



Genetically modified crops: current status and future prospects

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Abstract

Main conclusion While transgenic technology has heralded a new era in crop improvement, several concerns have precluded their widespread acceptance. Alternative technologies, such as cisgenesis and genome-editing may address many of such issues and facilitate the development of genetically engineered crop varieties with multiple favourable traits.

Abstract Genetic engineering and plant transformation have played a pivotal role in crop improvement via introducing beneficial foreign gene(s) or silencing the expression of endogenous gene(s) in crop plants. Genetically modified crops possess one or more useful traits, such as, herbicide tolerance, insect resistance, abiotic stress tolerance, disease resistance, and nutritional improvement. To date, nearly 525 different transgenic events in 32 crops have been approved for cultivation in different parts of the world. The adoption of transgenic technology has been shown to increase crop yields, reduce pesticide and insecticide use, reduce CO₂ emissions, and decrease the cost of crop production. However, widespread adoption of transgenic crops carrying foreign genes faces roadblocks due to concerns of potential toxicity and allergenicity to human beings, potential environmental risks, such as chances of gene flow, adverse effects on non-target organisms, evolution of resistance in weeds and insects etc. These concerns have prompted the adoption of alternative technologies like cisgenesis, intragenesis, and most recently, genome editing. Some of these alternative technologies can be utilized to develop crop plants that are free from any foreign gene hence, it is expected that such crops might achieve higher consumer acceptance as compared to the transgenic crops and would get faster regulatory approvals. In this review, we present a comprehensive update on the current status of the genetically modified (GM) crops under cultivation. We also discuss the issues affecting widespread adoption of transgenic GM crops and comment upon the recent tools and techniques developed to address some of these concerns.

Keywords GM crops · Transgenics · Public concerns · Cisgenesis · Intragenesis · Genome editing

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Introduction

Genetically modified (GM) crops are such crop plants whose genome is modified using genetic engineering techniques to improve the existing traits or for introduction of a new trait that does not occur naturally in the given crop species. The plants produced by the insertion of specific segments of foreign nucleic acid/gene sequence into its genome using transformation methods (such as *Agrobacterium*-mediated transformation or direct gene transfer) are known as transgenic plants (Griffiths et al. 2005). The inserted gene, also known as transgene, may come from an unrelated plant, bacteria, virus, fungus, or an animal species (Fig. 1). Thus, the advent of genetic transformation overcomes the major limitation of conventional plant breeding in which sexual

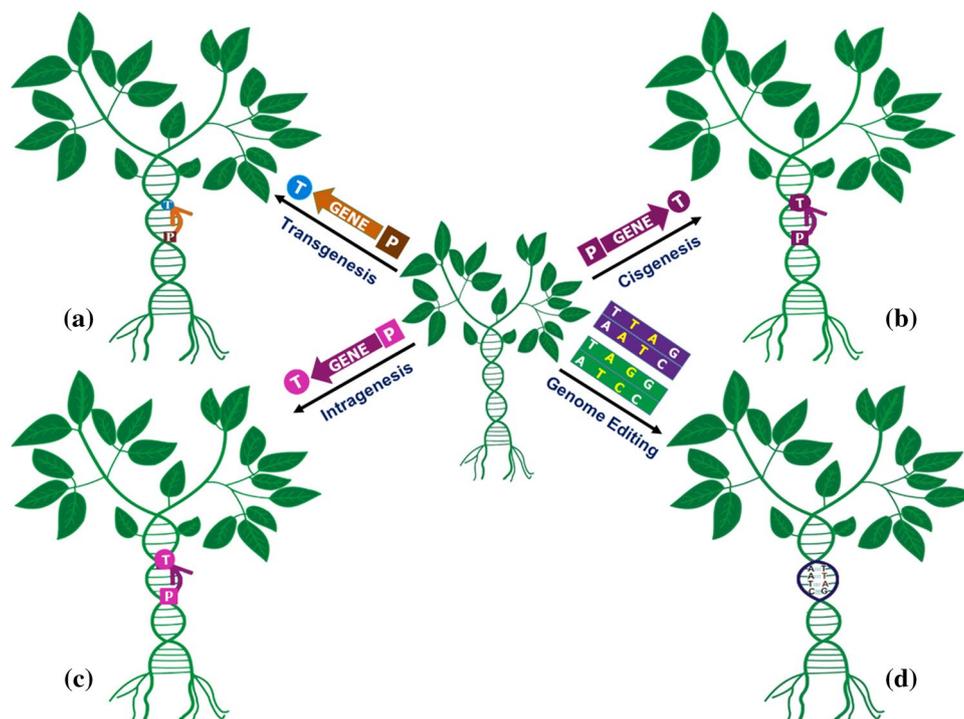


Fig. 1 Illustration of various genetic engineering techniques utilised for generation of improved crop plants. Letter P and T represents promoter and terminator, respectively. **a** Transgenesis: insertion of recombinant genetic elements in which one or more components (gene, promoter and terminator) are taken from sexually incompatible gene pool. **b** Cisgenesis: insertion of an identical copy of a complete genetic element, including gene, promoter and terminator from the same gene, within the sexually compatible gene pool. **c** Intragenesis: insertion of recombinant genetic elements in which one or more

