Improving Economically Valuable Traits in Crops

Subjects: Plant Sciences
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The purpose of crop quality improvement is to control and enhance the genetic characteristics of crops through breeding technology to boost the production performance of crops and to improve quality indicators such as palatability and nutrients. The development and improvement of molecular biology methods have led to the creation of new technologies that make it possible to modify plant genomes by transferring and integrating into the genomes' heterologous genes from various expression systems (genetic engineering), as well as inducing knockouts of one or more target genes of interest (genomic editing). The development of genome-editing methods is a new milestone in the development of modern breeding methods and certainly relies on the knowledge and technologies developed for transgenesis.

crops traits transgenesis genome gene editing

1. Introduction

The purpose of crop quality improvement is to control and enhance the genetic characteristics of crops through breeding technology to boost the production performance of crops and to improve quality indicators such as palatability and nutrients. Genetic traits determined by hereditary information encoded in DNA and determining phenotypic traits are inherited from parents from generation to generation. At the same time, genetic information is constantly subject to changes due to the presence of spontaneous or induced mutations, errors that occur during replication, the activity of mobile elements, the processes of meiotic crossing over, and cross-fertilization. In addition, there are a number of pathogenic and symbiotic bacteria capable of transferring part of their DNA into the genome of a plant cell [1]. Bacteria thereby influence the metabolism of the plant cell, forcing it to produce the substances it needs. Thus, it can be said that the plant genome is constantly being modified.

Improving the economically valuable traits of plants is also based on introducing various modifications to the genome of the plant cell. The history of plant mutagenesis dates back to 300 BC, as humans have used natural mutations generated in nature for selective breeding. Plant breeding involves systematic selection among the entire population of plants of samples bearing target properties. Thus, it is estimated that humans have been successfully breeding plants for over ten thousand years [2] when seeds of plants with favorable features were saved for the next plantation, a practice known as domestication. Among the various mutations that can either improve or worsen some plant characteristics, the breeder also selects the most interesting and important ones and uses them in his breeding work.

One of the most important achievements of the early to mid-20th century that should be considered is the development of methods for induced mutagenesis. A large number of varieties of cultivated plants grown today were obtained precisely as a result of induced mutations. To date, more than 3400 varieties obtained by mutagenesis have been registered, belonging to more than 200 different plant species (according to The Mutant Varieties Database, a joint initiative of the FAO/IAEA (International Atomic Energy Agency/Food and Agriculture Organization of the United Nations) https://nucleus.iaea.org/sites/mvd, (accessed on 29 November 2023). The most significant advances in plant breeding techniques have been achieved as knowledge and understanding of plants and their genetic structure have accumulated. The most important stage in plant breeding was the Green Revolution, which made it possible to dramatically increase the productivity of agricultural crops through the development of high-yielding varieties of cereals, particularly dwarf wheat and rice. Norman Borlaug, Nobel Prize laureate and father of the Green Revolution, emphasized that the key to the success of these semi-dwarf varieties was their wide adaptability, short plant height, high sensitivity to fertilizers, and resistance to disease, which ultimately made it possible to obtain more yield at a lower cost [2]. Induced mutagenesis has been most widely used to modify the genome of cereals [3]. Among horticultural species, the greatest success of induced mutagenesis was achieved in ornamental plants, especially chrysanthemums and roses [4][5][6].

However, the methods of traditional selection and chemically/physically induced mutagenesis have a number of disadvantages. The use of traditional selection is a very long and labor-intensive process, and in addition, the researcher is limited by the set of genes that are present in the genome of a given species. As for mutagenesis, although it still plays a significant role, it produces random mutation events, is hazardous to humans, is not eco-friendly, and its dose rate differs for each genotype and requires standardization [7].

In the second half of the 20th century, with an increase in the quantity and quality of food consumption, a revolution in plant breeding occurred, the key achievements of which were achieved in the creation of hybrids and transgenesis [2]. Transgenesis and editing, which appeared later, make it possible to obtain targeted changes in the genome in a shorter time, without the series of backcrossing and lengthy selection of successful events, as well as with a more predictable result, without destroying the existing combinations of genes in a particular variety.

2. Modifying Plant Genomes Using Gene Engineering Technologies

As data accumulated on the organization of the gene and the functioning of genetic information in the cell, new technologies for genome modification began to appear. Transgenesis became the main one for a long time. Transgenesis changes the genetic information of a plant cell, resulting in a so-called genetically modified organism (GMO) that carries in its genome a fragment of foreign DNA that gives the plant new useful traits that cannot be obtained by conventional breeding methods. Transgenesis serves not only for the transfer of genes originating from any other organisms (bacteria, animals, viruses, other plants, etc.) into the plant genome but also as an improved method of induced mutagenesis and a tool for manipulating the level of expression of host cell genes (gene silencing) [7]. Transgenic crops are now widespread globally and are increasingly accepted as food and feed. The development of genetic engineering, the emergence of PCR, and the simplification and improvement of sequencing methods have contributed to the wide spread of transgenesis technologies in the world.

To create transgenic plants, mainly two methods are used—agrobacterium transfer carried out using the soil bacterium *Agrobacterium tumefaciens* and transfer using bioballistics [8][9]. The bacterium *A. tumefaciens* naturally infects the wound of a plant to develop crown gall disease. This is possible because Agrobacterium carries the tumor-inducing (Ti) plasmid, which has a virulence (vir) region and a T-DNA (transfer DNA), which actually transferred from the bacterium to the plant. During the process of transformation, multiple components of the Ti plasmid work together for the effective transfer of the gene of interest into the plant cells. Agrobacterium-mediated transformation in our days is a simple and inexpensive biological technique, which can be applied in plants as well. The transformation results in either a single or low copy number of T-DNA insertions, which prevent homology-dependent gene silencing or the rearrangement of inserted genes by recombination. This is advantageous over methods that insert target sequences in multiple copies. However, it can be applied successfully more toward dicot plants than that of monocots; monocots are generally hard to transform by this method [9][10][11][12][13]. There are a huge number of examples of the use of agrobacterial transformation for the delivery of transgenic constructs into the genome of horticultural species. To date, protocols have been developed for many species, including fruit trees and many others [14]; for species that have difficulties with in vitro cultivation, methods of tissue culture-independent agrobacterial transformation have been developed [15]. Some of them are shown in **Table 1**.

