Chapter 10
Genetic Engineering: A Powerful Tool for Crop Improvement

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Abstract Rising population, changing climatic conditions, and various biotic and abiotic stresses are contributors to lowering crop yields. This, in turn, has augmented the number of people suffering from malnutrition. The applications of genetic engineering including genome editing are important as it can complement modern breeding activities to mitigate the effects of changing environment and boost crop production. The genetically modified (GM) crops thus offer one or more advantageous attributes, such as herbicide resistance, tolerance against pests and pathogens, and nutritional enhancement. The discovery of the natural ability of *Agrobacterium tumefaciens* to transfer a segment of its DNA (T-DNA) into the host was one of the breakthroughs of the twentieth century. It marked the beginning of achieving successful genetic transformation in a wide range of plants. Further, with the advent of technologies like zinc-finger nucleases (ZFNs), transcription activator-like effector nucleases (TALENs), and clustered regularly interspaced short palindromic repeats (CRISPR)/Cas, it has been possible to overcome the limitations of conventional breeding techniques. The synergism of scientific skills with sophisticated technologies resulted in many successful GM crops that were resistant to insects, pests, and weeds and enriched in micronutrients like vitamins and various minerals. Although not all GM crops have been commercialized, a few like soybean, papaya, maize, cotton, common bean, sweet potato, cowpea, etc. are practising. Recently, genome-edited crops are also approved for commercialization. The technology holds immense promise to achieve UN's sustainable development goals (SDGs) to fight hunger, attain food security, enhance nutrition, and promote sustainable agriculture.

Keywords Genetic engineering · GM crops · Food security · Biofortification

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10.1 Introduction

Genetic engineering has played a critical role in crop improvement either by improving the pre-existing traits or by introducing new desirable traits to improve crop production. GM crops offer one or more beneficial attributes, such as herbicide resistance, tolerance against pest/pathogens, and nutritional improvement (Kumar et al. 2020). Some of the famous instances of GM crops showcasing the potential to avert the challenges in agriculture include Bt cotton for insect tolerance and golden rice for improved vitamin A content (Qaim and Kouser 2013). Adoption of GM crops has revealed that the use of GM crops could boost the crop yield by 22% and lower the use of pesticides by 37% (Taheri et al. 2017). GM crops are the ones whose genomes have been modified such that economically important traits of the plants could be improved along with their yield. In this process, plants have been produced by inserting specific segments of foreign DNA or nucleic acid into their genome using the transformation methods like direct gene transfer or by *Agrobacterium*-mediated transformation (Griffiths et al. 2005). Such crops are referred to as transgenic crops, and the gene that has been inserted is known as a transgene (Kumar et al. 2020). The development of genetic transformation techniques marked the beginning of exponential growth in the field of plant research and offered a major advantage over conventional plant breeding technologies as compatibility was no longer a requirement. This breakthrough can be credited to the natural ability of *Agrobacterium tumefaciens* to insert its T-DNA into the host. The achievements in this field extend from the development of the first transgenic antibiotic-resistant tobacco and petunia (Fraley et al. 1983; Herrera-Estrella et al. 1983) to the commercialization of glyphosate-resistant soybean, bromoxynil herbicide-resistant cotton, and Bt maize (James 1998). The new editing techniques, like zinc-finger nucleases (ZFNs), transcription activator-like effector nucleases (TALENs), and clustered regularly interspaced short palindromic repeats (CRISPR)/Cas, have further utilized site-specific nucleases that have helped in addressing many concerns related to the unpredictability and inefficiency associated with conventional mutagenesis and transgenesis methods (Kumar et al. 2020). Thus, it is evident that traditional transgenic technologies along with genome-editing tools technology can prove useful in not only boosting agricultural productivity but also reducing dependency on agrochemicals, minimizing the environmental footprint of agriculture (Kumar et al. 2020).

Attaining food security is one of the prime concerns for any country. However, the growing population, diminishing arable land, and changing climate have widened the gap between population growth and food security (Islam and Karim 2019). Therefore, technological innovations in agriculture sector are necessary to sustainably increase production and decrease food losses (UNCTAD 2017). GM crops could positively impact food security by increasing the availability as well as improving the quality of food and influencing the farmers' socio-economically (Juma 2011). The recent global pandemic has triggered a serious social and economic crisis, presenting profound threats to nutritional status and food security around the world, especially in countries with low income (FAO 2020). Of particular concern is the rise in malnutrition, occurring due to changes in the availability, accessibility, and affordability of nutritious foods during this unprecedented situation. More women and children are suffering from malnutrition due to the declining quality of their diet, which is one of the worst repercussions of this outbreak (Osendarp et al. 2021; The World Bank Report 2021). In the uncertain circumstances provoked by the COVID-19 pandemic, where it is expected to decrease financial security and exacerbate all forms of malnutrition, wise adoption of GM crops can play a pivotal role in nutritional improvement (Wesseler and Purnhagen 2020; Gbashi et al. 2021). Furthermore, benefits like better agricultural yield, farm profits, and reduction of post-harvest losses could contribute to reducing food insecurity (Gbashi et al. 2021). The unanticipated chain of events during the pandemic has caused a shortage of workforce, restrictions on transportation, limited market operations, and the crumbling of the food supply chain, increasing our dependency on domestic food systems. Thus, the current situation necessitates the enabling of the environment, improving the resilience and nutrition sensitivity of the local food systems. Additionally, the threat to the food security scenario across the world has also encouraged people to accept and appreciate the need for every nation to adopt technologies that can support the farmers to boost yields with scarce resources. A pandemic like the current one may have an extensive and long-term influence on the agriculture and food industry.

This chapter highlights the importance of GM crops in agriculture by emphasizing the traits like herbicide tolerance, insect resistance, virus resistance, and biofortification. Different techniques utilized for the development of GM crops have also been exemplified in the text. Furthermore, the chapter also focused on the commercially available GM crops and the benefits of GM crops for addressing issues like food insecurity.

10.2 Pandemic and GM Crops

Like any other industry, the sudden outbreak of the COVID-19 pandemic had severely affected the agricultural sector. The situation of a pandemic can be regarded as the wake-up call for all the key stakeholders associated with this industry to reflect on the prevailing strategies such that the loopholes could be recognized. Just like human health, plant health must also be protected and secured by adopting preventive measures (Lamichhane and Reay-Jones 2021). During the time of Covid-19, the imposition of quarantine measures along with the lockdown had affected the mobilization of farmers and other people leading to a shortage of labourers as well as farm operators, especially in countries whose agricultural system is labour-intensive. This had not only affected the planting of the seasonal crops but also upsurge the loss of crops due to biotic and abiotic stress as the farmers were unable to carry out interventions like mechanical weeding or spraying pesticides. Moreover, activities like capacity development initiatives and pest management activities were severely affected in the agricultural sector due to the pandemic (FAO 2020).

In addition, equipment shortages during the time of the COVID-19 pandemic have also affected timely crop protection. For instance, a shortage in the fogging equipment was reported due to their utilization in disinfecting the areas for reducing the spread of the corona virus. Additionally, shortages in the respiratory protective equipment used by the pesticides handlers had also been reported in the United States. Under such conditions, not only did the health risk of the applicator increase but suboptimal applications of the pesticides had also upsurged the crop damage (Lamichhane and Reay-Jones 2021).

The outbreak of the pandemic has made it clear that it is crucial to make the agricultural system more resilient. In this context, one of the most important strategies that can be used is to develop GM crops that are self-sustainable and has the potential to overcome biotic and abiotic threats. Therefore, by using herbicide-resistant, virus-resistant, and drought-resistant GM crops, it could be hoped to minimize the loss of the crops and food insecurity despite measures like lockdown and social distancing. Therefore, the development of GM crops must be augmented so that crop yields could be improved irrespective of the pandemic. Furthermore, the outbreak of the Covid-19 pandemic had played a crucial role in increasing the burden of the malnutrition epidemic (Littlejohn and Finlay 2021). Further, Gastélum-Estrada et al. (2021) revealed that nutrients, such as vitamin C, D, and selenium, have the potential to reduce the risk as well as amelioration of COVID-19. These micronutrients are present in small quantities in most foods; however, the natural concentrations are not at par in meeting the needs of the human body. Henceforth, for meeting the immune-modulating needs, biofortification must be done for the foods like chickpea, tomato, wheat, and others such that their consumption could help in enhancing the immune system of humans.