components (gene, promoter and terminator) are isolated from different genes within the sexually compatible gene pool. **d** Genome editing: introduction of targeted mutation at specific loci in the genome. Nucleotide pairs with green background colour represent original base pairs in DNA of plant whilst nucleotide pairs with violet background colour represent desired modifications in base pairs (T:A to A:T and C:G to T:A mutation at second and third position, respectively, in base pair is shown) to be done

compatibility between species is prerequisite to cross them. In the year 1977, the natural ability of *Agrobacterium tumefaciens* to stably insert Ti plasmid DNA (T-DNA) into host plant cell genome was discovered (Chilton et al. 1977), and hence, Ti plasmid was proposed as a vector to introduce foreign genes into plant cells. This study led the breakthrough related to development of transgenic plants. Subsequently, specific gene sequence was first reported to be transferred to plant cell using recombinant DNA and transformation technique (Herrera-Estrella et al. 1983a; Bevan et al. 1983; Fraley et al. 1983; Murai et al. 1983). The first transgenic plants, viz., antibiotic-resistant tobacco and petunia, were developed in the same year (Fraley et al. 1983; Herrera-Estrella et al. 1983b). Murai et al. (1983) reported the expression of ‘phaseolin’ gene from bean in sunflower and thus their study demonstrated the expression of a plant gene even upon transfer to a taxonomically distinct angiosperm family. In 1994, transgenic tomato, ‘Flavr Savr’ with the property of longer shelf life or delayed ripening developed by Calgene (Monsanto), was approved by Food and Drug Administration (FDA) for sale in the USA. Later on, several transgenic

crops, such as canola with modified oil composition, *Bt* Potato, *Bt* maize, *Bt* cotton, bromoxynil herbicide-resistant cotton, and glyphosate-resistant soybeans, etc., received approval for commercialization (James 1997). The commercialised transgenic crops have chiefly deployed microbial genes and/or genetic elements (Kumar et al. 2018). To date, a total of 525 transgenic events in 32 crops have been commercialised (ISAAA database 2019). Of these, maize accounts for the maximum number of events (238), followed by cotton (61), potato (49), Argentine canola (42), soybean (41), carnation (19) and others.

The cultivation of transgenic crops has appreciably increased world agricultural productivity in the past two decades. A global meta-analysis of the impact of transgenic crop adoption has estimated that on an average transgenic technology has increased crop yields by 22% which has led to an estimated 68% increase in farmer profits (Klumper and Qaim 2014). However, crops with foreign gene(s) have remained the subject of some concerns owing to chances of gene flow between transgenic crops and its wild relatives, the possibility of lateral transfer of antibiotic resistance genes

to microbes in the environment and potential adverse health effects, such as toxicity and allergenicity to humans. Owing to these, transgenic crops have been facing lack of public acceptance in many parts of the world which, in turn, has precluded their widespread adoption. To overcome concerns related to foreign gene insertion, two new techniques, namely cisgenesis and intragenesis, were developed as an alternative to transgenesis. In both these techniques, genetic elements being deployed for crop improvement via transformation belong to same or closely related species, i.e. from sexually compatible gene pool (Fig. 1). Besides, the advent of the breakthrough technology of genome editing in recent years has enabled modifying the crop genomes with an unprecedented ease, accuracy and precision. A set of new editing techniques, using various site-specific nucleases (SSNs), viz., Zinc Finger Nucleases (ZFNs), Transcription Activator-Like Effector Nucleases (TALENs) and Clustered Regularly Interspaced Short Palindromic Repeats (CRISPR)/Cas system, have, to some extent, addressed the concerns regarding the unpredictability and inefficiency associated with conventional random mutagenesis and transgenesis. These gene editing tools have the potential to address many of the regulatory issues associated with transgenics and hence are set to contribute to develop improved varieties through interventions like targeted mutagenesis, precise editing of endogenous gene and site-specific insertion of a trait gene.

In spite of biosafety and environmental concerns, the transgenic technology has been a method of choice for rapid development of improved crop plants and stacking of multiple favourable traits. In the last 22 years, the global area of transgenic crops has increased significantly from 1.7 million hectare in 1996 to 191.7 million hectares in 2018, i.e. around 113-fold increases (ISAAA 2018). There were 95.9 million hectares (50%) of transgenic soybean, 58.9 million hectares (31%) of transgenic maize, 24.9 million hectares (13%) of transgenic cotton, 10.1 million hectares (5.3%) of transgenic canola and 1.9 million hectares (<1%) of other transgenic crops (ISAAA 2018). Thus, transgenic technology is regarded as the fastest crop technology to be adopted in modern agriculture. In 2017–18, these crops were planted by approximately 17 Mio. farmers in 26 countries and their estimated global market value was US\$18.2 billion (ISAAA 2018). The major traits, for which transgenic crops have been developed and approved for commercialization, include herbicide tolerance (HT), insect resistance (IR), disease resistance, abiotic stress tolerance and nutritional enhancement. Amongst these, herbicide tolerance is the most common one which accounts for about 88.7 million hectares or 47% of the total area occupied by transgenic crops in the year 2017. After HT, transgenic crops with stacked traits (crops with two or more traits) and IR trait occupies approximately 41% and 12% of the global transgenic crop area, respectively. The

remaining traits account for less than 1% of the total area occupied by transgenic crops (ISAAA 2017).