Table 1. A list of selected examples of transgenic and gene-edited horticulture crops.

Plant	Specific Trait	Target Gene	Transgenic	Gene-Edited	Used Transgenesis	Reference
			Herbicide Resistant			
Savoy cabbage (*Brassica oleracea* var. *sabauda*)	phosphinothricin (L-PPT) resistant	*bar*	yes	no	Yes *A. tumefaciens*	[16]
Sweet potato (*Ipomoea batatas* L. Lam.)	phosphinothricin (L-PPT) resistant	*bar*	yes	no	Yes Bioballistic	[17]
Potato (*Solanum tuberosum*)	glyphosate tolerance	*EPSPS*	yes	no	Yes *A. tumefaciens*	[18]
Easter lily *(Lilium longiflorum* Thunb.)	phosphinothricin (L-PPT) resistant	*bar*	yes	no	Yes Bioballistic	[19]
Tomato (*Solanum lycopersicum* L.)	chlorsulfuron-tolerant plants	*SlALS1*, *SlALS2*	no	Cas9 +	Yes *A. tumefaciens*	[20]

Plant	Specific Trait	Target Gene	Transgenic	Gene-Edited	Used Transgenesis	Reference
Potato (*Solanum tuberosum*)		*StALS1*, *StALS2*		Base editor		
Watermelon (*Citrullus lanatus* (Thunb.))	chlorsulfuron-tolerant plants	*ALS*	no	Cas9 + Base editor	Yes *A. tumefaciens*	[21]
Cassava (*Manihot esculenta*)	glyphosate tolerance	*EPSPS*	no	Cas9, HDR editing	Yes *A. tumefaciens*	[22]
Lettuce (*Lactuca sativa* L.)	paraquat	uORF of *LsGGP1* and *LsGGP2*	no	Cas9	Yes *A. tumefaciens*	[23]
			Pathogen Resistance			
Tomato (*Solanum lycopersicum* L.)	*tomato yellow leaf curl virus* inactivation	coat protein, replicase	yes	Cas9	Yes	[24]
Papaya (*Carica papaya* L.)	resistance to *papaya ringspot virus*	coat protein gene	yes	no	Yes *A. tumefaciens* Bioballistic	[25]
Tomato (*Solanum lycopersicum* L.)	resistance to larvae of *Helicoverpa armigera* and *Spodoptera litura*	*cry1Ab*	yes	no	Yes *A. tumefaciens*	[26]
Mustard, (*Brassica juncea* L.)	resistance to fungal pathogens	*NPR1*	yes	no	Yes *A. tumefaciens*	[27]
Chrysanthemum (*Chrysanthemum morifolium*)	resistance to *Spodoptera exigu*, *Aphis gossypii*	*CaXMT1*, *CaMXM1* *CaDXMT1*	yes	no	Yes *A. tumefaciens*	[28]
Cucumber (*Cucumis sativus* L.)	resistance to *cucumber vein yellowing virus*, *zucchini yellow mosaic virus*, *papaya ringspot mosaic virus-W*	*eIF4E*	yes	Cas9	Yes *A. tumefaciens*	[29]

Plant	Specific Trait	Target Gene	Transgenic	Gene-Edited	Used Transgenesis	Reference
Banana (*Musa* spp.)	inactivation of *banana streak virus*	ORFs of banana streak virus	yes	Cas9	Yes *A. tumefaciens*	[30]
Chilli pepper (*Capsicum annuum* L.)	resistance to *Colletotrichum truncatum*	*CaERF28*	no	Cas9	Yes *A. tumefaciens*	[31]
Grape (grape cultivar *Chardonnay*) Apple (apple cultivar *Golden delicious*)	resistance to powdery mildew and fire blight disease	*MLO-7* *DIPM-1*, *DIPM- 2*, *DIPM-4*	no	Cas9	No (RNP)	[32]
Tomato (*Solanum lycopersicum* L.)	tomato yellow leaf curl virus inactivation	coat protein, replicase	yes	Cas9	Yes	[24]
Abiotic Stress Resistance						
Apple (*Malus pumila Mill.*)	adaptation to cold and drought stress	*Osmyb4*	yes	no	Yes *A. tumefaciens*	[33]
Chilli pepper (*Capsicum annum*.)	improved salt tolerance	osmotin	yes	no	Yes *A. tumefaciens*	[34]
Grape (*Vitis vinifera* L.)	improved cold-resistance	*AtDREB1b*	yes	no	Yes *A. tumefaciens*	[35]
Grape (*Vitis vinifera* L.)	resistance to drought stress	*VaNCED1*	yes	no	Yes *A. tumefaciens*	[36]
Potato (*Solanum tuberosum*)	improved resistance to salt and drought stress	*SOD*, *APX*, *codA* under SWPA2 promoter	yes	no	Yes *A. tumefaciens*	[37]
Eggplant (*Solanum melongena* L.)	salinity tolerance	*TaNHX2*	yes	no	Yes *A. tumefaciens*	[38]
Tomato (*Solanum lycopersicum* L.)	improved salt tolerance	*SlABIG1*	no	Cas9	-	[39]

