is created, it is made up of several repeats of TALEs, each of which recognizes a different nucleotide in the DNA sequence that is being targeted. The specificity of TALENs is achieved by the use of repeat variable di-residues (RVDs) that may be customized. Since various RVDs recognize different nucleotides, it is possible to construct TALENs that are extremely particular [66]. TALENs make it possible to disrupt genes in a targeted manner by inducing double-strand breaks (DSBs), which in turn makes it possible to knock out genes or cause mutations that result in loss of function. The examination of gene function and the identification of genes associated with a variety of horticultural characteristics are both made easier by this approach. Through the use of TALENs, the SlAN2 gene in tomatoes has been knocked out, which has shed light on the functional role that it plays in the ripening process of fruit [67]. Additionally, they have been exploited for the purpose of gene deletion in citrus crops, with a particular focus on genes related to disease resistance. When it comes to grapevine, TALENs have been utilized for the purpose of researching gene function, with a particular emphasis on disease resistance.

However, the design and assembly of TALENs can be lengthy and technically demanding, which makes them less scalable and restricts their general acceptance in horticulture crop research. Therefore, TALENs are not widely used in crop research [68]. Multiple cloning processes are required for the assembly of TALEN constructs. These steps can be prone to mistakes and inefficiencies, which might result in a decreased transformation efficiency or difficulty in getting functioning TALEN constructs. TALENs are able to target certain DNA sequences because their recognition process is dependent on their RVDs, which bind to particular nucleotides [69]. This restricts their flexibility in targeting specific DNA sequences. Targeting repetitive or GC-rich regions presents hurdles for TALENs because it may be difficult to construct unique RVDs for sequences that have such a high concentration of GC. In spite of the fact that TALENs have a generally higher target specificity in comparison to earlier genome editing technologies, they are nevertheless capable of exhibiting off-target

effects. These effects can be attributable to partial complementarity between the TALEN and undesired DNA sequences [70].

When it comes to horticultural crops, the efficient delivery and transformation of TALENs presents a number of obstacles. While certain TALENs have better rates of success, others provide challenges. Each individual horticultural crop requires optimization and evaluation of the efficiency of TALEN delivery and transformation in order to be successful [71]. The effectiveness of TALEN delivery and transformation can also be affected by the genetic variety that exists within a crop species as well as the tissue specialization of the crop. Continuous attempts are being made to improve the effectiveness of TALEN delivery and transformation in horticulture crops by optimizing protocols, developing tissue-specific approaches, and making technological improvements. The initial development of TALENs was met with difficulties that were associated with the laborious and time-consuming process of constructing custom-engineered TALE repeat arrays for the purpose of recognizing particular DNA sequences [72].

2.3.2 ZFNs (Zinc Finger Nucleases)

There is a class of designed nucleases known as zinc finger nucleases (ZFNs), which have been utilized for the purpose of genome editing in horticultural crops. The nucleases in question are made up of two primary components: zinc finger proteins (ZFPs) and a nuclease domain derived from FokI endonuclease. Both of these components are capable of recognizing DNA based on its sequence [73]. There are three DNA bases that are targeted by each zinc finger module, and the application of numerous modules enables accurate targeting of particular DNA sequences. ZFNs are commonly used in pairs, with each pair focusing on a single strand of DNA. ZFNs bind to their respective target sites, which results in the dimerization of the FokI nuclease domain. This results in the formation of a functional nuclease complex that has the ability to induce double-strand breaks (DSBs) at the particular target site [74]. This is accomplished through ZFN-mediated site-specific mutagenesis, which makes use of non-homologous end joining (NHEJ) repair pathways in order to introduce mutations. ZFNs make it possible to modify specific sites. Arabidopsis and tobacco are two examples of plant species that have shown that it is possible to successfully target both transgenic sequences and native sequences respectively. Additionally, ZFNs have been utilized in the process of removing transgenes from tobacco plants by the utilization of NHEJ-mediated repairs. This has resulted in shortened alterations at the targeted sites as well as the deletion of transgenes [75].