Whilst gene function studies and proof-of-concept studies for several other traits in different crop varieties using the transgenic technology are promising, many such studies do not get translated into the commercial release of a new transgenic variety. Besides, even amongst the commercialised varieties, there have been reports of variation in the degree of improvement of a given trait in a transgenic variety. Against this background, the present review aims to summarise the current status of commercially cultivated transgenic crops possessing various traits, public concerns and potential biosafety issues related to the use of transgenic food crops and recent advances in genetic engineering tools for plants. We also comment on the future prospects of engineered crops developed using genome editing tools.

Herbicide-tolerant transgenic crops

Weeds compete with crop plants for nutrients, water, sunlight and space, and hence lead to significant yield losses. Owing to crop yield loss by weeds, active management through various strategies such as use of herbicides is needed. However, because most weeds are herbaceous plants, selective killing of the weeds whilst protecting the crop plant is not always possible. Therefore, developing herbicide tolerance trait in the main crop is a potential solution which can facilitate flexible use of robust non-selective and broad spectrum herbicides. The herbicides available for killing the weeds have two different modes of actions, selective or non-selective. Amongst the non-selective ones, glyphosate and glufosinate are the most extensively used herbicides. Notably, most of the herbicide-tolerant (HT) transgenic plants have been developed so as to tolerate glyphosate and glufosinate. Glyphosate specifically inhibits 5-enolpyruvyl shikimate-3-phosphate synthase (EPSPS) enzyme, the key enzyme involved in the shikimate pathway of aromatic amino acid biosynthesis. As the shikimate pathway is not present in the animal kingdom, glyphosate is not harmful for human beings, birds, insects and other animals. The development of glyphosate-resistant transgenic crops has relied on the heterologous expression of a glyphosate-insensitive form of *epsps* obtained from either *A. tumefaciens* strain CP4 (Barry et al. 1997; Padgett et al. 1995), or mutant version of maize *epsps* or chemically synthesized gene similar to *epsps grg23* gene of *Arthrobacter globiformis*. In 1996, glyphosate-tolerant (“Roundup Ready”) soybean harbouring *cp4epsps* gene was commercialised as the first herbicide-tolerant transgenic crop. Most of the commercialised glyphosate-resistant crops harbour this gene (Dill et al. 2008). Besides, a few commercialised transgenic crops express either glyphosate oxidoreductase (GOX) encoding gene derived from *Ochrobactrum anthropi* or glyphosate acetyltransferase (GAT) encoding

gene derived from *Bacillus licheniformis*. Both of these are glyphosate-degrading enzymes which detoxify glyphosate to non-toxic byproducts.

The other non-selective herbicide is glufosinate (or phosphinothricin) which competitively inhibits glutamine synthetase enzyme (Lea et al. 1984). This enzyme plays a role in the conversion of glutamate and ammonia into glutamine. Inhibition of this enzyme by glufosinate leads to accumulation of ammonia which inhibits photosystem I and II reactions (Tachibana et al. 1986; Sauer et al. 1987). Two different bacterial genes, namely *pat* and *bar*, from *Streptomyces* spp. were utilised for developing glufosinate-resistant crops. Both of these genes encode phosphinothricin acetyl transferase (PAT) enzyme which detoxifies this herbicide by acetylation. Beside the above-mentioned herbicides, HT transgenic crops specific to other herbicides, such as 2,4-D, dicamba, isoxaflutole, mesotrione, oxynil and sulfonylurea, have been commercialised recently. So far (1996–2018), a total of 351 HT events have been approved for cultivation (ISAAA database 2019). Amongst these, the maximum number of HT events (210) have been commercialised in maize, followed by Argentine canola (34), soybean (33), potato (4), carnation (4), rice (3), sugarbeet (3), wheat (1) and others (Fig. 2). The HT transgenic crops occupy the highest area amongst the commercialised transgenic crops.