Plant	Specific Trait	Target Gene	Transgenic	Gene-Edited	Used Transgenesis	Reference
Potato (*Solanum tuberosum*)	resistance to abiotic stress and viruses	Coilin	no	Cas9	No, RNP Bioballistic Vacuum infiltration	[40]
Ethiopian mustard (*Brassica carinata*)	reduced root length under phosphorus stress	*BcFLA1*	-	Cas9	Yes *A. tumefaciens*	[41]
Lettuce (*Lactuca sativa* L.)	high temperature resistance	*LsNCED4*	yes	Cas9	Yes *A. tumefaciens*	[42]
Potato (*Solanum tuberosum*)	improved cold stress resistance	*VInv*	no	Cas9	No *A. tumefaciens* Transient expression	[43]
		Enhanced Quality				
Tomato (*Solanum lycopersicum* L.)	enhanced fruit softening	*LeEXP1*	yes	no	Yes *A. tumefaciens*	[44]
Apple (*Malus domestica*)	non-browning	*PPO*	yes	no	Yes *A. tumefaciens*	[45]
Potato (*Solanum tuberosum*) Tomato (*Solanum lycopersicum* L.) Strawberry (*Fragaria vesca*)	higher vitamin C	*GGP* or *VTC2*	yes	no	Yes *A. tumefaciens*	[46]
Orchid (Oncidium Gower Ramsey)	early flowering	*OMADS1*	yes	no	Yes *A. tumefaciens*	[47]
Tomato (*Solanum lycopersicum* L.)	high ɣ-aminobutyric acid (GABA)	*SlGAD2* *SlGAD3*	-	Cas9	Yes *A. tumefaciens*	[48]
Tomato (*Solanum lycopersicum* L.)	high lycopene	*SGR1*, *LCY-E*, *Blc*, *LCY-B1*, *LCY-B2*	-	Cas9	Yes *A. tumefaciens*	[49]
Potato (*Solanum*	high amylopectin starch	*GBSS*	no	Cas9	No *A.*	[50]

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51, 195–217.

Plant	Specific Trait	Target Gene	Transgenic	Gene-Edited	Used Transgenesis	Reference
tuberosum)					*tumefaciens* transient expression	
Potato (*Solanum tuberosum*)	non-browning	*StPPO2*	no	Cas9	No, RNP, PEG transfection	[51]
Banana (Cavendish banana cultivar (cv.) *Grand Naine*)	β-carotene-enriched	*LCYε*	-	Cas9	Yes *A. tumefaciens*	[52]
Strawberry (*Fragaria vesca*)	improvement of sugar content	uORF of *FvebZIPs1*.1	-	Cas9 + Base editor	Yes *A. tumefaciens*	[53]
Watermellon (*Citrullus lanatus* (Thunb.))	albino phenotype	*ClPDS*	-	Cas9	Yes *A. tumefaciens*	[54]

Bioballistics is a physicochemical method that bombards microcarriers containing genes of interest at high speed on plant cell walls using the so-called gene gun [8][9]. Nanoparticles coated with DNA are accelerated with gas pressure and shot using a gene gun into plant tissue kept in a Petri dish. The gene construct can be a circular or linear plasmid or a linear expression cassette. Factors that affect successful transformation include the size and density of the microcarriers, velocity of the microcarriers at the point of impact, nature of the plant tissue to be transformed, and suitable pre-culture or pretreatment of the target plant explants [9]. However, this technique requires expensive equipment. In addition, the transformation with the gene gun often gives rise to chimeric plants that consist of both transformed and non-transformed cells. If the reproductive cell line of such chimeras does not contain the target gene, then the next generation of such a plant would not be transformed. Transformation by this method can result in multiple copies of target DNA being inserted randomly anywhere in the plant genome. However, this method poses less physiological risk to the plant cell since there is no need for microbial intermediaries (Agrobacterium strains) and it requires less additional DNA. Moreover, it can adapt to both monocots and dicot plants. Examples of the successful use of bioballistics for the transformation of horticultural crops are also presented in **Table 1**.

A huge number of technologies have been developed to increase the efficiency of the transformation and regeneration of transgenic plants. These include various new delivery methods such as the use of nanoparticles, magnetofection, viruses, etc. [8][55], as well as the use of genes involved in embryogenesis or meristem maintenance, such as *BBM*, *GRF*, and *WUS2* [11][56][57].

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from random to targeted genome editing. Plant Biotechnol. Rep. 2019, 13, 423–445.

8. Rustgi, S.; Naveed, S.; Windham, J.; Zhang, H.; Demirer, G.S. Plant biomacromolecule delivery methods in the 21st century. Front. Genome Ed. 2022, 4, 1011934.
9. Sathishkumar, R.; Kumar, S.R.; Hema, J.; Baskar, V. Advances in Plant Transgenics: Methods and Applications; Springer: Berlin/Heidelberg, Germany, 2019; ISBN 9789811396243.
10. Mirzaee, M.; Osmani, Z.; Frébortová, J.; Frébort, I. Recent advances in molecular farming using monocot plants. Biotechnol. Adv. 2022, 58, 107913.
11. Su, W.; Xu, M.; Radani, Y.; Yang, L. Technological Development and Application of Plant Genetic Transformation. Int. J. Mol. Sci. 2023, 24, 10646.
12. Ahmad, N.; Jianyu, L.; Xu, T.; Noman, M.; Jameel, A.; Na, Y.; Yuanyuan, D.; Nan, W.; Xiaowei, L.; Fawei, W.; et al. Overexpression of a Novel Cytochrome P450 Promotes Flavonoid Biosynthesis and Osmotic Stress Tolerance in Transgenic Arabidopsis. Genes 2019, 10, 756.
13. Noman, M.; Jameel, A.; Qiang, W.-D.; Ahmad, N.; Liu, W.-C.; Wang, F.-W.; Li, H.-Y. Overexpression of GmCAMTA12 Enhanced Drought Tolerance in Arabidopsis and Soybean. Int. J. Mol. Sci. 2019, 20, 4849.
14. Song, G.Q.; Prieto, H.; Orbovic, V. Agrobacterium-mediated transformation of tree fruit crops: Methods, progress, and challenges. Front. Plant Sci. 2019, 10, 226.
15. Hao, S.; Zhang, Y.; Li, R.; Qu, P.; Cheng, C. Agrobacterium-mediated in planta transformation of horticultural plants: Current status and future prospects. Sci. Hortic. 2024, 325, 112693.