10.3 Abiotic Stress and GM Crops

A deleterious profusion of various environmental factors, which can be summarised as abiotic stress, such as flood, heat, cold, drought, etc., imposes a detrimental impact on the overall growth of the crops and also on the grain yield of the crop plants. The plants cope up themselves with the existing obliterated condition by changing the physiological mechanism and altering the signalling cascades, regulatory proteins, and modification of the antioxidant defence system ultimately to limitise the cellular homeostatic condition. The changes that the plant made to adapt with the changing environmental regarding the abiotic stresses ultimately helps to minimise the loss of the plant along with its yield; also the near-optimal conditions facilitate them for better growth and development. At the molecular level, several arrays of genes get expressed variably due to the abiotic stresses, and also several disruptions of normal functioning can be seen due to this factor.

Many of the reported deficits of the crop plants, like cold, heat, and water-deficit of rice, water-deficit of maize, etc., have been studied through mitigation of the effects of the bacterial cold shock proteins (*csp*), as mentioned in the study of Castiglioni et al. (2008). The whole study summarises that maintaining the RNA stability and protein translation proved to be effective in the maintenance of the cellular functions during dehydration stress conditions, where *cspA* and *cspB* gene were used as a bacterial RNA chaperone extracted, respectively, from *E. coli* and a soil bacterium *B. subtilis*. The phenotypic behaviour of the transgenic maize was normal under adequate-watered condition but was showing better adaptation in water scarcity condition. Here, the RNA chaperone helps in stabilising the mis-folded RNA structures.

Another example of drought stress tolerance can be sugarcane, which was approved by Indonesia as commercial cultivation in 2013 (Waltz 2014). The gene *betA* extracted from *E. coli* and *Rhizobium meliloti* codes for choline dehydrogenase and catalyses formation of the osmoprotectant compound glycinebetaine, which helps in adapting to the water stress (Takabe et al. (1998); Khan et al. (2009); Singh et al. (2015)). It was inferred by another study of Chen and Murata (2002) and Singh et al. (2015) that the accumulation of the compatible solutes designated as the osmoprotectants, which comprise non-reducing sugars like fructan, trehalose, mannitol, and sorbitol, along with the proline and glycinebetaine, aids the survival of the plants under osmotic stress. In 2016, Nahar et al. have stated that increased titre of glycinebetaine helps in stabilising the enzymes and the protein structures, thus maintaining the cellular integrity during the stress condition. According to Waltz (2014), these transgenic sugarcane plants can produce 10–30% higher sugar than that of the non-transgenic plants under drought conditions in field trial.

Heat as an abiotic stress is also vulnerable to the crop plants, and as a by-product of this particular stress, abundant number of reactive oxygen species (ROS), such as hydrogen peroxide and superoxide, are produced inside the plant body, which hampers in the growth and development and declines the yield of the crop. Scavenging the ROS and restricting it to denature the enzymes and damaging the internal cellular components are straightforward approaches to tolerate the detrimental effects of heat stress (Chaitanya et al. 2002). Overexpression of the cytosolic ascorbate peroxidise (*cAPX*) gene to enhance tolerance to heat stress is already reported in transgenic apple and tomato, which seems to withstand a temperature of 40 degree Celsius in field condition as documented by Wisniewski et al. (2002) and Wang et al. (2005), respectively.

SAMDC (S-adenosyl-l-methionine decarboxylase) is one of the regulatory target enzymes in polyamine biosynthesis, and a related study on a tomato plant for enhancing polyamines production was done, where Chen and Xiong (2009) overexpressed *SAMDC* cDNA isolated from *Saccharomyces cerevisiae* and the transgenic lines was found to produce about 1.7–2.4-fold higher levels of spermidine and spermine along with enhanced antioxidant enzyme activity for better protection of membrane lipid peroxidation when compared to wild-type plants, which ultimately can withstand a temperature up to 38 degree Celsius. It is quite obvious that proline works as an important osmoprotectant, which protects cells from damage under heat stress, and according to Boston et al. (1996), HSPs facilitate correct protein folding, assembly, and translocation and provide stability to the integral proteins and cell membranes under heat stress; therefore, in 2014, Song et al., in his experiment, had overexpressed *CgHSP70* gene in chrysanthemum. The result in the transgenic lines showed increased peroxidase activity and higher proline content along with reduced malondialdehyde content.

Salinity or salt stress stands out to be the prevalent and one of the important abiotic stresses in the crop plants, which is engulfing around 20% of the agricultural land according to Rengasamy (2005). Here, *osmotin* comes out to be the important pathogenesis-related protein, which is solely for the plants to manage various abiotic stresses. Chilli plants are vulnerable to the salinity stress, and on the same hand, they are also very inept to genetic transformations and tissue culture, which also prevents the scope of genetic transformation in the chilli plants to withstand the salinity stress, but it has been made possible by Subramanyam et al. (2011), when he had successfully made the chilli pepper (*Capsicum annum L.* cv. Aiswarya 2103) by ectopic expression of tobacco *osmotin* gene via *Agrobacterium tumefaciens*-mediated gene transfer technique. The transgenic pepper plants also showed increased levels of chlorophyll, proline, glycine betaine, ascorbate peroxidase (*APX*), superoxide dismutase (*SOD*), glutathione reductase (*GR*), and relative water content (*RWC*) in biochemical analysis and survived in salinity level up to 300 mM NaCl concentration. Also previously in 2008, Husaini and Abdin have overexpressed the tobacco osmotin gene in strawberry plants (*Fragaria ananassa* Duch.), which also gave a positive result to withstand the salinity stress.

10.4 Biotic Stress and GM Crops

The drastically declining yield and productivity of the crops due to biotic is a major concern faced by the agricultural sector across the globe. Some of the biotic constraints responsible for crop destruction include infestation of crops by pests, diseases, weeds, and herbicides. This is the reason that has led to the emergence of conventional breeding methods, such that better crop varieties could be developed with superior traits. This was the only and most reliable method that could lead to crop protection, improvement, as well as quality management. However, the limitations of the conventional methods led to the development of genetic engineering techniques. This, in turn, allowed the scientists to tailor the plant varieties for expressing economically important traits (Tohidfar and Khosravi 2015; Parmar et al. 2017). Unlike abiotic stress, biotic stresses cause their hosts to directly face deprivation of nutrients thus leading to a reduction in the vigour and often the death of the plant. This is the major reason that causes pre-harvest and post-harvest losses in the field (Singla and Krattinger 2016). Additionally, the limited availability of agricultural resources along with insufficient nutritious food has increased the issue of food insecurity and malnutrition across the globe (Chrispeels and Sadava 2003). Thus, it is imperative to minimise the damage caused by the different stresses and to improve the quality of the food being produced for consumption. This, in turn, will resolve half of the food issues that are being faced by the rising population of the world.

Here, in this chapter, emphasis has been led to illuminate the ways genetic engineering could aid in reducing the biotic stresses (insect, weeds, and pathogens) and improving the nutrition profile of the crops (biofortification).

10.4.1 Herbicide Resistance

Herbicides are widely utilised in the farmlands across the globe for increasing crop yield along with farm labour efficiency (Schütte et al. 2017). However, a serious concern associated with the usage of herbicide is that other than affecting the target crops, it could also drift and run off towards the non-target plants. This, in turn, has deteriorated the ecological adaptability of the plants. Thus, during the development of GM crops, researchers had focused on involving the herbicide-resistant trait (Table 10.1). GM crops having resistance against herbicides like glyphosate and glufosinate-ammonium have been developed by plant scientists. Out of these two herbicides, the underlying mechanism of herbicide glyphosate is to inhibit 5-enolpyruvylshikimate-3-phosphate synthase (EPSPS). This enzyme is involved in carrying out the biosynthesis of aromatic acids and phenolics via the shikimate pathway in plants (OCED 1999). As a result, protein synthesis, production of the phenolics, defence molecules, salicylic acid, and lignin derivatives all get impacted. On the other hand, glufosinate ammonia is the racemic mixture of D- and L-isomers of phosphinothricin (PPT) and is found to inhibit the activity of glutamine synthetase via L-isomer. This, in turn, leads to the accretion of a lethal level of ammonia, ultimately causing death in plants (OCED 1999). Thus, the mode of action for herbicide-tolerant crops to get rid of the herbicides (either at the tissue or cellular level of the target plant) occurs in two ways, i.e. (i) the modification/alteration of an enzyme or other herbicidal target in plants in order to render its insensitive to the action of herbicide (ii) and the use of enzyme/enzyme system to degrade or detoxify the herbicide before they can act on the plant (Botterman and Leemans 1988). Some examples of the GM crops that are resistant to glyphosate herbicide include alfalfa, canola, maize, and soybean (Kumar et al. 2020). The molecular mechanism that has been used in the development of the above-mentioned crops is by creating modification at the target site of the herbicide such that the herbicide is unable to bind to its target. In most of the cases, the resistance mechanism is based on the heterologous expression of glyphosate-insensitive forms of *epsps* (epsps derived from *A. tumefaciens* strain CP4, mutant version of maize epsps, chemically synthesized gene similar to *epsps*grg23 gene of *Arthrobacter globiformis*) (Barry et al. 1997; Padgette et al. 1995).