ZFNs have the ability to promote site-specific homology-directed repair (HDR) in tobacco and corn plants, which makes it easier for donor DNA to be accurately integrated into the genomes of these plants. When it comes to successful site-specific mutagenesis, it is absolutely necessary to have efficient expression of ZFNs in regenerating cells or tissues. There have been attempts made to achieve high levels of ZFN expression in Arabidopsis plants by the use of transgenic techniques, which has resulted in the production of altered seeds [76]. In order to design zinc finger nanoparticles (ZFNs), it is necessary to custom-engineer zinc finger proteins (ZFPs) that are unique to a particular DNA sequence. This procedure requires expertise in both protein engineering and DNA binding specificity. As a result of this design complexity, the widespread use of ZFNs is hampered, and their applicability is limited to a wider range of target sequences in horticulture crops [77].

In order to achieve successful mutagenesis, it is vital to distribute ZFNs into plant cells in an effective manner. However, the different cell types, tissue architectures, and cell wall compositions that are present in horticulture crops can provide obstacles. It is crucial to tailor delivery strategies, such as Agrobacterium-mediated transformation or particle bombardment, to each crop in order to achieve efficient delivery [78].

ZFN platforms that are modular have been developed in order to solve the restrictions that were present in the early stages of ZFN development. These platforms offer enhanced design flexibility and make it easier to manufacture ZFNs that are customized to a variety of target sites in horticultural crops. ZFN improvements have mostly focused on improving specificity, which has led to a reduction in off-target effects, as well as an improvement in precision and safety in ZFN-mediated genome editing in horticultural crops [79].

2.3.3 CRISPR/Cas systems

In the natural world, bacterial and archaeal species employ adaptive immune systems that are based on clustered regularly interspaced palindromic repeats (CRISPR) and CRISPR-associated protein (Cas) in order to protect themselves from foreign genetic material. With the ability to target DNA, RNA, or both for degradation, approximately forty percent of bacteria and the majority of archaea possess several CRISPR/Cas systems [80]. This allows them to protect themselves from the introduction of foreign genetic material. The adaptation phase occurs when a phage infects a bacterium that is equipped with CRISPR. During this phase, the bacteria receives bits of the phage DNA that are contained inside the CRISPR array. With the most recent acquisition being the one that is closest to the leader sequence, which acts as a promoter, the order of acquisitions is determined.

During the biogenesis phase, the CRISPR array undergoes transcription, which results in the production of mature RNAs in the form of crRNAs [81]. Cas9 makes use of these crRNAs as guides in order to target the phage genome during subsequent invasions and confer immunity to the bacterial cell. This phase is referred to as the interference or immunity phase. There are two primary categories of CRISPR systems, which are referred to as classes I and II. Class I systems are made up of a multicomponent system that is made up of several effectors, whereas class II systems (types II, V, and VI) are made up of a single-component system and a single effector that is guided by the crRNA [82]. Cas9 and a single guide RNA (sgRNA) molecule are the two components that make up the CRISPR/Cas9 system, which is classified as a class II system composed of two components. It is required to ensure that the delivery of the genome-engineering reagents to the proper species is achievable in order to achieve high-efficiency genome engineering in any eukaryotic cell. Additionally, it is necessary to ensure that editing of the target genome is both highly specific and efficient [83]. The development of high-efficiency genome-engineering tools requires significant research in a number of crucial areas, including editing specificity and reagent delivery. A significant amount of attention is being paid to the development of delivery platforms for genome-engineering reagents in plants. The delivery platforms should ideally be designed for distribution into germline cells in order to avoid the necessity of tissue culture and regeneration following editing. A variety of delivery platforms are available, such as bacterial and viral vectors, as well as physical distribution into various types of cells [84].