Widespread cultivation of HT transgenic crops has benefitted farmers in many ways, viz., yield increase due to more efficacious and simplified weed management, and reduction in overall cost for weed management (Brookes and Barfoot 2018). It has been estimated that in glyphosate-resistant soybean, 38% of the economic benefit has been due to increase in yield whilst the remaining 62% has been due to reduction in cost for weed management (Green 2012; Brookes and Barfoot 2018). Besides, adoption of HT crops has reduced the environmental impact of weed management. For example, the profile of herbicide use changed towards more environmentally benign herbicides, such as glyphosate and glufosinate which are rapidly broken down after application. Furthermore, adoption of the HT technology facilitated switching from conventional plough-based to reduced, minimum or no tillage production systems which resulted in lower greenhouse gas emissions owing to reduced usage of tractors (Brookes and Barfoot 2017).

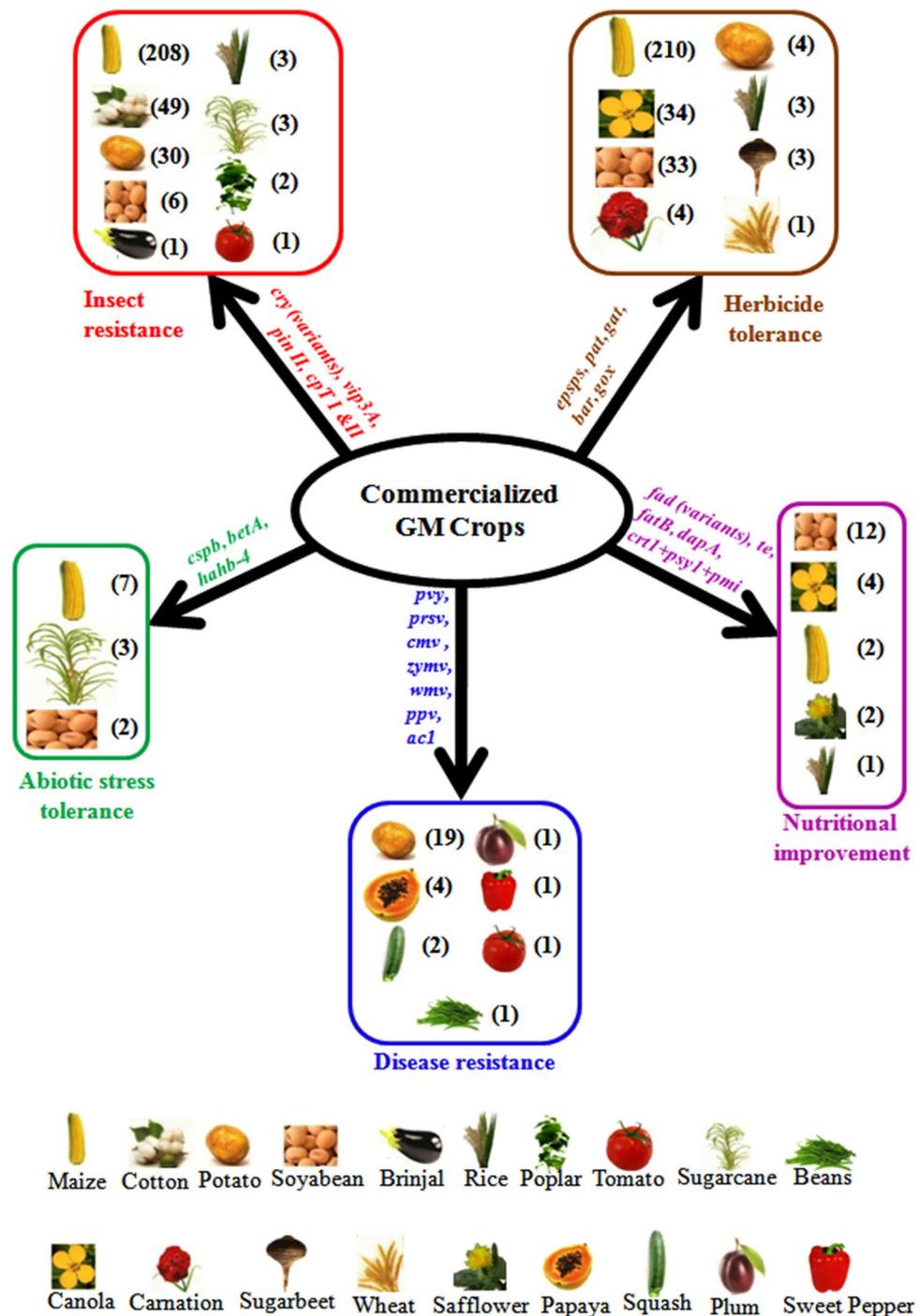
Insect-resistant transgenic crops

Insect pests and diseases cause severe crop loss. There are about 67,000 species of insects that cause losses to economically important crops. They damage crops by sucking sap or chewing plant parts like leaves, stems, and roots. Besides, insects also act as carriers of various plant pathogens which are transferred to plants at the time of feeding (Rahman et al. 2012). For the control and management of insect