In addition, herbicide-degrading enzymes were explored to generate herbicide resistance in plants. Such enzymes are extracted from bacteria residing in soil and water. One such gene is *atzA*, which is plasmid-borne. This gene is present in the

Table 10.1 Summary of 32 researches published between 1999 and 2020 using 18 techniques related to GM for enhancing herbicide tolerance in 11 crops

Technique and crop	Year
Base-editing-mediated gene evolution	
Rice	2020
BE	
Rice	2017, 2018
CRISPR-Cas9	
Maize	2020
Rice	2019
Tomato	2018
CRISPR-Cas9-based cytosine base editing	
Rice	2016
CRISPR-Cpf1	
Rice	2015
CRISPR/Cas9 SDN1	
Linum	2016
Tomato	2019
Watermelon	2018
CRISPR/Cas9 SDN2	
Rice	2016, 2017
Soybean	2015
CRISPR/Cas9 SDN3	
Cassava	2018
CRISPR/Cas9, TALENs SDN2	
Potato	2016
CRISPR/Cas-mediated base editing	
Maize	2019
Oilseed rape	2018
CRISPRnCas9-RT	
Maize	2020
Rice	2020
Meganuclease SDN3	
Cotton	2013
ODM	
Canola	2015
Maize	1999, 2000
Rice	2004
SDN1, SDN2, SDN3	
Maize	2015
SDN1, SDN2, SDN4	
Maize	2016
SDN3	
Soybean	2017

(continued)

Table 10.1 (continued)

Technique and crop	Year
TALENs SDN2	
Rice	2015
ZFN SDN3	
Maize	2013

gram-negative bacteria *Pseudomonas* sp. strain ADP. The gene encodes atrazine chlorohydrolase that catalyses the hydrolytic dechlorination of atrazine (Wang et al. 2005). Other genes that have been introduced into the plants for the development of the transgenic lines include soybean cytochrome P450 monooxygenase (Siminszky et al. 1999), glutamylcysteine synthetase (Gullner et al. 2001), *bar*, and *pat* genes (Lutz et al. 2001). Apart from this, GM crops have also been developed that are resistant to two herbicides. One of the GM plants that have been developed by introducing the genes tolerant to bensulfuron-methyl (BM) and glufosinate herbicides is rice AHAS (acetohydroxyacid synthase). The development of dual herbicide resistance confers the advantage that the chance of the evolution of weed resistance to herbicide within a short period decreases (Green and Castle 2010). Additionally, photoperiod-sensitive genic male-sterile transgenic rice has been developed that is tolerant to glyphosate and glufosinate herbicides (Deng et al. 2014). Other examples include the development of herbicide-tolerant tobacco by the introduction of the chimeric psbA gene [resistant to atrazine] (Cheung et al. 1988) and protoporphyrinogen oxidase [resistant to diphenyl ether] (Lermontova and Grimm 2000).

10.4.2 Insect Resistance

Among the various biotic stresses, the effect of insects has always been a major concern for farmers (Manosathiyadevan et al. 2017). This is because the quantity of crops damaged by insects is equal to 42% of the direct calorie consumed by humans worldwide every year. The rate of damage increases with the increase in the temperature of the climate. Studies have revealed that a warmer climate increases the metabolic rate of insects (Petersen et al. 2000). Thus, it leads to an augmentation of the food consumption rate among the insects. Apart from this, the population growth rate of insects also increases with the change in temperature (Deutsch et al. 2008). This had necessitated the development of plant lines that would be resistant to insects. In this regard, *Bt* genes that confer resistance to *Bacillus thuringiensis* have been extensively studied and introduced in economically important plants, like maize, cotton, and others. The GM crops express *Cry* genes that encode *Cry* protein. The *Cry* protein is responsible for pore formation in the cell membranes of the insect midgut, which finally leads to paralysis and death in the insects (Labbé et al. 2017). Some of the examples of the crops that have been made insect resistant with the help of the *Bt* gene, include maize, rice, cotton, and potato. GM maize has been developed with the integration of *Cry1Ab*, *Cry1Ac,* or *Cry9C* for protecting against *Ostrinia nubilalis* (Lepidoptera: European corn borer and Crambidae) and *Sesamia nonagrioides* (Lepidoptera: Noctuidae, Mediterranean corn borer). Further, *Cry1F* gene had been used for protecting maize against *Spodoptera frugiperda* (Lepidoptera: Noctuidae). On the other hand, the development of the GM maize with *Cry3Bb, Cry34Ab*, and *Cry35Ab* has helped the plant to get protected from rootworms belonging to the genus *Diabrotica* (Coleoptera: Chrysomelidae). For protecting cotton plants from getting affected by *Heliothis virescens, Pectinophora gossypiella, Helicoverpa zea, and Helicoverpa armigera,* GM cotton had been developed by inserting either *Cry1Ac* or by the stacking of *Cry1Ac* and *Cry1Ab* (Romeis et al. 2008). Apart from this, other genes for insect resistance that have been introduced into GE crops include Vip proteins (source of isolation: *B. thuringiensis* and *B. cereus*), lectins (source of isolation: plants like *Nicotiana tabacum* and *Oryza sativa)*, and protease inhibitors (source of isolation: plants, bacteria, and fungus). Vip gene encodes vegetative insecticidal proteins and has been successfully inserted for the development of transgenic cotton and maize (Chakroun et al. 2016).

Likewise, lectins interact with various glycoproteins or glycan structures, which can interfere with a variety of physiological functions in insects (Macedo et al. 2015). Orysata (agglutinin, a type of lectin isolated from the seedlings of *Oryza sativa*) was successfully expressed in transgenic tobacco and found to exhibit insecticidal activity against beet armyworm, the green peach aphid, and the pea aphid (Al Atalah et al. 2014). Similarly, protease inhibitors (PIs) work by inhibiting the proteolytic enzymes present in the guts of insects preventing them to procure the amino acids necessary for their growth and development (Broadway and Duffey 1986). For instance, PIs like trypsin inhibitor (Hilder et al. 1987) and potato protease inhibitor II (Duan et al. 1996; Majeed et al. 2011) were successfully explored to impart insect resistance in tobacco, rice, and cotton, respectively (Jagdish et al. 2020; Quilis et al. 2014; Dunse et al. 2010).

10.4.3 Virus Resistance

Plant viruses are another biotic factor that has been causing severe crop losses across the globe. As per the reports from FAO, 20% to 40% of the global crop production is lost to pests (FAO 2019). Every year, plant diseases cost the global economy approximately $220 billion. The expeditious distribution of the global plant virus diseases is the outcome of the rapid expansion of the international trade in the plant as well as plant produce (Jones 2021). This, in turn, has facilitated the introduction of virus diseases in parts of the world where they were not present earlier. All this has occurred for three main reasons. Firstly, trade globalisation that involves international agreements over free trade has led to the transfer of crops from one continent to another. Secondly, lower subsidies for developed countries had let the developing countries expand trade in international crop produce. Lastly, a well-developed and efficient transportation system with loosening plant quarantine facilities has further facilitated crop trading (Jones 2009). Thus, the movement of the plants from their domestication centre to other regions for monoculture had also led to the emergence of new virus diseases. In addition to this, the rapid change in climatic conditions has also been found to be one of the major reasons that have made it difficult to manage virus-caused pandemics (Jones and Naidu 2019). To get rid of the virus-mediated destruction, transgenic plants resistant to the virus are being developed at a swift pace by plant researchers. In this context, the gene silencing mechanism has been used for the development of transgenic plants, where genetic constructs having similar sequence identity as that of the pathogen genes are used for eliciting RNAi (RNA interference) in plants for silencing the gene. This method, where a particular gene in the pathogen is silenced, has been used to transform papaya with the coat protein gene of the papaya ring spot virus (PRSV) (Ferreira et al. 2002; Gonsalves et al. 2004). This method has also been successfully applied for developing GM cassava (Odipio et al. 2014), summer squash (Klas et al. 2011), and soybean (Abbas et al. 2021). The other methods that have been used for introducing virus resistance in plants are by genetically modifying targets of pathogen virulence factors so that host resistance could be increased without the insertion of any transgene or any exogenous biochemical pathway in the plants (Vincelli 2016). Apart from this, attempts have been made to develop virus-resistant plants to transform plants with satellite RNAs (satRNA). These are the small RNAs that depend upon the helper virus for replication and get encapsidated in the particles of the helper virus (Tien and Wu 1991). The transgenic expression of satRNA has helped in decreasing the virus systems by integrating DNA-copy of the responsible satRNA. The attempt had been successfully made in tobacco plants where a DNA copy of satRNA of cucumber mosaic virus (CMV) was introduced which helped in restricting the replication of CMV (Kundu and Mandal 2001). Similarly, resistance to plum pox virus and CMV was conferred in *Prunus domestica* (Ravelonandro et al. 1997), *Capsicum annuum* (Zhu et al. 1996) and *Solanum lycopersicum* (Yang et al. 1997), utilising viral coat protein (cp). Other advances in the realm of virus-resistant technologies include the use of the PRSV replicase (rep) gene and antisense technology to confer resistance in papaya (Guo et al. 2009) and common bean (Faria et al. 2006), respectively.