As a whole, bio-transformation methods were successfully used for the transformation of many horticultural crops (**Table 1**). In general, traits affecting the production of the crop are preferred over the traits involved in the modification of the final product. In this case, two main categories of GM crop traits can be distinguished. Firstly, it is tolerance to the application of specific herbicides. The most commonly developed trait has been tolerance to glyphosate, followed by glufosinate (phosphinothricin). Such traits were successfully inserted in potato [18] and sweet potato [17], cabbage [16], ornamental plants [19], as well as in many cereal crops [58][59]. Since 2016, crops with additional tolerance to active ingredients like 2,4-D and dicamba have been introduced, mostly in North America [60]. In 2020, ~90% of all corn, cotton, and soybeans planted in the U.S. were GM variants tolerant to one or more herbicides [61]. Secondly, it is resistance to specific insect pests of eggplant, potato, tomato, broccoli, and other crops [62]. This GM insect resistance (other name "Bt" technology—from Bacillus thuringiensis) offers farmers resistance in the plants to major pests, such as stem and stalk borers, earworms, cutworms, and rootworms in maize, bollworm/budworm in cotton, caterpillars in soybeans, and the fruit and shoot borer in eggplant [63][64][65].

The size of the area occupied by GM varieties grown in the world in 2022 was estimated at 202.2 million hectares. They are mainly occupied by soybeans (98.9 million hectares), corn (66.2 million hectares), cotton (25.4 million hectares), and rapeseed (9.9 million hectares) [66]. Despite the fact that the same use of plants resistant to various pests made it possible to reduce the amount of pesticides used in the areas where these plants are grown by 7.2% [58], society is wary of transgenic plants. An analysis of 2 million cases mentioning GMOs in various social networks and web resources in 2019–2021 and an assessment of their emotional connotation showed that 54% of mentions can be classified as neutral, 32% as negative, and only 14% as positive [67]. GMO organisms are perceived ambiguously by society, which stems from the fact that obtaining state registration for a GMO variety in some countries is significantly difficult or completely impossible.

Despite all the successes of transgenesis, the impact on public opinion complicates the use of this technology for improving crops. But since the demand for the development of new varieties still exists, a new technology has emerged in response to this request—gene editing. Unlike transgenesis, the insertion of foreign genes into the genome using gene editing is significantly difficult but gene editing allows you to make small changes to the target DNA sequence, thereby performing site-specific mutagenesis. In the last ten years, gene editing has been gaining popularity as a safe and cheap technique allowing you to quickly develop a new variety that meets all regulatory requirements [68].

3. Genome-Editing Technologies

With the development of genetic engineering methods and the accumulation of data on plant genomes, gene-editing technologies began to develop—making it possible to perform site-specific changes in the target site of the genome. The first methods that appeared were zinc-finger nuclease (ZFN) and, later, transcription activator-like effector nucleases (TALEN). Both TALEN and ZFN are composed of repeated tandem sequences of DNA-binding domains and an attached Fok1 nuclease protein; such a recombinant protein can be targeted to recognize a target DNA sequence and, therefore, create double-strand breaks (DSBs) at the target site. For each target site, a new TALEN or ZFN protein must be prepared to recognize the target DNA sequence, which requires labor-intensive

16. Sretenović-Rajičić, T.; Ninković, S.; Vinterhalter, B.; Miljuš-Đukić, J.; Vinterhalter, D. Introduction of resistance to herbicide Basta® in Savoy cabbage. Biol. Plant. 2004, 48, 431–436.
17. Yi, G.; Shin, Y.M.; Choe, G.; Shin, B.; Kim, Y.S.; Kim, K.M. Production of herbicide-resistant sweet potato plants transformed with the bar gene. Biotechnol. Lett. 2007, 29, 669–675.
18. Bakhsh, A.; Hussain, T.; Rahamkulov, I.; Demirel, U.; Çaliskan, M.E. Transgenic potato lines expressing CP4-EPSP synthase exhibit resistance against glyphosate. Plant Cell Tissue Organ Cult. 2020, 140, 23–34.
19. Benedito, V.A.; van Kronenburg-van der Ven, B.C.E.; van Tuyl, J.M.; Angenent, G.C.; Krens, F.A. Transformation of Lilium longiflorum via particle bombardment and generation of herbicide-resistant plants. Crop Breed. Appl. Biotechnol. 2005, 5, 259–264.
20. Veillet, F.; Perrot, L.; Chauvin, L.; Kermarrec, M.P.; Guyon-Debast, A.; Chauvin, J.E.; Nogué, F.; Mazier, M. Transgene-free genome editing in tomato and potato plants using Agrobacterium-mediated delivery of a CRISPR/Cas9 cytidine base editor. Int. J. Mol. Sci. 2019, 20, 402.
21. Tian, S.; Jiang, L.; Cui, X.; Zhang, J.; Guo, S.; Li, M.; Zhang, H.; Ren, Y.; Gong, G.; Zong, M.; et al. Engineering herbicide-resistant watermelon variety through CRISPR/Cas9-mediated base-editing. Plant Cell Rep. 2018, 37, 1353–1356.
22. Hummel, A.W.; Chauhan, R.D.; Cermak, T.; Mutka, A.M.; Vijayaraghavan, A.; Boyher, A.; Starker, C.G.; Bart, R.; Voytas, D.F.; Taylor, N.J. Allele exchange at the EPSPS locus confers glyphosate tolerance in cassava. Plant Biotechnol. J. 2018, 16, 1275–1282.
23. Zhang, H.; Si, X.; Ji, X.; Fan, R.; Liu, J.; Chen, K.; Wang, D.; Gao, C. Genome editing of upstream open reading frames enables translational control in plants. Nat. Biotechnol. 2018, 36, 894–900.
24. Tashkandi, M.; Ali, Z.; Aljedaani, F.; Shami, A.; Mahfouz, M.M. Engineering resistance against Tomato yellow leaf curl virus via the CRISPR/Cas9 system in tomato. Plant Signal. Behav. 2018, 13, e1525996.
25. Ye, C.; Li, H. 20 years of transgenic research in China for resistance to Papaya ringspot virus. Transgenic Plant J. 2010, 4, 58–63.
26. Koul, B.; Srivastava, S.; Sanyal, I.; Tripathi, B.; Sharma, V.; Amla, D.V. Transgenic tomato line expressing modified Bacillus thuringiensis cry1Ab gene showing complete resistance to two lepidopteran pests. SpringerPlus 2014, 3, 84.
27. Ali, S.; Mir, Z.A.; Tyagi, A.; Mehari, H.; Meena, R.P.; Bhat, J.A.; Yadav, P.; Papalou, P.; Rawat, S.; Grover, A. Overexpression of NPR1 in brassica juncea confers broad spectrum resistance to fungal pathogens. Front. Plant Sci. 2017, 8, 1693.
28. Kim, Y.S.; Lim, S.; Kang, K.K.; Jung, Y.J.; Lee, Y.H.; Choi, Y.E.; Sano, H. Resistance against beet armyworms and cotton aphids in caffeine-producing transgenic chrysanthemum. Plant Biotechnol.