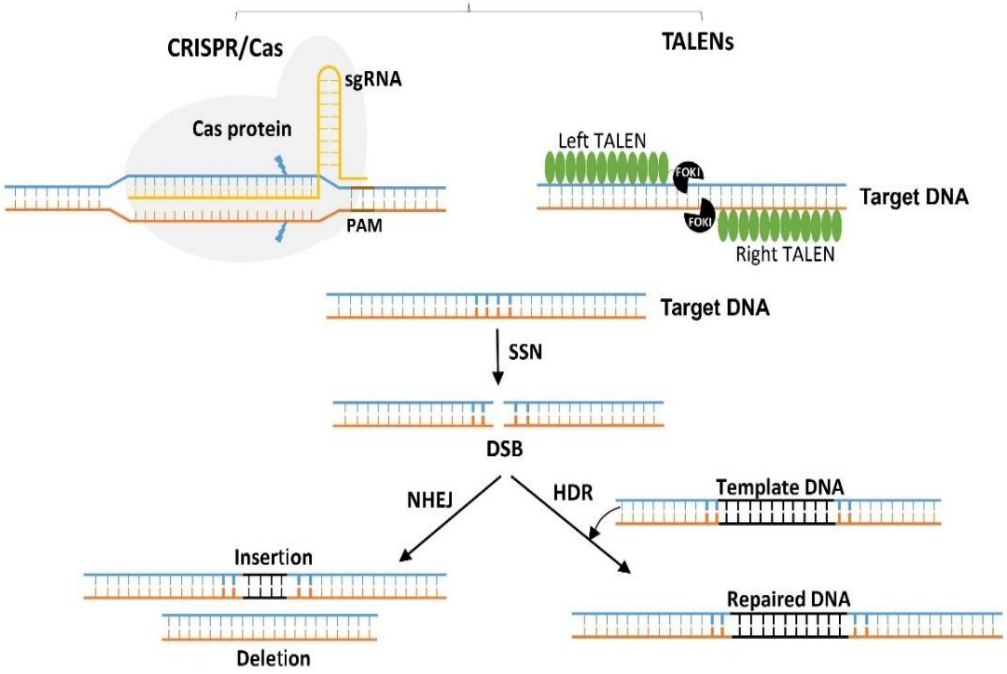

Fig. 7. Efficient site-specific nucleases (SSN)