10.4.4 Biofortification

Biofortification is the process of the development of crops with enhanced nutritional values either by conventional selective breeding or by genetic engineering. As per the estimation, more than 800 million people across the globe are malnourished, of which 98% are in developing countries (Sinha et al. 2019). Besides, more than two billion people are experiencing hidden hunger that is caused by the poor intake of key micronutrients via their daily diet (Gillespie et al. 2016). Henceforth, the most feasible and cost-effective way that could help in providing micronutrients to the population of the developing countries is by the development of biofortified crops. Another major benefit that could be achieved by the production of biofortified crops is that biofortified seeds are found to have an indirect impact on agriculture. This is because higher concentrations of minerals are found to provide better protection against diverse biotic and abiotic stresses that have been affecting the crops and their productivity (Welch and Graham 2004). According to the World Health Organisation (WHO), malnutrition includes different forms of undernutrition, such as wasting, stunting, being underweight, inadequate minerals or vitamins, and diet-related non-communicable diseases. As per WHO, 1.9 billion people are either obsessed or overweight, 462 million are underweight, 149 million children below the age of 5 are stunted, 45 million are wasted, and 38.9 million were obese in the year 2020. In addition to this, nearly 45% of the deaths among the children who were under the age of 5 years were related to undernutrition. Various methods have been used for biofortifying the crops, such as via agronomic practices, plant breeding, and genetic engineering. Amid all these methods, genetic engineering is highly popular as it aids in the development of new cultivars with traits of interest. In addition to this, this technique also uses an enlarged gene pool for facilitating the transfer and expression of desirable traits from one species to another. Apart from this, if a particular micronutrient cannot be produced naturally in the crops, then the only method that could aid in fortifying those crops is genetic engineering (Pérez-Massot et al. 2013). Moreover, the transgenic approach provides the scope to insert novel genes, overexpress, or down-regulate the genes that are present in the plants. This transgenic approach has been used for the development of iron bio-fortified crops by inserting the iron-binding protein gene lactoferrin in crops like rice under the control of endospermic promoters, such that iron could be accumulated in the seeds that are consumed by humans. This helped in the development of the rice variety that had 120% more iron content in it and was suitable for meeting the iron needs of children but not adults (Suzuki et al. 2001). Parallel to this, to increase the iron content in the rice by two- to threefold, transgenic rice containing soybean ferritin was developed under the control of GluB-1, the rice seed-storage protein glutelin promoter (Lucca et al. 2001), and an increase of 3.7-fold was achieved indica cv. IR68144 seeds (Vasconcelos et al. 2003). Other than this, zinc had been fortified in the plants either by introducing zinc-binding protein-specific coding sequence, by overexpression of the zinc-storage protein and by enhancing the expression of zinc uptaking proteins. In addition to this, by introducing a protein having the capability to reduce anti-nutrient content, it becomes possible to increase the bioavailability of zinc. In this context, overexpression of the exogenous HvNAS1 (barley NAS gene) in *Arabidopsis* and tobacco had helped in increasing the concentration of copper, iron, and zinc in both the plant's seeds (Kim et al. 2005). Similarly, the overexpression of endogenous NAS genes helped in ameliorating the concentration of sodium, iron, and zinc in the endosperm of the transgenic rice (Wirth et al. 2009). Apart from this, plants have been biofortified for iodine (Itoh et al. 2009), vitamin A (Wang et al. 2014), vitamin B (Chen and Xiong 2009), vitamin C (Scholes et al. 2012), and vitamin E (Tanaka et al. 2015). Henceforth, biofortification holds a great promise for improving the nutritional profile of the plants and ultimately in overcoming the issue of malnutrition.

10.5 Technologies Exploited for the Development of GM Crops

The constant efforts made by the plant biotechnologists had led to the development of methods like *Agrobacterium*-mediated transformation or biolistic transformation for introducing the desired gene into the plant cells. After the insertion, if the gene is stable, inherited, and expressed by the succeeding generations, then such plants are referred to as transgenic plants. Another gene-editing method that has been in the spotlight is RNA interference (RNAi), a technique where the structure and function of the hosts' genes are modified by the insertion of guide RNA. Other than this, the success achieved by the scientists in developing programmable nucleases, like (ZFNs), transcription activator-like effector nucleases (TALENs), and clustered regularly interspaced short palindromic repeat (CRISPR)–Cas-associated nucleases, has opened new dimensions in the field of gene editing.

In this section of the chapter, an overview of the diverse gene-editing technology and its impact on the development of GM plants has been provided.

10.5.1 Agrobacterium and Biolistic Methods

The natural ability of *Agrobacterium* species like *A. tumefaciens* and *A. rhizogenes* to transform the plants and induce crown gall tuber and hairy root disease, respectively, had let the researchers mimic the process for achieving the first plant transformation breakthrough in 1983. However, among the two species, it was *A. tumefaciens* that helped the scientists significantly to understand the mechanism of plant transformation by tracking the activities of Ti (tumour-inducing) plasmid. It is a soil-dwelling bacterium that transforms the plant by injecting ssDNA (also referred to as T-DNA) that leads to the induction of the tumour via the synthesis of the phytohormones. Followed by this, the bacterium also compels the plants to synthesise opines that are used as nutrients by the pathogens (Flores-Mireles et al. 2012). Further, the genes required for the pathogenic function, along with the T-DNA, are encoded on the Ti plasmid. Moreover, the virulence or *vir* genes plays a crucial role in the translocation of the T-DNA within the host nucleus, such that the integration of the tumorigenic genes into the host chromosomes could be achieved. However, during plant transformation, disarmed Ti plasmids are constructed by deleting the oncogenes while keeping opine biosynthetic genes (Pratiwi and Surya 2020). Henceforth, in the *Agrobacterium*-mediated transformation (AMT), requisite foreign DNA substitutes the T-DNA, and the bacterium itself is used as the vehicle for inserting the gene of interest into the host (Chilton et al. 1977). Additionally, while constructing Ti plasmid with genes of interest, selectable marker genes are also inserted for distinguishing transformed cells from normal cells (Matveeva and Lutova 2014). The application of the AMT for the development of transgenic plants has helped in the development of insect-resistant crops, like cotton, and herbicide-tolerant plants, like soybeans and corn. Some of the economically important plants that have been developed with the application of the AMT method include papaya lines resistant to ringspot virus. GM papaya played a vital role in saving the US papaya industry. In addition, AMT has also been used for enhancing the nutritional value of the plants, like beta-carotene in canola (Fujisawa et al. 2009), oil composition in corn (Mohammed and Abalaka 2011), and vitamin A in rice (Dubock 2019). The major advantage associated with the application of AMT is that it ameliorates the probability to achieve single-copy insertions successfully. However, certain limitations associated with the process include the demand for a long tissue culture period for recovering the transgenic plants, the low frequency of stably transformed plants, and a narrow range of genotypes that could be transformed within a species (Rahangdale et al. 2020).