genetic engineering and. is significantly limited by the widespread use of these gene-editing technologies [69][70]. However, there are examples of the successful use of ZFN to manipulate genes in tobacco, Arabidopsis, and maize [71][72][73], as well as for some horticulture species like tomato [74], potato [75], apple, and fig trees [76]. TALENs, which are easier to target to a specific DNA region because each TALEN domain recognizes one target nucleotide, as opposed to ZFN, where each domain recognizes a triplet of nucleotides, have been successfully used in horticultural crops. In potatoes, to change the functioning of the vacuolar invertase gene [77], in tomatoes, for targeted mutagenesis of the negative regulator of GA signaling gene PROCERA [78], and in cabbage, where the vernalization determinant allele of FRIGIDA was targeted [79]. However, the major drawback related to ZFNs and TALENs are their off-targeting effects, prolonged screening process, toxicity to the host cell, and complex genetic engineering procedures, limiting their applicability. The most widespread method of genome editing today is CRISPR technology; the first article on the successful application of this technology on plant cells was published in 2013 and the first edited plants were *Arabidopsis thaliana* and *Nicotiana benthamiana* [80]. Over the past ten years, a large number of examples of the use of CRISPR technology on various plant species, including horticultural species, have appeared; some of them, for example, tomatoes with a high GABA content [48], are already commercially available. This and other examples are presented in **Table 1**.

In natural conditions, CRISPR/Cas9 plays a part in bacterial immunity, providing bacteria with the ability to recognize and cut the nucleic acids of bacteriophages and plasmids [81][82]. Modified versions of the CRISPR/Cas9 editing tools used in the laboratory are typically a complex consisting of two components: the Cas9 endonuclease protein and a single guide RNA (sgRNA) with 20-nucleotide homology to the target DNA region [83][84][85]. The Cas9 endonuclease binds to the protospacer adjacent motif (PAM) DNA sequence (for Cas9, the PAM site is NGG) and the sgRNA complementarily binds to the DNA sequence adjacent to the PAM site, and if the binding is successful, Cas9 carves out a DSB in the target site [84][86]. DSBs caused by the Cas9 endonuclease lead to the activation of DNA repair systems, which can take two pathways: the error-prone nonhomologous end-joining (NHEJ) or homology-directed repair (HDR). Errors in the DNA repair system result in deletions, insertions, or substitutions of DNA at DSB sites, which in turn disrupt gene function or cause a reading frameshift, known as a gene mutation or knockout [83][84][85]. As a result of DSB repair via the NHEJ pathway, insertions/deletions (indels) of several bases are usually observed during genome editing. The use of the mechanism of HDR, in turn, makes it possible, using editing systems, to replace individual nucleotides in the DNA sequence and even obtain a site-specific insertion of a gene or group of genes.

At the moment, editing technologies have become so widely developed that they make it possible to influence any stage of the implementation of genetic information in a cell—at the level of transcription, translation, post-translational changes, the epigenetic level, etc. [87]. Over the past 10 years, a number of different CRISPR-based tools have been developed, allowing editing at almost any desired location in the genome. Some examples include DNA base editors [88][89], epigenetic modifiers [90][91], prime editors [89][92][93], and transcription regulators [91][94]. The fusion of various additional molecules with partially disrupted (nickase Cas9, nCas9) or nuclease-deficient (dead Cas9, dCas9) Cas9 has been used as a vehicle to deliver the CRISPR fusion protein to the target genomic site [91][95]. RNA-targeting Cas proteins also enable a variety of RNA manipulations beyond simple RNA editing, such as

2011, 28, 393–395.