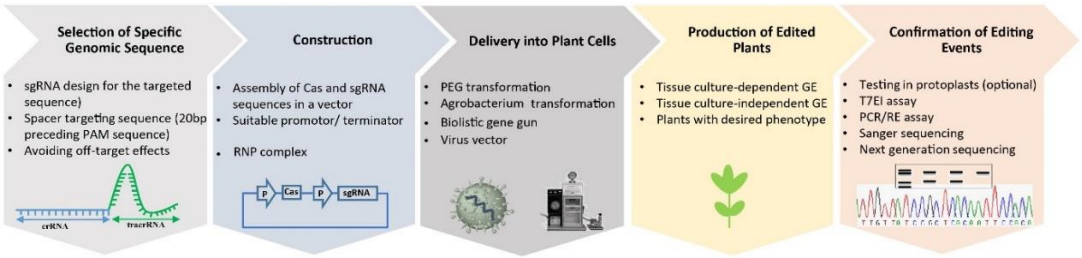

Fig. 8. Bacterial and viral vectors

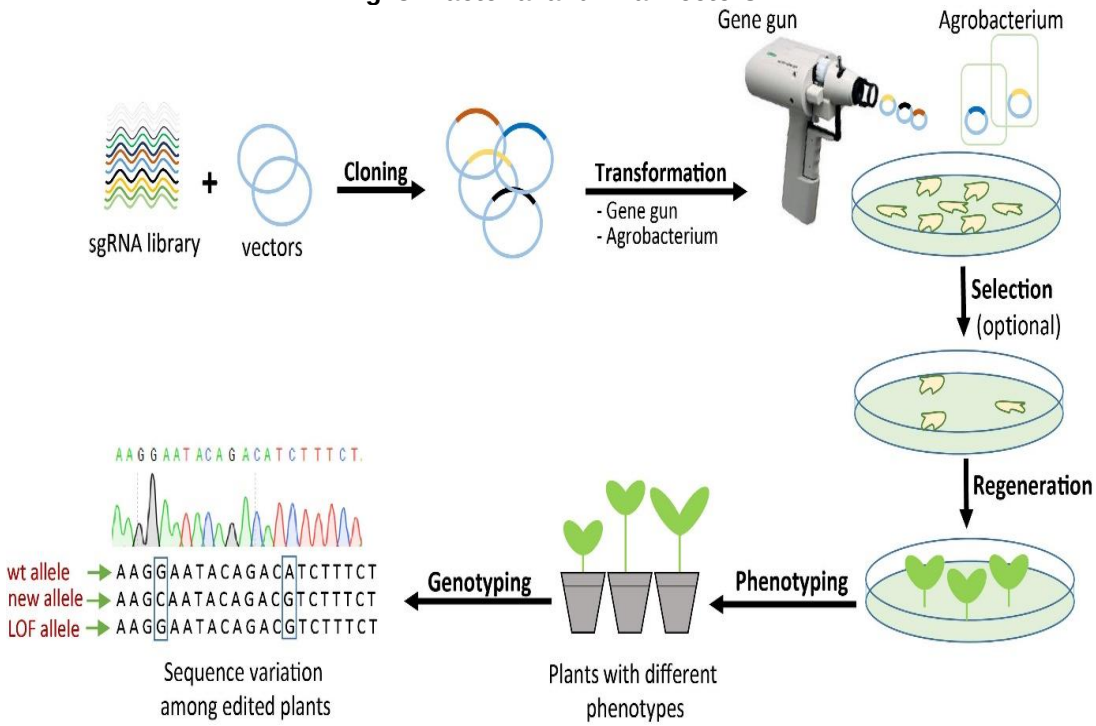

Fig. 9. Genetic engineering's in horticulture crops

For the time being, the process of engineering the CRISPR/Cas9 system consists of merely engineering the sgRNA molecule. This molecule not only provides targeting specificity but also has the potential to provide a template for HDR. In order to transfer small guide RNA (sgRNA) into plants that express Cas9, a technique has been created that makes use of a virus as the transmission vehicle. This strategy requires the production of a Cas9 overexpression line in a model plant species such as *Nicotiana benthamiana or Arabidopsis thaliana*, followed by the delivery of small guide RNAs (sgRNAs) through the use of Tobacco rattle mosaic virus (TRV) [85].

(1) tissue-culture-free genome editing, in which the CRISPR/Cas9 machinery is active in the germline; and (2) tissue-culture-dependent genome engineering. Both of these alternatives are made available by the viral delivery method. There are RNA viruses that are capable of infecting germline cells, albeit at a low frequency [86]. This would allow for the recovery of progeny that carry the genetic change that was intended. Because it is able to transfer a portion of its genome, known as transfer DNA (T-DNA), into the genome of a plant, Agrobacterium is considered to be a natural genetic engineer among the prokaryotic vectors. The virulence proteins, which are encoded by the Ti plasmid and promote DNA nicking, processing, transfer, and integration into the plant genome, are responsible for this fascinating interkingdom DNA transfer [87]. They are also responsible for facilitating genome integration. Some of these proteins could be used to transport ribonucleoproteins (RNPs) from the bacterium into the nucleus

of the plant cell. This would be an intriguing possibility because it would make it possible to produce the CRISPR/Cas9 machinery in bacteria and then transport it intact into plant cells. This would enable researchers to recover seed progeny that carries the desired gene edits without the need for traditional tissue culture [88].