The biolistic method also referred to as particle bombardment aids in delivering the desired DNA directly into the plant cell. In this method, small metal particles (either tungsten or gold) coated with DNA are fired into the plant cells by accelerating them to high speed and releasing high-pressure helium in the gene gun (Bhatia et al. 2015). The target cells for gene gun bombardment are usually the totipotent cells that consist of either embryogenic suspension culture or embryogenic callus that is derived from the recipient plant (George et al. 2009). The metals used in the process are not lethal and safely deliver the DNA on the construct within the nucleus of the cell, aiding in its integration by recombination with the chromosomes. Thereafter, the transformed cells are induced to form plants under selection. This helps only those plants to survive that are expressing the selectable marker and the gene of interest (George et al. 2009). The exogenous DNA used for transformation consists of a plant expression cassette that is inserted in a vector based on a high-copy number bacterial cloning plasmid. With the aid of this process, Datta et al. (2003) were able to develop Golden Indian Rice lines that consisted of genes required for extending the existing carotenoid metabolic pathway (psy, crtI, and lcy), along with selectable marker gene (phospho-mannose isomerase or hygromycin phosphotransferase). Apart from this, the gene gun method was also used for the development of transgenic soybean, cotton plants, and beans by promoting multiple shoot induction from the embryonic axes of the mature seeds (Homrich et al. 2012). Further, the biolistic method is also being readily used in breeding tropical and subtropical fruit trees. One such breeding had been done for transforming banana cultivars, where several genes were transferred. One of the major advantages of using the biolistic method is that it aided in overcoming the host-range limitation of AMT. This is the reason that has led to the genetic transformation of a large number of plants, like corn, cotton, soybean, and wheat (Nicholas et al. 2017). The other advantages of the technique include the ability to transform a large number of cells and tissues, delivery of multiple plasmids for achieving high frequencies of co-transformation, delivery of large DNA fragments, and delivery of mRNA or protein (Nicholas et al. 2017). Additionally, in the biolistic technique, delivery of the desired genes could be done without the availability of a vector. Despite such advantages, the technique also suffers from certain limitations, like messy integration patterns, high input cost, low throughput, and inefficiency in controlling the cellular target.

10.5.2 RNA Interference

Another scientific breakthrough that helped in improving the crops genetically is RNA interference (RNAi). This technique was developed in 1998 and since then has become an approach of choice for plant scientists, as both desirable and undesirable genes could be manipulated for improving the novel traits of the plants. One of the significant features of RNAi is that it could be used for predicting the effects of off-target silencing making this technique more specific as well as effective. The degradation of RNA is triggered by introducing double-stranded RNA through transgenes. This dsRNA is thereafter cleaved by an enzyme called dicer that leads to the formation of duplexes of 21 nucleotides (nt) with symmetric 2 nt $3'$ overhangs. These duplexes are termed small interfering RNAs (siRNAs) and are responsible for the degradation process as well as suppression or alteration of the gene expression. This technique had been first utilized for developing plants resistant to viruses as the engineered antiviral strategies are found to mimic the natural RNA silencing process. This was first revealed in the case of potato virus Y-resistant plants that expressed RNA transcripts of a viral proteinase gene (Mansoor et al. 2006). Thereafter, immunity in plants against viruses, such as *Tomato spotted wilt virus, Rice tungro bacilliform virus*, *Cucumber mosaic virus, Tobacco mosaic virus*, *Bean golden mosaic virus*, and others, were observed (ISAAA, Pocket K No. 34). The significant usage of this improved technology has been demonstrated by enhancing the nutritional value, quality, and resistance towards pests and diseases in different crop plants. For example, *Lycopersicon esculentum* (tomato) with enhanced carotenoid and flavonoid was developed using the RNAi technique. In this regard, Davuluri et al. (2005) combined the RNAi technique with a fruit-specific promoter for suppressing *DET1*, an endogenous photomorphogenesis regulatory gene in tomatoes. Further, the RNAi approach was used for enhancing the amylose content by $>70\%$ in wheat by suppressing expression of SBEIIa and SBEIIb simultaneously (Regina et al. 2006). Similarly, the amylose content of sweet potato (*Ipomoea batatas*) was also increased using this approach. Furthermore, Sunilkumar et al. 2006, made a successful attempt to develop gossypol-free cottonseed by disrupting gossypol biosynthesis in cottonseed. This disruption was made possible by the interference in the expression of the δ-cadinene synthase gene at the time of seed development. In this process, a tissue-specific promoter was used. The transgenic cottonseed with 99% less gossypol made the crop suitable for human consumption This success led to the application of the RNAi approach in food sources, like *Lathyrus sativus*, cassava, and fava beans (Tang et al. 2007). Additionally, plants can also be triggered to silence essential genes by altering them to produce dsRNA in response to insect and parasitic nematodes. Using this approach, resistant varieties, like root-knot nematode (Huang et al. 2006), cotton bollworm (Baum et al. 2007), and corn rootworm (Mao et al. 2007), had been developed.

10.5.3 Genome-Editing Technologies

Extensive research is being conducted for improving the genetic makeup of the plants such that better outputs could be achieved for meeting the growing demand for agricultural output. In this regard, various gene-editing techniques have been developed and put into practice for remodelling the future of crops. Some of these include zinc-finger nucleases (ZFNs), engineered endonucleases/meganucleases, TAL effector nucleases (TALENs), and clustered regularly interspaced to short palindromic repeats (CRISPR). Among these techniques, ZFNs stirred up the genome manipulation research area as they aided in targeting the protein reagents. ZFNs are the DNA binding domains that can identify three base pairs at the target site (Ahmar et al. 2020). The next site-driven mutagenesis genome-editing system is TALENs, which resembles the technique of ZFNs. However, the major difference between the two techniques is that TALENs can target one site (Table 10.2). Further, research in the field of genome editing led to the development of CRISPR technology, such as CRISPR/Cas9 and CRISPR/Cpf1 (Nadakuduti and Enciso-Rodrı́guez 2021). Although the technique was first used in the prokaryotes, its application in the eukaryotes in the latter stages had revolutionized crop genome editing by facilitating specific changes in the crops. The simplicity of the tool and the minimal requirement of an RNA guide concerning the target DNA make it very efficient in terms of usage and genome modification (Ahmar et al. 2020).

10.5.3.1 Zinc-Finger Nucleases (ZFNs)

Zinc-finger nucleases (ZFNs) were first developed by using chemically engineered nucleases and since then it has been one of the most potential genome-editing tools that target the double-strand breaks (Durai et al. 2005). The application of this technique led to the discovery of the functional Cys2-His2 zinc-finger domain (Gaj et al. 2013). Structurally, ZFNs are composed of two domains. Firstly, the DNA-binding domain consists of 300–600 zinc-finger repeats. These repeats can monitor and read between 9 and 18 bp (Carlson et al. 2012). Secondly, a DNA cleavage domain (non-specific) of the type II restriction endonuclease enzyme Fok1 (Carroll et al. 2006). Further, ZFNs consist of two monomers that flank reversely between 5 and 6 bp of the target DNA and are attributed to their respective target sequence (Carroll et al. 2006). During the cleavage process, the flanking sequences of Fok1 domains slice the DNA. The zinc-finger domain monitors sequences of 24–30 bp that have either specific or rare targeting sites within the genome (Gaj et al. 2012).

Table 10.2 Comparison between ZFN, TALEN, and CRISPR/Cas9 technology

	ZFN	TALEN	CRISPR/Cas9
Recognition site	Zinc-finger protein	RVD tandem repeat region of TALE protein	Single-strand guide RNA
Modification pattern	Fok1 nuclease	Fok1 nuclease	Cas9 nuclease
Target sequence size	Typically, 9–18 bp per ZFN monomer, 18–36 bp per ZFN pair	Typically, 14–20 bp per TALEN monomer, 28–40 bp per TALEN pair	Typically, 20 bp guide sequence + PAM sequence
Specificity	Tolerating a small number of positional mismatches	Tolerating a small number of positional mismatches	Tolerating positional/multiple consecutive mismatches
Mode of action	Double-strand breaks in the target DNA	Double-strand breaks in the target DNA	Double-strand breaks or single-strand nicks in the target DNA
Target recognition Efficiency	High	High	High
Length of the target Sequence (bp)	24–36	24–59	20–22
Targeting limitations	Difficult to target non-G-rich sites	5′ targeted base must be a T for each TALEN monomer	Targeted site must precede a PAM sequence
Mutation rate	High	Middle	Low
Off-target effects	Low off-target effect	Least off-target Activities	Shows least off-target activities
Difficulties of engineering	Requiring substantial protein engineering	Requiring complex molecular cloning methods	Using standard cloning procedures and oligo synthesis
Difficulties of delivering	Relatively easy as the small size of ZFN expression elements is suitable for a variety of viral vectors	Difficult due to the large size of functional components	Moderate as the commonly used SpCas9 is large and may cause packaging problems for viral vectors such as AAV, but smaller orthologs exist
Cost of Development	High	Higher	Low

[a]The table was modified from Li et al. 2020, and Ahmar et al. 2020

This gene-editing technique has been successfully used to alter plants like maize, *Arabidopsis*, *Glycine max*, *Nicotiana*, petunia, rice, apple, rapeseed, and fig (Martínez-Fortún et al. 2017; Ahmar et al. 2020). In one such application, disruption of maize gene *ZmIPK1* was performed by inserting the PAT gene cascade. This resulted in the development of maize seeds that were tolerant to herbicides and possessed an altered inositol phosphate profile (Shukla et al. 2009). Furthermore, Cantos et al. (2014) identified safe regions for gene integration in rice such that they could serve as reliable loci for gene integration as well as trait stacking. However, the design of ZFNs is highly complicated and challenging. Moreover, the efficacy associated with ZFNs is low (Zhang et al. 2018a, b).