pests, farmers rely on expensive chemically synthesised insecticides. This method of crop protection is not environment friendly and imparts economic burden on the farmers. Therefore, to overcome these drawbacks of insecticide use, newer technologies such as genetic modification of crops to enhance their resistance against insects have gained popularity. So far, ten insect-resistant transgenic crops have been commercialised for cultivation. Majority of these commercialised crops have been transformed with insecticidal genes (most commonly different variants of *cry* gene, and in a few events *vip* gene) which control the harmful insects attacking crops (Kereses et al. 2008). Insect-resistant transgenic crops have the second largest area under cultivation—23.3 million hectares in 2017 (ISAAA 2017). In all, 304 events have been approved worldwide for cultivation. Out of this, 208 events comprising various IR genes in maize have been approved for cultivation depending upon the prevalence of insect pests. Other commercialised crops having various IR genes are cotton (49 events), potato (30 events), soybean (6), rice (3), sugarcane (3), poplar (2), brinjal (1), and tomato (1) (Fig. 2). A summary of commercially released insect-resistant (IR) transgenic crops is presented in Supplementary Table 1.

The *cry* genes from soil bacteria *Bacillus thuringiensis* (Bt) are amongst the few highly exploited genes for developing insect-resistant transgenic crops. The *cry* genes produce Cry protein, which forms crystalline inclusions in bacterial spores. The Cry protein imparts insecticidal activities to *B. thuringiensis*. The Cry toxin fragment consists of three domains. The first causes pore formation; the second facilitates receptor binding and the third is instrumental in protecting the toxin from proteases. The toxin binds to specific receptors and gets inserted in the cell membrane of the epithelial cells lining the insect midgut. Upon binding to the receptor, the domain I gets inserted into the membrane leading to pore formation eventually leading to insect paralysis and death. *Cry* genes from different isolates of *B. thuringiensis* provide resistance against wide variety of insect pests, i.e. Lepidopterans, Coleopterans and Dipterans (McPherson et al. 1988; Yamamoto and McLaughlin 1981). Many variants of *cry* genes have been reported and used in gene stacking to confer stable insect resistance. An added advantage of using the *cry* genes is the non-toxicity of the Cry protein to mammals. Cotton was the first commercially successful crop in which *cry* genes were incorporated to provide resistance against lepidopteron insect pest (Perlak et al. 1991). After the success of transgenic cotton, *cry* genes have been incorporated in many crops, viz., potato (Adang et al. 1993), rice (Fujimoto et al. 1993; Wunn et al. 1996), canola (Tabashnik et al. 1993; Stewart et al. 1996; Ramachandran et al. 1998; Halfill et al. 2001), soybean (Parrott et al. 1994; Dufourmantel et al. 2005; Dang and Wei 2007), maize (Koziel et al. 1993; Vaughn et al. 2005; Gassmann et al. 2011), chickpea

Fig. 2 Diagrammatic representation of commercialised transgenic (GM) crops with various improved traits viz., insect resistance, herbicide tolerance, nutritional improvement, disease resistance and abiotic stress tolerance. Numbers in parentheses represent numbers of commercialised transgenic events in the particular crop. The trait-wise major genes employed for GM crop development are given beside the arrows. *cry* (variants)—crystal proteins; *vip3a*—vegetative insecticidal protein; *pin II*—proteinase inhibitor II; *pin I* and *pin II*—Cowpea trypsin inhibitor; *esps*—5-enolpyruvylshikimate-3-phosphate synthase; *pat*—phosphinothricin acetyltransferase; *gat*—glyphosate acetyltransferase; *bar*—bialaphos resistance; *gox*—glyphosate oxidoreductase; *fad* (variants)—flavin adenine dinucleotide; *te*—thioesterases; *fatB*—dapA-dihydrodipicolinate synthase; *crtI*—Calreticulin; *psyl*—phytoene synthase; *pmi*—phosphomannose isomerase; *pvv*—coat protein of *potato virus Y*; *prsv*—coat protein of *papaya ring spot virus*; *cmv*—coat protein of *cucumber mosaic virus*; *zymv*—coat protein of *zucchini yellow mosaic virus*; *wmv*—coat protein of *watermelon mosaic virus*; *ppv*—coat protein of *plum pox virus*; *Ac1*—encoding viral replication protein (Rep) from *bean golden mosaic virus*



(Sanyal et al. 2005; Indurker et al. 2007; Acharjee et al. 2010; Mehrotra et al. 2011), alfalfa (Tohidfar et al. 2013), and tomato (Mandaokar et al. 2000; Kumar and Kumar 2004; Koul et al. 2014). Apart from *cry*, other insecticidal genes such as *vip* genes which encode vegetative insecticidal proteins have been deployed in commercialised crops. The *vip* genes were isolated from *Bacillus* species (*B. thuringiensis* and *B. cereus*) (Fang et al. 2007). To date, *vip3A(a)* and *vip3Aa20* genes have been heterologously expressed in

cotton and maize, respectively (Supplementary Table 1 and ISAAA database 2019).