29. Chandrasekaran, J.; Brumin, M.; Wolf, D.; Leibman, D.; Klap, C.; Pearlsman, M.; Sherman, A.; Arazi, T.; Gal-On, A. Development of broad virus resistance in non-transgenic cucumber using CRISPR/Cas9 technology. Mol. Plant Pathol. 2016, 17, 1140–1153.
30. Tripathi, J.N.; Ntui, V.O.; Ron, M.; Muiruri, S.K.; Britt, A.; Tripathi, L. CRISPR/Cas9 editing of endogenous banana streak virus in the B genome of Musa spp. overcomes a major challenge in banana breeding. Commun. Biol. 2019, 2, 46.
31. Mishra, R.; Mohanty, J.N.; Mahanty, B.; Joshi, R.K. A single transcript CRISPR/Cas9 mediated mutagenesis of CaERF28 confers anthracnose resistance in chilli pepper (Capsicum annuum L.). Planta 2021, 254, 5.
32. Malnoy, M.; Viola, R.; Jung, M.-H.; Koo, O.-J.; Kim, S.; Kim, J.-S.; Velasco, R.; Nagamangala Kanchiswamy, C. DNA-Free Genetically Edited Grapevine and Apple Protoplast Using CRISPR/Cas9 Ribonucleoproteins. Front. Plant Sci. 2016, 7, 1904.
33. Pasquali, G.; Biricolti, S.; Locatelli, F.; Baldoni, E.; Mattana, M. Osmyb4 expression improves adaptive responses to drought and cold stress in transgenic apples. Plant Cell Rep. 2008, 27, 1677–1686.
34. Subramanyam, K.; Sailaja, K.V.; Subramanyam, K.; Muralidhara Rao, D.; Lakshmidevi, K. Ectopic expression of an osmotin gene leads to enhanced salt tolerance in transgenic chilli pepper (Capsicum annum L.). Plant Cell Tissue Organ Cult. 2011, 105, 181–192.
35. Jin, W.; Dong, J.; Hu, Y.; Lin, Z.; Xu, X.; Han, Z. Improved cold-resistant performance in transgenic grape (Vitis vinifera L.) overexpressing cold-inducible transcription factors AtDREB1b. HortScience 2009, 44, 35–39.
36. He, R.; Zhuang, Y.; Cai, Y.; Agüero, C.B.; Liu, S.; Wu, J.; Deng, S.; Walker, M.A.; Lu, J.; Zhang, Y. Overexpression of 9-cis-epoxycarotenoid dioxygenase cisgene in grapevine increases drought tolerance and results in pleiotropic effects. Front. Plant Sci. 2018, 9, 970.
37. Ahmad, R.; Kim, Y.H.; Kim, M.D.; Kwon, S.Y.; Cho, K.; Lee, H.S.; Kwak, S.S. Simultaneous expression of choline oxidase, superoxide dismutase and ascorbate peroxidase in potato plant chloroplasts provides synergistically enhanced protection against various abiotic stresses. Physiol. Plant. 2010, 138, 520–533.
38. Yarra, R.; Kirti, P.B. Expressing class I wheat NHX (TaNHX2) gene in eggplant (Solanum melongena L.) improves plant performance under saline condition. Funct. Integr. Genom. 2019, 19, 541–554.
39. Ding, F.; Qiang, X.; Jia, Z.; Li, L.; Hu, J.; Yin, M.; Xia, S.; Chen, B.; Qi, J.; Li, Q.; et al. Knockout of a novel salt responsive gene SlABIG1 enhance salinity tolerance in tomato. Environ. Exp. Bot. 2022, 200, 104903.

RNA degradation, detection of nucleic acids, and pathogens, single RNA base editing, and live imaging of RNA, which can be read in more detail in recently published reviews [87][96].

40. Makhotenko, A.V.; Khromov, A.V.; Snigir, E.A.; Makarova, S.S.; Makarov, V.V.; Suprunova, T.P.; Kalinina, N.O.; Taliansky, M.E. Functional Analysis of Coilin in Virus Resistance and Stress Tolerance of Potato Solanum tuberosum using CRISPR-Cas9 Editing. Dokl. Biochem. Biophys. 2019, 484, 88–91.
41. Kirchner, T.W.; Niehaus, M.; Rössig, K.L.; Lauterbach, T.; Herde, M.; Küster, H.; Schenk, M.K. Molecular background of pi deficiency-induced root hair growth in brassica carinata—A fasciclin-like arabinogalactan protein is involved. Front. Plant Sci. 2018, 9, 1372.
42. Bertier, L.D.; Ron, M.; Huo, H.; Bradford, K.J.; Britt, A.B.; Michelmore, R.W. High-resolution analysis of the efficiency, heritability, and editing outcomes of CRISPR/Cas9-induced modifications of NCED4 in lettuce (Lactuca sativa). G3 Genes Genomes Genet. 2018, 8, 1513–1521.
43. Teper-Bamnolker, P.; Roitman, M.; Katar, O.; Peleg, N.; Aruchamy, K.; Suher, S.; Doron-Faigenboim, A.; Leibman, D.; Omid, A.; Belausov, E.; et al. An alternative pathway to plant cold tolerance in the absence of vacuolar invertase activity. Plant J. 2023, 113, 327–341.
44. Kaur, P.; Samuel, D.V.K.; Bansal, K.C. Fruit-specific Over-expression of LeEXP1 Gene in Tomato Alters Fruit Texture. J. Plant Biochem. Biotechnol. 2010, 19, 177–183.
45. Murata, M.; Nishimura, M.; Murai, N.; Haruta, M.; Homma, S.; Itoh, Y. A Transgenic Apple Callus Showing Reduced Polyphenol Oxidase Activity and Lower Browning Potential. Biosci. Biotechnol. Biochem. 2001, 65, 383–388.
46. Bulley, S.; Wright, M.; Rommens, C.; Yan, H.; Rassam, M.; Lin-Wang, K.; Andre, C.; Brewster, D.; Karunairetnam, S.; Allan, A.C.; et al. Enhancing ascorbate in fruits and tubers through over-expression of the l-galactose pathway gene GDP-l-galactose phosphorylase. Plant Biotechnol. J. 2012, 10, 390–397.
47. Thiruvengadam, M.; Chung, I.M.; Yang, C.H. Overexpression of Oncidium MADS box (OMADS1) gene promotes early flowering in transgenic orchid (oncidium gower ramsey). Acta Physiol. Plant. 2012, 34, 1295–1302.
48. Nonaka, S.; Arai, C.; Takayama, M.; Matsukura, C.; Ezura, H. Efficient increase of Γ-aminobutyric acid (GABA) content in tomato fruits by targeted mutagenesis. Sci. Rep. 2017, 7, 7057.
49. Li, X.; Wang, Y.; Chen, S.; Tian, H.; Fu, D.; Zhu, B.; Luo, Y.; Zhu, H. Lycopene is enriched in tomato fruit by CRISPR/Cas9-mediated multiplex genome editing. Front. Plant Sci. 2018, 9, 559.
50. Andersson, M.; Turesson, H.; Nicolia, A.; Fält, A.S.; Samuelsson, M.; Hofvander, P. Efficient targeted multiallelic mutagenesis in tetraploid potato (Solanum tuberosum) by transient CRISPR-Cas9 expression in protoplasts. Plant Cell Rep. 2017, 36, 117–128.
51. González, M.N.; Massa, G.A.; Andersson, M.; Turesson, H.; Olsson, N.; Fält, A.S.; Storani, L.; Décima Oneto, C.A.; Hofvander, P.; Feingold, S.E. Reduced Enzymatic Browning in Potato

Tubers by Specific Editing of a Polyphenol Oxidase Gene via Ribonucleoprotein Complexes Delivery of the CRISPR/Cas9 System. Front. Plant Sci. 2020, 10, 1649.