10.5.3.2 Transcriptional Activator-like Effector Nucleases (TALENs)

The amalgamation of the FokI cleavage domain with the transcription activator-like effectors (TALE) protein's DNA-binding domain led to the development of TALEN. In the TALEN system, the involved proteins have the central domain that is responsible for DNA binding and nuclear localization sequence (Schornack et al. 2006). The significance of the proteins in binding to the DNA was first observed in 2007. This protein consists of a repeated sequence of 34 amino acids, where each repeat perceives three nucleotides in the target DNA (Römer et al. 2007). Like ZFNs, this gene-editing technique also targets DSBs for initiating pathways responsible for DNA damage and modification (Gaj et al. 2013). It has been used in plants like *Arabidopsis*, *Brachypodium*, barley, flax, maize, *Nicotiana*, potato, tomato, sugarcane, rice, rapeseed, soybean, and wheat (Martínez-Fortún et al. 2017; Jansing et al. 2019). Amid all these plants, the first application of TALEN was done for improving rice by disrupting OsSWEET14, a bacterial blight susceptibility gene (Li et al. 2012). Similarly, three TaMLO homeologs were knocked out for creating powdery mildew-resistant wheat (Wang et al. 2014). Other achievements include the alteration of nutritional profiles in crops using the TALEN genome-editing system. Examples include the generation of soybean by disrupting fatty acid desaturase (FAD) genes such that oleic acid content could be alleviated and linoleic acid could be reduced. This, in turn, helped in improving the shelf life as well as heat stability of the soybean oil (Haun et al. 2014; Demorest et al. 2016). Furthermore, the vacuolar invertase (*VInv*) gene was knocked out for developing potato tubers with low or negligible levels of reducing sugar such that their quality does not get influenced during cold storage (Clasen et al. 2016).

10.5.3.3 CRISPR/Cas Technology

The discovery of CRISPR/Cas9 technology is considered to be one of the most prominent breakthroughs of the twentieth century as it is highly efficient as well as a simple tool that could lead to gene modification both in animals and plants (Barrangou and Doudna 2016). The tool relies on the signal of RNA-guided nucleases and has gained stardom due to its versatility, adequacy, potency, and simplicity (Gasiunas et al. 2012). The complex formed by Cas9 protein and guide RNA is responsible for locating and cleaving target DNA. DNA cleavage at the target site is usually 3 bp upstream of the protospacer adjacent motif (PAM) site. This process of breakage of double-stranded DNA leads to the activation of the DNA repair mechanism, non-homologous end joining (NHEJ), and homology-directed repair (HDR) (Symington and Gautier 2011). However, NHEJ mediates the relegation of the broken DNA directly in the absence of a homologous template. This, in turn, leads to insertions and deletions (InDels), or substitutions at the site of breakage. On the contrary, HDR is capable of adding new alleles, inserting new sequences of insert, and correcting the existing ones in the presence of the donor DNA (Zha et al. 2009). As the integration of the DNA within the plant genome occurs at a low frequency, the expression of CRISPR/Cas9 through transgenesis offers a better scope (Hilscher et al. 2016). After the transformation, two methods of selection are considered, viz. antibiotic and herbicide resistance, which aids in the regeneration of the explants expressing the CRISPR/Cas9 system functionally. This technique is being frequently used for gene knockouts and production of null alleles either by the insertion of indels, such that frameshift mutation could be achieved, or by introducing premature stop codons. With the application of this system, genome modification of plants, like cotton, rice, maize, wheat, soybean, grapefruit, potato, tomato, lettuce, oranges, and watermelon, have been done successfully (Zhang et al. 2016; Ricroch et al. 2017). Some of the prominent plant breeding activities conducted by the researchers using CRISPR technology include *LAZY1* gene knockout in rice for generating tiller-spreading phenotype for increasing the crop yield (Miao et al. 2013), mutation of *Gn1a, DEP1*, and *GS3* genes of the rice cultivar Zhonghua11 was achieved for enhancing the grain size and number along with dense erect panicles (Li et al. 2016). Another attempt in rice was made by Sun et al. (2017) for improving the fine structure of the rice grain along with nutritional properties. In this context, the SBEIIb gene was mutated for achieving a long chain of amylopectin. Other than this, with the aid of CRISPR/Cas9, the GW2 gene was disrupted, which led to the increase in the grain weight and protein content of the wheat (Zhang et al. 2018a, b). In addition to this, Jiang et al. 2017, used this gene-editing technology to improve oleic acid content in *Camelina sativa* by targeting FAD2. This also helped in lessening polyunsaturated fatty acids in the oilseed plant. Further, maize waxy gene Wx1 was knocked out for eliminating the expression of the granule-bound starch synthase (GBSS) gene, leading to the development of amylopectin rather than amylose. This led to improved digestibility of the maize along with made the maize species potential for bio-industrial application (Pioneer Dupont 2016). Additionally, wheat resistant to powdery mildew (Zhang et al. 2017), rice resistant to bacterial blight (Wang et al. 2016; Zhou et al. 2015), and tomatoes resistant to powdery mildew (Ortigosa et al. 2019) were developed.

10.5.3.4 New Tools for Genome Editing

Recently, there have been many new additions to the classic CRISPR toolbox, which have offered a multitude of applications in genome editing and beyond. For instance, the adoption of Cas9 variants, like SpCas9-VQR (PAM: NGA), SpCas9-EQR (PAM: NGAG), Cas9 NG (PAM: NG), and xCas9 3.7(PAM: NG/GAA/GAT), helped to overcome the limitation displayed by CRISPR/Cas9 system, i.e. the “NGG” sequence as PAM requirement, which reduces the target recognition sites.

These variants have been successfully used in plant-like *Physcomitrella*, *Arabidopsis*, rice, tomato, and potato (Zhang et al. 2019). The use of nucleases like Cas12/Cpf1 (class 2, type V CRISPR systems) has led to flexibility in base editing and epigenetic modulation. Cpf1 has several advantages over Cas9, including its ability to target T-rich motifs (PAM: TTTV, where V = A, C, or G), the lack of a necessity for trans-activating crRNA, its ability to generate a staggering double-strand break (4–5 nt 5′ overhangs), and the capability for both RNA processing and DNA nuclease activity (Safari et al. 2019). Another recent addition is the CRISPR-CasΦ protein (type V, CRISPR system), which displays the advantage of being smaller in size (~70 kilodalton) and requiring minimal PAM sequence of 5′-TBN-3 (where B = G, T, or C) for execution. However, CRISPR-CasΦ protein showed a low frequency of editing (0.85%) when it was used to edit the phytoene desaturase (*PDS*) gene in *Arabidopsis* protoplasts (Pausch et al. 2020).

Further, the recent development of base editors has allowed all combinations of precise base conversions without requiring DSB of DNA. Base editors use a catalytically hindered dead Cas9, dCas9 (D10A and H840A), or, usually a nickase, nCas9 (D10A), to precisely convert one target DNA nucleotide to another (Nadakuduti and Enciso-Rodríguez 2021). Individual nicks generated by base editors are repaired by a more precise base excision repair pathway, which minimizes the error-prone gene editing mediated by DSBs and NHEJ (Dianov and Hübscher 2013). The classic base editors are the cytosine base editors that catalyse C-to-T using a cytosine deaminase bound to nCas9 (Komor et al. 2016; Nishida et al. 2016) and adenine base editors that catalyse A-to-G conversions using an evolved DNA processing deoxyadenosine deaminase tethered to nCas9 (Gaudelli et al. 2017). Both cytosine base editors and adenine base editors have been optimized and utilized for genome editing in various plant species (Shimatani et al. 2017; Shan and Voytas 2018). The base editing in both cases is limited to transition mutation. However, recently, glycosylase base editors were developed that could mediate mutations, such as C-to-A and C-to-G, making transversion mutation feasible for base editors (Nadakuduti and Enciso-Rodríguez 2021).

Another breakthrough technology that allowed targeted insertions/deletions or a precise transition/transversion mutation at targeted genomic loci is prime editing (PE). The protein component of a prime editor compromises an altered form of the Cas9 enzyme (cuts DNA) and reverse transcriptase (produces complementary DNA from an RNA template). The RNA component here is the prime editing guide RNA (pegRNA) that recognises the targeted DNA site and has information for the desired edit. The section of the pegRNA that encodes the altered DNA sequence is directly copied into the target site by the reverse transcriptase, resulting in a new flap of DNA that carries the edit. The cell replaces the original DNA sequence on both strands of the DNA double helix when it integrates this altered flap (Nadakuduti and Enciso-Rodríguez 2021). The application of the prime editing technique is still in its infant stage. However, it has been greatly exploited in *Oryza sativa* (Zafar et al. 2020) and *Solanum Lycopersicum* (Lu et al. 2021).