Beside *cry* and *vip* genes, protease inhibitor (PI) encoding genes from different sources, such as plants, bacteria and fungi, have also been used to engineer plants for insect resistance. Protease inhibitors are naturally occurring defense-related proteins in plants which are released in response to any physical injury or insect attack. Protease inhibitors work by inhibiting the proteolytic enzymes present in the

insects and their larvae gut, thus rendering the insect unable to acquire amino acids necessary for its growth and development (Broadway and Duffey 1986). Protease inhibitors like trypsin inhibitor (encoded by the gene *CpTI*) (Hilder et al. 1987) and potato protease inhibitor II (encoded by the gene *pinII*) (Duan et al. 1996; Majeed et al. 2011) inhibit insect digestive enzymes. The *cptII* and potato protease inhibitor II gene have been utilised to provide resistance against insects in tobacco, and rice and cotton, respectively. To the best of our knowledge, there are only three commercially approved events in which protease inhibitor encoding genes were utilised to confer resistance against a wide range of insect pests, and these are *cptI* gene from *Vigna unguiculata* introduced in cotton, *api* gene (encoding the Arrowhead Protease Inhibitor) from *Sagittaria sagittifolia* introduced in poplar and *pinII* gene from *Solanum tuberosum* introduced in maize (ISAAA database 2019).

Abiotic stress-tolerant transgenic crops

A plethora of environmental factors referred to as abiotic stresses, such as drought, heat, cold, flooding, salinity, etc., exert a negative impact on growth and development of crop plants, leading to reduction in grain yield (reviewed by Suzuki et al. 2014). With the ever-changing climatic conditions, the impact of these abiotic stresses is believed to be increasing (Tuteja and Gill 2014). To cope up with abiotic stresses, plants alter their metabolism in many ways, such as by activating signalling cascades and regulatory proteins (for example, transcription factors and heat shock factors), activating/modifying antioxidant defence system to maintain cellular homeostasis, synthesising and accumulating compatible solutes (polyamines, sugars, betains, proline, etc.) which assist in osmotic adjustment, etc. (Rizhsky et al. 2004; Gao et al. 2007; Koussevitzky et al. 2008; Gill and Tuteja 2010; Rasmussen et al. 2013; Gupta et al. 2015; Onaga and Wydra 2016; reviewed by Raza et al. 2019). These adaptive changes in plants in response to abiotic stresses, in turn, help in minimising the adverse effects on plants by maintaining the near-optimal conditions for plant growth and development. At the molecular level, abiotic stresses cause alterations in expression of an array of genes. Therefore, abiotic stress adaptation requires interplay of many gene networks. Due to complexity of the trait, lesser number of events conferring abiotic stress tolerance has been commercialised as compared to traits like herbicide, insect and disease resistance. To date, seven, three and two events pertaining to abiotic stress tolerance have been commercialised in maize, sugarcane and soybean, respectively (ISAAA database 2019) (Fig. 2).

Castiglioni et al. (2008) demonstrated the use of bacterial cold shock proteins (*csp*) towards mitigation of the effects of abiotic stresses, such as cold in Arabidopsis; cold, heat and

water-deficit in rice; and water-deficit in maize. In this study, *cspA* gene from *E. coli* and *cspB* gene from soil bacterium, *B. subtilis*, were used. Further, it was demonstrated that the use of cold shock proteins for transgenic plants did not lead to pleiotropic effects. The transgenic maize displayed normal phenotype under well-watered conditions, and showed better adaptation during water-scarce conditions. The cold shock proteins used are a class of bacterial RNA chaperones. Like the protein chaperones, RNA chaperones resolve the misfolded RNA structures into stable forms. Hence, they contribute towards maintaining cellular functions during dehydration stress conditions by maintaining RNA stability and protein translation (Castiglioni et al. 2008). Karlsson et al. (2002) reported the presence of a homolog of *E. coli* CSPA in wheat (*Triticum aestivum*). The said homolog, WCSP1, was found to have two RNA-binding domains and the concentration of the protein was found at elevated levels upon cold treatment. Similarly, GRP2, an RNA-binding protein from Arabidopsis (*Arabidopsis thaliana*), has been reported to have dual role in adaptation to cold and salt stress (Kim et al. 2007). Kim et al. (2013a, b) demonstrated the use of the cold shock protein, Csp3, for salt and drought tolerance. The CspB protein has been used in Monsanto's drought-tolerant transgenic maize hybrids commercialised as Genuity® DroughtGuard™ (MON 87460 event) launched in the United States in 2013 (James 2013). Other six events in maize have been created involving stacking of herbicide-resistance and/or insect-resistance events with drought stress tolerance (ISAAA database 2019). The drought-tolerant maize thus developed showed appreciable reduction in water loss via transpiration under stress conditions thereby reducing the requirement of water for the plant. This variety aimed to solve two of the most devastating problems in the Sub-Saharan Africa region, viz., drought and insect pest by developing stacked drought-tolerant and insect-resistant maize (James 2013, 2015). In 2017, transgenic maize with stacked drought tolerance and insect resistance (*Bt*) were planted for demonstration by limited number of smallholder farmers and promising results were found (ISAAA 2017).