2.3.4 Challenges for genetic engineering's in horticulture crops

- A significant problem that is related with CRISPR-Cas9 gene editing is the possibility of off-target effects. These effects might lead to unwanted genetic alterations, which can have unanticipated ramifications for the phenotypic of the crop as well as the genomic stability of the crop. In order to lessen the impact of these consequences, the current efforts are concentrated on strengthening the specificity of Cas9 and refining the design of gRNA. There are a number of factors that can influence off-target effects [89]. These factors include the degree of similarity between the target site and off-target sites, the length and structure of the gRNA, the effectiveness of the Cas9 enzyme, and the delivery strategy that is utilized.
- The detection and evaluation of off-target effects of CRISPR-Cas9 gene editing is accomplished through the utilization of a variety of techniques, including as whole-genome sequencing, targeted deep sequencing, and computational analysis. Researchers are able to evaluate the specificity of CRISPR-Cas9 editing and discover potential alterations that are not specific to the target gene with the assistance of these technologies [90]. Cas9 variants with improved fidelity, such as high-fidelity Cas9 (HiFi Cas9) and enhanced-specificity Cas9 (eSpCas9), have been developed over the years, which has allowed for significant progress to be made in the enhancement of the specificity of CRISPR-Cas9, which has enabled the reduction of off-target effects even while maintaining editing efficiency [91].
- It is absolutely necessary to ensure that CRISPR-Cas9 components are delivered into plant cells in an effective manner in order to achieve successful genome editing. It is important to note that the process of transformation for horticultural crops can be extremely difficult, particularly in species that are resistant to change or those that have genomes that are complicated. In order to promote wider applications of CRISPR-Cas9 across a variety of horticulture crops, ongoing research is concentrating on improving delivery techniques and enhancing transformation efficiency [92]. The use of *Agrobacterium tumefaciens* as a method for introducing CRISPR-Cas9 components into plant cells is a common practice. This method also helps to facilitate the transfer of genetic material and makes it possible to transport CRISPR-Cas9 into the plant genome [93].
- These species-specific needs, which include tissue culture techniques, regeneration capacity, and sensitivity to transformation methods, all play a role in determining the degree to which horticultural crop species are able to undergo transformation effectively. There are some constraints associated with each delivery strategy for CRISPR-Cas9, including the species of plant, the kind of tissue, the regeneration methods, and the specific components of the CRISPR-Cas9 system [94]. These limits can be broken down into several categories. Certain genotypes of horticultural crops inherently provide difficulties for transformation because of their low capacity for regeneration or their high levels of tissue browning or necrosis.
- The non-coding sections of the genome are crucial for the control of genes and the growth of plants [95]. Off-target effects that occur in these regions have the ability to alter gene expression and regulatory networks, which can result in undesired changes in the physiology and development of plants. In order to reduce the likelihood of unintentional changes in gene regulation, it is essential to carry out study and acquire a comprehensive understanding of the potential off-target effects that may occur in non-coding areas [96].
- The identification of potential off-target sites in non-coding areas is a challenging endeavour because, in comparison to coding regions, non-coding regions have a greater number of putative target sites of interest. Tools from the field of bioinformatics are frequently utilized for the

purpose of predicting off-target locations; however, the accuracy of these tools may be reduced for non-coding regions. There are structural differences and repetitive sequences in the genome, both of which significantly complicate the process of off-target prediction [97].

- There are problems involved in achieving the stable inheritance of CRISPR-edited traits through sexual reproduction in horticulture crops. This is because it is vital to ensure that modified traits are present in germ cells and that they are reliably transmitted to succeeding generations [98]. A number of different approaches, including the screening and selection of edited lines, as well as the exploration of gene drive systems, are now being investigated in order to improve the inheritance and segregation of CRISPR-edited characteristics.
- Achieving extensive and efficient editing of all target sites in every plant cell presents obstacles [99]. This is because there are some cells in which successful editing may not take place, which results in a combination of cells that have been altered and cells that have not been edited within a single plant. Understanding and taking into consideration the effects of the genetic background of horticulture crops is essential for accurate prediction of trait inheritance [100]. The genetic background of horticultural crops plays a role in the expression and inheritance of altered traits. In order to solve these issues and enhance the efficient inheritance and spread of desirable features in horticultural crops modified using CRISPR-Cas9, advancements in genomics, molecular breeding, and genetic analytic methodologies have been made [101].