10.6 Commercial GM Crops

The increasing awareness about the significance of transgenic crops along with the improvement in the techniques in terms of accuracy and precision has led many transgenic crops to be accepted at the commercial level. Adoption of biotech crop have been increasing in the past 25 years with the USA at 95% (average for soybeans, maize, and canola adoption), Brazil (94%), Argentina (~100%), Canada (90%), and India (94%) (ISAAA 2019a). In the year 2019, 190.4 million hectares of land have been used to plant the GE crops by 1.7 million farmers in 29 countries, compared to 2015 when only 28 countries grew GE crops on nearly 179.7 million hectares (ISAAA 2019b). In the past 22 years, the growth of transgenic plants has increased by several folds. As per the available reports previously in 1996, approximately 1.7 million hectares of land were used for the development of transgenic crops; contrarily in 2018, approximately 191.7 million hectares had been used for growing transgenic crops (ISAAA 2018). Concerning the crops, it was found that 95.9 million hectares were used to grow transgenic soybean which accounts for 50%, 58.9 million hectares were used to grow transgenic maize (31%), transgenic cotton occupied 24.9 million hectares (13%), transgenic canola occupied 5.3% that is 10.1 million hectares, and other transgenic crops were grown in remaining 1.9 million hectares (ISAAA 2018). And in the context of transgenic events, a total of 525 in 32 different crops have been commercialized (ISAAA 2019b). Out of this these, maize is the most exploited crop accounting for the maximum number of events (238 events), followed by cotton (61 events), potato (49 events), Argentine canola (42 events), soybean (41 events), carnation (19 events), and others (Kumar et al. 2020). The details of the most commercially exploited crop (corn) along with their applicants, traits, and date of effectiveness are listed in Table 10.3. The process of commercialization initiated with transgenic tomato called Flavr Savr was launched in the United States (Calgene company) in 1994. The advantage of this transgenic product was that it aided in slowing down the post-harvest ripening of tomatoes (Bruening and Lyons 2000). However, papaya plants resistant to papaya ringspot virus (PRSV) have been declared to be the first successful application of GE technology in the fruit crop at the commercial scale (Gonsalves 1998). The production of papaya was affected adversely in Hawaii due to the actions of the papaya ringspot virus (PRSV), which was detected in the Puna district of Hawaii in 1992 (Gonsalves 1998; Fuchs and Gonsalves 2007). This, in turn, emphasised the development of GE papaya varieties that are resistant to the virus. As a result of which, the first PRSV-resistant papaya plants were obtained by bombarding the PRSV code protein gene (Fitch et al. 1992). These transgenic papaya plants have been commercialised so that they could reach the end users in Hawaii (Tripathi et al. 2008). In addition to this, "Sunset" papaya was transformed with a gene that had been derived from a Hawaiian strain for producing the transgenic papaya "SunUp". This transgenic variety was completely resistant to PRSV. Followed by this, "SunUp" was crossed with "Kapoho," which was a non-engineered cultivar for obtaining yellow fresh papaya called "rainbow". This transgenic line was also

Table 10.3 List of the GM varieties of corn

Applicant	Phenotype
Dow	2,4-D and ACCase-inhibitor tolerant
Bayer/Genective	Herbicide tolerant
Monsanto	Male sterile
Pioneer	Insect resistant and glufosinate tolerant
Stine seed	Herbicide tolerant
Syngenta	Rootworm resistant
Monsanto	Drought tolerant
Pioneer	Male sterile, fertility restored, visual marker
Syngenta	Thermostable alpha-amylase
Syngenta	Moth and butterfly resistant
Pioneer	Herbicide and imidazolinone tolerant
Monsanto	European corn borer resistant
Syngenta	Corn rootworm protected
Monsanto	High lysine
Monsanto	Corn rootworm resistant
Dow	Corn rootworm resistant
Dow	Moth and butterfly resistant and phosphinothricin tolerant
Monsanto	Corn rootworm resistant
Mycogen c/o Dow and Pioneer	Moth and butterfly resistant Phosphinothricin tolerant
Monsanto	Herbicide tolerant
AgrEvo	Phosphinothricin tolerant and male sterile
Pioneer	Male sterile and phosphinothricin tolerant
AgrEvo	Phosphinothricin tolerant and moth and butterfly resistant
Monsanto	Herbicide tolerant
Monsanto	Herbicide tolerant and European corn borer resistant
DeKalb	European corn borer resistant
Northrup king	European corn borer resistant
Monsanto	European corn borer resistant
Plant genetic systems	Male sterileMS3
DeKalb	Glufosinate tolerant
Monsanto	Moth and butterfly resistant
AgrEvo	Glufosinate tolerant
Ciba seeds	Moth and butterfly resistant

[a] Table is adopted from Johnson and O'Connor 2015

resistant to PRSV (Gonsalves et al. 2004). Other transgenic varieties of papaya include Huanong No. 1 papaya, which is resistant to the four predominant PRSV strains, namely, Ys, Vb, Sm, and Lc24, which were found in South China. Moreover, this GM variety of papaya produced bigger and thicker flesh in the fruit (Lobato-Gómez et al. 2021). Besides papaya, in 1996, the first corn variety that was resistant to glyphosate herbicide was commercialised by Monsanto with the name "Roundup Ready Corn" as the corn variety could tolerate the use of roundup (Gutterson 2020). Followed by this, Liberty Link corn resistance to glufosinate was also developed by Bayer CropScience (CASE M.8084). The popularity of GM corn increased gradually; as a result of which, in 2011, 14 countries were involved in the production of herbicide-resistant GM crops, and by the next year, the European Union had authorised 26 varieties of herbicide-resistant GM maize. In 2013, Monsanto also launched the first transgenic drought-tolerance corn hybrids under the trade name of DroughtGard (DiLeo 2012). In the United States, GM corn is being readily used for the production of different ingredients that are used in processing strings in fruits, such as high fructose corn syrup and cornstarch. Apart from this, the major parts of GM corn are used to feed livestock, and some of them are also converted into biofuel. In addition to this, specific maize strains have been GM or engineered for expressing agriculturally desirable traits such as resistance to pests and herbicides. Additionally, GM sweet corn varieties have also been developed by Syngenta and Monsanto and have been commercialised under the trade name "Attribute" (Shelton et al. 2013) and Performance Series™ (Dively et al. 2021), respectively, which are both insect resistant. Other than this, one of the most popular maize varieties resistant to insects is Bt corn. This had been GM for expressing more than one protein-like delta-endotoxin from the bacterium *Bacillus thuringiensis*. The development of Bt corn relied significantly on the success rate of Bt cotton, which was first commercialized in 1996. At that time, Bt cotton cultivation was approved in countries, like the United States, Mexico, and others (Rocha-Munive et al. 2018), and the Bt cotton that produced *Cry1Ac* toxin had high activity on tobacco budworm and pink bollworm (Layton et al. 1997). However, in India, Bt cotton was approved only in 2002 after a thorough study conducted by ICAR. Thereafter, Bt brinjal resistant to brinjal shoot fly received the approval for commercialisation in 2009. Apart from the above-mentioned plants, GM legumes are also being commercialised. Monsanto had developed a GM soybean called Roundup Ready®, which was made commercially available, as it provided tolerance to the herbicide glyphosate (Roundup™). Further, it also could reduce the population of weeds. Gradually, other transgenic soybeans like Liberty Link® soybean (Meyer and Norsworthy 2020), soybeans containing the *Arabpdiosis*csr1–2 gene (Gabard et al. 1989), soybeans resistant to dicamba (Soltani et al. 2020), and many more were developed. Additionally, Bt soybean was also developed for protecting it against lepidopteron species. In 1994, soybean containing synthetic Bt (*Cry1Ac*) was developed (Martins-Salles et al. 2017). The other leguminous plant that has been commercialised is *Phaseolus vulgaris*, which showed resistance against the bean golden mosaic virus (Kumar et al. 2020). Moreover, the GM cowpea, Pod Borer-Resistant Cowpea (PBR Cowpea—event AAT709A) is resistant to *Maruca vitrata* has also been commercialized in Nigeria (ISAAA, GM approval database). The resistance was conferred by the presence of Cry1Ab protein. Another commercially grown fruit GM plant is the apple. Okanagan Specialty Fruits developed Arctic® Apple events GD743 that had limited quinone biosynthesis. This was achieved by targeting four PPO genes of apple using RNAi technology (ISAAA, GM approval database). An intensive amount of research is being carried out for developing transgenic plants with enhanced traits as it holds a great promise for the future of a nation by letting the country overcome the issue of food insecurities. However, it is