52. Kaur, N.; Alok, A.; Shivani; Kumar, P.; Kaur, N.; Awasthi, P.; Chaturvedi, S.; Pandey, P.; Pandey, A.; Pandey, A.K.; et al. CRISPR/Cas9 directed editing of lycopene epsilon-cyclase modulates metabolic flux for β-carotene biosynthesis in banana fruit. Metab. Eng. 2020, 59, 76–86.
53. Xing, S.; Chen, K.; Zhu, H.; Zhang, R.; Zhang, H.; Li, B.; Gao, C. Fine-tuning sugar content in strawberry. Genome Biol. 2020, 21, 230.
54. Tian, S.; Jiang, L.; Gao, Q.; Zhang, J.; Zong, M.; Zhang, H.; Ren, Y.; Guo, S.; Gong, G.; Liu, F.; et al. Efficient CRISPR/Cas9-based gene knockout in watermelon. Plant Cell Rep. 2017, 36, 399–406.
55. Demirer, G.S.; Zhang, H.; Goh, N.S.; González-Grandío, E.; Landry, M.P. Carbon nanotube–mediated DNA delivery without transgene integration in intact plants. Nat. Protoc. 2019, 14, 2954–2971.
56. Peterson, D.; Barone, P.; Lenderts, B.; Schwartz, C.; Feigenbutz, L.; St. Clair, G.; Jones, S.; Svitashev, S. Advances in Agrobacterium transformation and vector design result in high frequency targeted gene insertion in maize. Plant Biotechnol. J. 2021, 19, 2000–2010.
57. Laforest, L.C.; Nadakuduti, S.S. Advances in Delivery Mechanisms of CRISPR Gene-Editing Reagents in Plants. Front. Genome Ed. 2022, 4, 830178.
58. Brookes, G. Genetically Modified (GM) Crop Use 1996–2020: Environmental Impacts Associated with Pesticide Use Change Associated with Pesticide Use Change. GM Crops Food 2022, 13, 262–289.
59. Nandula, V.K. Herbicide Resistance Traits in Maize and Soybean: Current Status and Future Outlook. Plants 2019, 8, 337.
60. Wright, T.R.; Shan, G.; Walsh, T.A.; Lira, J.M.; Cui, C.; Song, P.; Zhuang, M.; Arnold, N.L.; Lin, G.; Yau, K.; et al. Robust crop resistance to broadleaf and grass herbicides provided by aryloxyalkanoate dioxygenase transgenes. Proc. Natl. Acad. Sci. USA 2010, 107, 20240–20245.
61. Sharkey, S.M.; Williams, B.J.; Parker, K.M. Herbicide Drift from Genetically Engineered Herbicide-Tolerant Crops. Environ. Sci. Technol. 2021, 55, 1559–15568.
62. Gatehouse, A.M.R.; Ferry, N.; Edwards, M.G.; Bell, H.A. Insect-resistant biotech crops and their impacts on beneficial arthropods. Philos. Trans. R. Soc. B Biol. Sci. 2011, 366, 1438–1452.
63. Petek, M.; Gerasymenko, I.; Juter, M.; Kallam, K.; Gim, E.M.; Gondolf, J.; Nordmann, A.; Gruden, K.; Orzaez, D. Insect pest management in the age of synthetic biology. Plant Biotechnol. J. 2022, 20, 25–36.

64. Paper, R.; Kumar, S.; Chandra, A.; Pandey, K.C.; Grassland, I. Bacillus thuringiensis (Bt) transgenic crop: An environment friendly insect-pest management strategy. J. Environ. Biol. 2008, 29, 641–653.

65. Alemu, M. Trends of Biotechnology Applications in Pest Management: A Review Role of Plant Protection for Improving. Int. J. Appl. Sci. Biotechnol. 2020, 8, 108–131.

66. AgbioInvestor. Global GM Crop Area Review May 2023; AgbioInvestor: Pathhead, UK, 2023.

67. Sohi, M.; Pitesky, M. Analyzing public sentiment toward GMOs via social media between 2019–2021. GM Crops Food 2023, 14, 2190294.

68. Teferra, T.F. Should we still worry about the safety of GMO foods? Why and why not? A review. Food Sci. Nutr. 2021, 9, 5324–5331.

69. Mahfouz, M.M.; Li, L. TALE nucleases and next generation GM crops. GM Crops 2011, 2, 99–103.

70. Wood, A.J.; Lo, T.-W.; Zeitler, B.; Pickle, C.S.; Ralston, E.J.; Lee, A.H.; Amora, R.; Miller, J.C.; Leung, E.; Meng, X.; et al. Targeted genome editing across species using ZFNs and TALENs. Science 2011, 333, 307.

71. Shukla, V.K.; Doyon, Y.; Miller, J.C.; Dekelver, R.C.; Moehle, E.A.; Worden, S.E.; Mitchell, J.C.; Arnold, N.L.; Gopalan, S.; Meng, X.; et al. Precise genome modification in the crop species Zea mays using zinc-finger nucleases. Nature 2009, 459, 437–441.

72. Townsend, J.A.; Wright, D.A.; Winfrey, R.J.; Fu, F.; Maeder, M.L.; Joung, J.K.; Voytas, D.F. High-frequency modification of plant genes using engineered zinc-finger nucleases. Nature 2009, 459, 442–445.

73. Osakabe, K.; Osakabe, Y.; Toki, S. Site-directed mutagenesis in Arabidopsis using custom-designed zinc finger nucleases. Proc. Natl. Acad. Sci. USA 2010, 107, 12034–12039.

74. Hilioti, Z.; Ganopoulos, I.; Ajith, S.; Bossis, I.; Tsaftaris, A. A novel arrangement of zinc finger nuclease system for in vivo targeted genome engineering: The tomato LEC1-LIKE4 gene case. Plant Cell Rep. 2016, 35, 2241–2255.