2.4 Economics and Socio Impact of Genetically Modified Crops

According to the findings of a study conducted by PG Economics, farmers all over the world who utilize genetically modified (GM) seeds have not only improved the environmental sustainability of their operations but have also realized economic gains that averaged more than one hundred dollars per hectare in 2014 [102]. Approximately two-thirds of these advantages are a result of increased yields and additional production, with the most significant gains being experienced by farmers in poor nations. Farmers are increasingly adopting conservation tillage practices, building their weed management practices around more benign herbicides, and replacing pesticide use with insect-resistant genetically modified crops, all of which are beneficial to the environment [103].

The usage of pesticides has decreased by about 581 million kilos as a result of crop biotechnology. Additionally, farmers are spending less time on the tractor, which results in a reduction in the amount of fossil fuels burned, which ultimately leads to a reduction in carbon dioxide emissions [104]. As a result of farmers cultivating herbicide-tolerant crops switching to no-till cropping systems, the condition of the soil has improved, and farmers have been able to switch to herbicides that are less harmful to their plants in order to better control weeds. According to the paper titled "GM Crops: Global Socio-Economic and Environmental Impacts 1996-2014," the global economic benefits of genetically modified crops have reached a total of $150 billion from 1996 to 2014. Crop biotechnology was responsible for the additional production of 158.4 million metric tons of soybeans and 321.8 million tons of corn around the world between the years 1996 and 2014. Soybeans, maize, canola, and cotton are the four most important crops that are grown around the world. The direct worldwide farm income advantage from genetically modified crops was $17.7 billion, which is equivalent to having added 7.2% to the value of global plant production. Since 1996, there has been a rise of $150.3 billion in farm revenues, which has been almost evenly distributed between farmers in developing nations and farmers in industrialized countries [105].

By reducing the amount of damage caused by pests, the insect-resistant (IR) technology that is utilized in cotton and corn has regularly resulted in increased yields. The average yield gains for insect-resistant corn and insect-resistant cotton throughout the period of 1996-2014 have been +13.1 percent and +17.3 percent, respectively, when compared to conventional production systems. These gains have been achieved by all users of this technology. The technology that is herbicide tolerant (HT) has also led to enhanced output, improved weed management, and higher yields in certain regions. Additionally, it has assisted farmers in Argentina in producing soybeans as a "second crop" following wheat during the same growing season [106].

3. CONCLUSION

When compared to conventional breeding techniques, genome editing technologies offer a number of benefits in the field of horticulture crop development. These benefits include enhanced characteristics, an extended shelf life, and innovative colours and shapes. On the other hand, these technologies are confronted with obstacles like as lengthy breeding cycles, large heterozygosity's, and low frequencies of beneficial mutations, which contribute to the high resource requirements associated with the generation of new varieties. In addition to overcoming incompatibility barriers between species, transgenic technology can also be utilized to result in the generation of new varieties that possess the characteristics that are desired. On the other hand, the expenses and the amount of time required for transgenic crop varieties have increased as a result of public opposition and risk evaluations. When the genomic sequences of the genes that are being edited are known, CRISPR/Cas technologies promise to make gene editing methods more effective and precise. These technologies have the potential to develop novel kinds through mutation breeding; but, they also have the potential to be as direct and efficient as transgenic procedures, earning them the distinction of being termed non-transgenic crops. It is possible that these technologies could be classified as non-transgenic crops, which would make them more acceptable in nations where the public is opposed to being exposed to transgenic plants. It is confident that these obstacles will be overcome, and that genome-editing technologies, notably CRISPR/Cas, will be incorporated into the process of horticultural plant breeding regardless of the barriers that have been encountered. It is of the utmost importance to formulate a policy for this emerging biotechnology and to differentiate between conventional genetically modified species and creatures that have had their genomes edited. When used in conjunction with other breeding technologies, genome editing technology has the potential to produce fruits, vegetables, and decorative flowers that are more aesthetically pleasing, aesthetically pleasing, and nutritionally beneficial. This will make our lives more attractive, more fun, and healthier.

COMPETING INTERESTS

Author has declared that no competing interests exist.

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