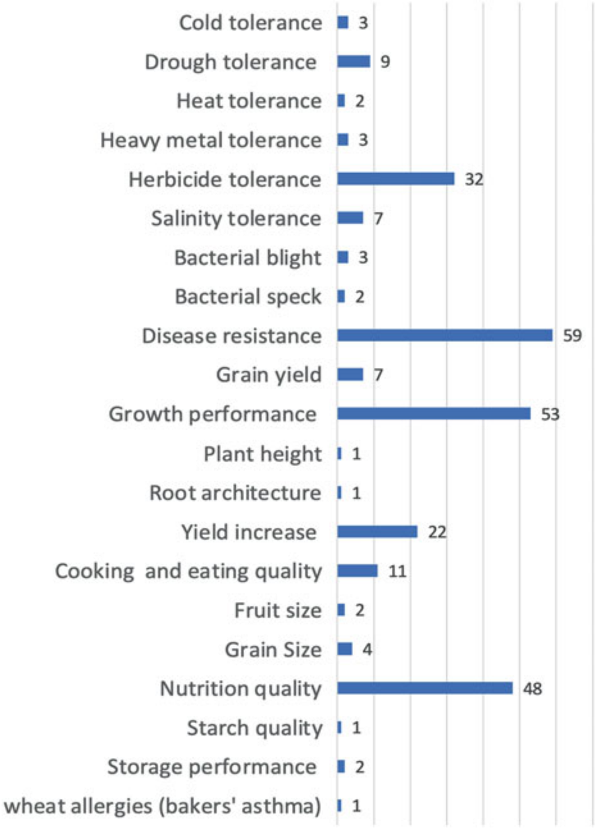

Fig. 10.1 Summary of 21traits (biotic, abiotic stresses, yield and nutritional quality). About 70% of the 273 were focused on enhancing disease resistance, growth performance, and nutrition quality and herbicide tolerance

unfortunate that some of those studies are not being translated into commercialization. Therefore, it is of utmost importance that based on the safety and efficiencies of the improved traits, genetically engineered crops must be commercialised at a larger scale (Fig. 10.1).

10.7 Benefits of GM Crop Cultivation

The gaining popularity of the GM crops itself signifies its potentiality to revolutionise the agricultural sector globally. The development of sophisticated technology like CRISPR and TALEN has helped in improving the agronomic traits of the economically important crops such that they provide benefits beyond being just edible products.

At the farm level, GM crops, like Bt cotton, Bt maize, GM tomatoes, apples, and others, have played a crucial role in reducing the negative impact of herbicides, pesticides, and fertilizers. This, in turn, had not only minimised the million-dollar losses that the farmers had to face but also helped in improving the water quality near the agricultural lands. In addition to this, the development of pest-resistant varieties has also improved the crop yield exponentially (Zilberman et al. 2018). In terms of figures, it has been found that the developing countries had received an extra income of $4.42 in 2018 due to their investment in GM crop seeds. On the other hand, in developed countries, the rise in income was found to be $3.24. The financial upsurge was not only observed at the industrial scale but also at the farm level. Expenses faced by the farmers owing to the utilisation of pesticides and herbicides have declined significantly due to the sowing of GM seeds for eggplant. In 2016, the farmers cultivating Bt eggplant in the 35 districts of Bangladesh were found to achieve direct income gains, as they had to spend 61% less on pesticides in comparison to those who used the conventional varieties (Shelton et al. 2018). Similarly, the sowing of Arctic® apples had improved the benefits for the retailers, because this variety of apples was found to be more suitable for mechanical harvesting. Moreover, they suffered less impact due to bin rubs, finger bruising, and other damages superficially. Thus, fewer amounts of fruit were wasted, and a significant decline was observed in pack-outs. Besides, Arctic® Golden variety also reduced the cost of production as it did not require warm packing. Hence, an ample amount of financial stress faced by the stakeholders of agriculture has been curbed by the cultivation of GM crops (Lobato-Gómez et al. 2021).

Further, the role of transgenic crops in achieving sustainable development has also been recognised. The usage of transgenic crops for biofuel production has helped in switching to the generation of greener energy and decreased the release of greenhouse gases that are responsible for the ongoing climate change. Moreover, as the transgenic crops enable wider utilisation of the conservation tillage systems; henceforth, it is further likely to reduce GHG emissions (Raymond Park et al. 2011). As per the available data, if GM crops were not developed and grown by 2018, then an additional 23 kilograms of carbon dioxide would have been emitted to the atmosphere. This number is equal to the addition of 15.3 million cars to the roads (Brookes 2020). Last but not the least, GE crops are playing a crucial role in helping countries to overcome the issue of food insecurities by minimising the yield loss due to biotic and abiotic factors, thus letting the nations meet the food needs of the people at the local and global level. Over the last 23 years, the utilisation of crop biotechnology had helped in producing 278 million soybeans, 498 million tonnes of corn, 14 million tonnes of canola, and 32.6 million tonnes of cotton lit additionally at the global scale (PG economics, 2014). In addition to this, biofortification has opened the doors to tackle the issue of malnutrition by incorporating essential nutrients within the plants. Thus, it has provided the scope of the farmers to improve the safety and quality of the food being produced in the farms. This, in turn, had further helped in improving both the economic as well as social situations of the farmers (Azadi et al. 2016). Tables 10.4 and 10.5 adopted from ISAAA, Pocket K No. 5, further illustrate the global impact of GM crops. In addition to this, it is also expected that the issue of food security that has been brought in due to the outbreak of the covid-19 pandemic could be improved with the adoption of GM crops (Petrova and

Table 10.4 Global farm income benefits from growing GE crops, 1996–2016 (US$ million)

GM trait	2016 increase in farm income	1996–2016 increase in farm income
HT soybean	4373.3	54,524.4
HT + IR soybean	2490.9	5211.5
HT maize	2104.9	13,108.1
HT cotton	130.1	1916.9
HT canola	509.9	5970.9
IR maize	4809.1	50,565.5
IR cotton	3695.2	53,986.9
Others	81.5	817.9
Totals	**18,194.9**	**186,102.1**

Note: *HT* herbicide tolerant, *IR* insect resistant, *others* virus-resistant papaya and squash and herbicide-tolerant sugar beet

Table 10.5 Impact of changes in the use of herbicides and insecticides in GE crops globally, 1996–2016

GM trait	Change in volume of AI used (million kg)	Change in field EIQ impact (million field EIQ/ha units)	% change in AI use on GE crops	% change in environmental impact associated with herbicide and insecticide use on GE crops
HT soybean	+13.0	-8526	+0.4	−13.4
HT + IR soybean	−7.4	−678	−6.1	−6.3
HT maize	−239.3	−7859	−8.1	−12.5
HT canola	−27.3	−931	−18.2	−29.7
HT cotton	−29.1	−706	−8.2	−10.7
IR maize	−92.1	−4142	−56.1	−58.6
IR cotton	−288.0	−12,762	−29.9	−32.3
HT sugar beet	+1.0	−43	+9.9	−19.4
Totals	**−671.2**	**−35,647**	**−8.2**	**−18.4**

Note: *HT* herbicide tolerant, *IR* insect resistant, *Ai* active ingredient, *EIQ* environmental impact quotient. (Environmental impact quotient (EIQ), a universal indicator where the various environmental impacts of individual pesticides are integrated into a single field value per hectare. This EIQ value is multiplied by the amount of pesticide active ingredient (ai) used per hectare to produce a field EIQ value)

[a]Tables 10.4 and 10.5 *are adopted from isaaa.org, Pocket K No. 5, 2020*

AbouRaya 2020). Moreover, it would also help in overcoming the issue of malnutrition that is being faced by women and children at the global level, especially in underdeveloped and developing countries (Qaim and Kouser 2013).

10.8 Conclusion

We discussed the multiple aspects of GM technologies and their advancements for the development of improved crop varieties. The utilisation of GM tools for quality improvement and yield enhancement in crops is essential to attain food security soon. The modern genetic/genome engineering techniques have revolutionised the trait enhancements in crops as they possess the ability to make precise and quick targeted modifications in genes of interest compared to conventional breeding techniques. Recent technologies like CRISPR/Cas have proved to be a fundamental breakthrough in the field of genome editing and are successfully explored for yield enhancement, quality improvement, and disease resistance. Furthermore, estimations imply that the adoption of genetic engineering technology has aided in the decline of the use of agrochemicals (pesticide and insecticide) as well as a reduction in environmental footprint and an increase in farmer revenue. Moreover, these new technologies have the potential to be adopted as a viable approach to achieve zero hunger and nutritional imbalance for the growing human population.

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