75. Butler, N.M.; Baltes, N.J.; Voytas, D.F.; Douches, D.S. Geminivirus-Mediated Genome Editing in Potato (Solanum tuberosum L.) Using Sequence-Specific Nucleases. Front. Plant Sci. 2016, 7, 1045.

76. Peer, R.; Rivlin, G.; Golobovitch, S.; Lapidot, M.; Gal-On, A.; Vainstein, A.; Tzfira, T.; Flaishman, M.A. Targeted mutagenesis using zinc-finger nucleases in perennial fruit trees. Planta 2015, 241, 941–951.

77. Clasen, B.M.; Stoddard, T.J.; Luo, S.; Demorest, Z.L.; Li, J.; Cedrone, F.; Tibebu, R.; Davison, S.; Ray, E.E.; Daulhac, A.; et al. Improving cold storage and processing traits in potato through

targeted gene knockout. Plant Biotechnol. J. 2016, 14, 169–176.

78. Lor, V.S.; Starker, C.G.; Voytas, D.F.; Weiss, D.; Olszewski, N.E. Targeted Mutagenesis of the Tomato PROCERA Gene Using Transcription Activator-like Effector Nucleases. Plant Physiol. 2014, 166, 1288–1291.
79. Sun, Z.; Li, N.; Huang, G.; Xu, J.; Pan, Y.; Wang, Z.; Tang, Q.; Song, M.; Wang, X. Site-Specific Gene Targeting Using Transcription Activator-like Effector (TALE)-Based Nuclease in Brassica oleracea. J. Integr. Plant Biol. 2013, 55, 1092–1103.
80. Li, J.-F.; Norville, J.E.; Aach, J.; McCormack, M.; Zhang, D.; Bush, J.; Church, G.M.; Sheen, J. Multiplex and homologous recombination–mediated genome editing in Arabidopsis and Nicotiana benthamiana using guide RNA and Cas9. Nat. Biotechnol. 2013, 31, 688–691.
81. Fineran, P.C.; Charpentier, E. Memory of viral infections by CRISPR-Cas adaptive immune systems: Acquisition of new information. Virology 2012, 434, 202–209.
82. Garneau, J.E.; Dupuis, M.È.; Villion, M.; Romero, D.A.; Barrangou, R.; Boyaval, P.; Fremaux, C.; Horvath, P.; Magadán, A.H.; Moineau, S. The CRISPR/cas bacterial immune system cleaves bacteriophage and plasmid DNA. Nature 2010, 468, 67–71.
83. Mali, P.; Yang, L.; Esvelt, K.M.; Aach, J.; Guell, M.; DiCarlo, J.E.; Norville, J.E.; Church, G.M. RNA-guided human genome engineering via Cas9. Science 2013, 339, 823–826.
84. Jinek, M.; Chylinski, K.; Fonfara, I.; Hauer, M.; Doudna, J.A.; Charpentier, E. A programmable dual-RNA—Guided DNA endonuclease in adaptive bacterial immunity. Science 2012, 337, 816–822.
85. Cong, L.; Ran, F.A.; Cox, D.; Lin, S.; Barretto, R.; Habib, N.; Hsu, P.D.; Wu, X.; Jiang, W.; Marraffini, L.A.; et al. Multiplex genome engineering using CRISPR/Cas systems. Science 2013, 339, 819–823.
86. Li, W.; Teng, F.; Li, T.; Zhou, Q. Simultaneous generation and germline transmission of multiple gene mutations in rat using CRISPR-Cas systems. Nat. Biotechnol. 2013, 31, 684–686.
87. Pramanik, D.; Shelake, R.M.; Kim, M.J.; Kim, J.Y. CRISPR-Mediated Engineering across the Central Dogma in Plant Biology for Basic Research and Crop Improvement. Mol. Plant 2021, 14, 127–150.
88. Jeong, Y.K.; Song, B.; Bae, S. Current Status and Challenges of DNA Base Editing Tools. Mol. Ther. 2020, 28, 1938–1952.
89. Molla, K.A.; Sretenovic, S.; Bansal, K.C.; Qi, Y. Precise plant genome editing using base editors and prime editors. Nat. Plants 2021, 7, 1166–1187.
90. Qi, Q.; Hu, B.; Jiang, W.; Wang, Y.; Yan, J.; Ma, F.; Guan, Q.; Xu, J. Advances in Plant Epigenome Editing Research and Its Application in Plants. Int. J. Mol. Sci. 2023, 24, 3442.

91. Pan, C.; Sretenovic, S.; Qi, Y. CRISPR/dCas-mediated transcriptional and epigenetic regulation in plants. Curr. Opin. Plant Biol. 2021, 60, 101980.

92. Lin, Q.; Jin, S.; Zong, Y.; Yu, H.; Zhu, Z.; Liu, G.; Kou, L.; Wang, Y.; Qiu, J.L.; Li, J.; et al. High-efficiency prime editing with optimized, paired pegRNAs in plants. Nat. Biotechnol. 2021, 39, 923–927.

93. Huang, T.K.; Puchta, H. Novel CRISPR/Cas applications in plants: From prime editing to chromosome engineering. Transgenic Res. 2021, 30, 529–549.

94. Tang, X.; Zhang, Y. Beyond knockouts: Fine-tuning regulation of gene expression in plants with CRISPR-Cas-based promoter editing. New Phytol. 2023, 239, 868–874.

95. Moradpour, M.; Abdulah, S.N.A. CRISPR/dCas9 platforms in plants: Strategies and applications beyond genome editing. Plant Biotechnol. J. 2019, 18, 32–44.

96. Shelake, R.M.; Kadam, U.S.; Kumar, R.; Pramanik, D.; Singh, A.K.; Kim, J.-Y. Engineering drought and salinity tolerance traits in crops through CRISPR-mediated genome editing: Targets, tools, challenges, and perspectives. Plant Commun. 2022, 3, 100417.