Apart from chaperones, transcription factors (TFs) have also been used successfully for imparting abiotic stresses tolerance. One such class of TFs is the homeodomain-leucine zipper (HD-Zip) class of TFs, which are unique to plants. HD-Zip TFs possess highly conserved homeodomain (HD) and leucine zipper (Zip) motifs (Ariel et al. 2007; Perotti et al. 2017). These transcription factors have been shown to interact with abscisic acid-regulated developmental networks, which potentiate linkage of environmental dynamics to gene expression. For instance, *Hahb-4*, a *Helianthus annuus* (sunflower) homeobox-leucine zipper gene, is strongly and reversibly induced by water-deficit conditions and binds to *cis*-elements of genes regulated by dehydration (Gago et al. 2002). Over-expression of this TF driven by a

constitutive or its own promoter has been shown to impart drought tolerance (Dezar et al. 2005) and improves yield in control as well as in stress conditions (Chan et al. 2013a, b). An event with heterologous expression of sunflower *Hahb-4* in transgenic soybean has been approved for cultivation in Argentina in 2015 and in USA and Brazil in 2019 under the name Verdeca HB4 soybean (<https://www.isaaa.org/>). The transgenic HB4 soybean has demonstrated up to 14% yield increase under drought and low-water conditions during multi-location field trials for six seasons in Argentina and the USA.

Sugarcane is the other drought-tolerant transgenic crop approved for commercial cultivation in Indonesia in 2013. Three transgenic events have been approved which were created using *betA* gene from *E. coli* and *Rhizobium meliloti* (<https://www.isaaa.org/>). The *betA* gene codes for choline dehydrogenase, which catalyses formation of the osmoprotectant compound glycinebetaine, which aids adaptation to water stress (Takabe et al. 1998; Khan et al. 2009; Singh et al. 2015). It has been shown that accumulation of osmoprotectant or compatible solutes like non-reducing sugars (fructan, trehalose mannitol, and sorbitol), proline and glycinebetaine help plants to survive under osmotic stress (Chen and Murata 2002; Singh et al. 2015). These osmoprotectants may have a role in protecting cell membrane and maintaining its osmotic potential. Glycinebetaine (*N,N,N*-trimethyl glycine) is considered as the most compatible solute. Increase in its level help to stabilise enzymes and protein structures, and maintain integrity of the cell membrane during environmental stress condition (Nahar et al. 2016). These transgenic sugarcane plants can withstand water stress conditions up to 36 days (James 2015) and produce 10–30% higher sugar than the non-transgenic plants under drought conditions in field trial (Waltz 2014).

Disease-resistant transgenic crops

Diseases caused by pathogens, such as nematodes, fungi, bacteria and viruses, cause extensive loss in the crop yield. Plant diseases are often managed through application of agrochemicals. However, the environmental hazards caused by the use of agrochemicals warrant exploration of alternative strategies to tackle plant diseases. Further, there are possibilities of development of chemical-resistant pests due to indiscriminate use of chemicals. To overcome the challenges posed by plant pathogens, it is important to develop inherent disease resistance in crop plants. This requires delineating the genes responsible for disease resistance and transferring the same to plants through breeding or biotechnological approaches. So far, 29 transgenic events pertaining to resistance to various diseases have been commercialised globally; of which, 25 events confer resistance against viruses. Most events for disease-resistance trait have been reported

in potato (19 events) followed by four events in papaya, two events in squash and one event in bean, plum, sweet pepper and tomato each (Fig. 2). Most of the virus-resistant transgenic crops have been developed via gene silencing techniques, such as co-suppression/RNAi and antisense RNA targeted against viral genes (Tricoli et al. 1995; Yan et al. 2007). Four different transgenic approaches that have been employed successfully for developing virus resistance are expressing viral coat protein (*cp*) gene to confer resistance through "pathogen-derived resistance" mechanism; expressing defective viral replicase and/or helicase domain to confer resistance through gene silencing mechanism; expressing sense and antisense RNA strands of viral replication protein (Rep); and use of antisense RNA to degrade mRNA coding for an important viral protein.

In one event commercialised in squash, resistance was conferred against three viruses, viz., cucumber mosaic virus, zucchini yellow mosaic virus and watermelon mosaic poty virus 2 by expressing the viral coat protein (*cp*) gene as transgene (Marc and Dennis 1995). In papaya, the transgenic technology has been successfully used for conferring resistance against papaya ring spot virus (PRSV). Coat protein of PRSV was expressed to impart resistance against the virus through the pathogen-derived resistance mechanism and the event was commercialised under the trade name Rainbow, SunUp (Ferreira et al. 2002). An event was created and commercialised in deciduous tree, *Prunus domestica* using the coat protein of plum pox virus (PPV) (Ravelonandro et al. 1997; Malinowski et al. 2006). Similarly, resistance against cucumber mosaic virus (CMV) has been shown to be imparted in sweet pepper (Zhu et al. 1996) and tomato (Yang et al. 1995) via expressing viral coat protein. Amongst the examples of technologies using defective replicase gene for providing viral resistance, in one of the studies, the replicase (*rep*) gene of PRSV was used to generate virus-resistant plants and commercialised in an event named as Huanong No. 1 papaya (Guo et al. 2009). Besides, using sense and antisense RNA molecules against viral replicating protein (Rep), an event reported to confer resistance against bean golden mosaic virus in *Phaseolus vulgaris* was commercialised (Faria et al. 2006). In potato, out of 19 disease-resistant commercialised events reported, 18 are stacked either with insect-resistance (IR) or modified product quality trait. The disease-resistance and IR traits were stacked via expressing *cry3A* gene alongwith either potato virus Y (PVY) coat protein (*cp*) gene or gene encoding replicase (*plrv_orf1*) and helicase (*plrv_orf1*) domain of the potato leaf roll virus (PLRV) (Kaniewski et al. 1990; Thomas et al. 2000). Some multi-trait stacked events impart resistance against foliar leaf blight along with fewer black spots, decreased accumulation of reducing sugars and lower free asparagine content. A gene implicated in late blight resistance, *rpi-vnt1* from *Solanum venturii*, was used to confer resistance for foliar late blight

disease (Foster et al. 2009) and these events were commercialised under the names Innate® Acclimate and Innate® Hibernate (ISAAA database 2019).

Nutritionally improved transgenic crops

Provitamin A biofortified rice

Vitamin A deficiency (VAD) is a major public health concern and has been estimated to affect nearly one-third of preschool aged children and 15% pregnant women globally in 2005 (WHO 2009). The highest frequency of VAD in children is in developing parts of world, such as sub-Saharan Africa (48%) and South Asia (44%). The precursor molecule required for vitamin A biosynthesis is beta (β)-carotene, which does not occur naturally in edible parts of staple food crops such as rice. To combat vitamin A deficiency, transgenic rice enriched with provitamin A in its endosperm by engineering pathway for β -carotene biosynthesis was developed (Ye et al. 2000). This engineered rice was named as ‘Golden rice’ due to its yellow colour. Two foreign genes—the *psy* gene encoding for phytoene synthase from daffodil and the *crtI* gene encoding for carotene/phytoene desaturase from bacterium, *Erwinia uredovora*, which in turn, reconstitute the carotenoid biosynthetic pathway within the rice endosperm were introduced in the *japonica* rice cultivar Taipei309 (Ye et al. 2000). In this breakthrough study, up to 1.6 $\mu\text{g/g}$ total carotenoid accumulation was reported. However, it was too low an amount to solve the VAD problem. Later, Syngenta introduced the two transgenes- *psy* and *crtI* under the control of an endosperm-specific promoter in the American rice variety Cocodrie and developed Golden Rice 1 (GR1) which accumulates upto 6 $\mu\text{g/g}$ carotenoid in endosperm (Al-Babili and Beyer 2005). It was hypothesised that the *psy* transgene was the limiting step to achieve high carotenoid accumulation. Therefore, in 2005 Syngenta introduced maize *psy* gene (which has much higher activity than its daffodil orthologue) along with the bacterial *crtI* gene into the American rice variety Kaybonnet and produced a new version of engineered rice which was named as ‘Golden Rice 2’ (GR2) (Paine et al. 2005). Expression of both the transgenes in GR2 was driven by endosperm-specific glutelin-1 promoter from rice. The endosperm of GR2 plants accumulates up to 37 $\mu\text{g/g}$ (9–37 $\mu\text{g/g}$) total carotenoid which is about 23 times more than the original golden rice and 84% of which is β -carotene (Paine et al. 2005). Six GR2 events developed by Syngenta have been made available for use in public sector breeding programs by Humanitarian Board on Golden Rice. Recently, International Rice Research Institute, Philippines introduced maize *psy1* and *Pantoea ananatis* bacterium *crtI* genes into rice and the variant was named as Golden Rice 2E (GR2E). This transgenic rice also possesses the phosphomannose isomerase

(*pmi*) gene from *Escherichia coli* strain K-12 which acts as a selectable marker allowing transformed rice cells to grow on mannose as a carbon source. In 2017–18, one event of this provitamin A biofortified rice line GR2E was approved for use as food in Australia, New Zealand, Canada and the United States under the Golden Rice trade name (ISAAA database 2019) (Fig. 2).

Modified oil/fatty acid

Metabolic engineering of oilseed crops through the transgenic approach has been utilised extensively for improving the nutritional qualities of seed oil such as altering the endogenous fatty acid composition to make it free of trans-fats for health benefits and for increasing the shelf life of oils. Oils with low saturated fatty acid content and having a higher proportion of polyunsaturated fatty acids (PUFAs) are considered to be better for human consumption (WHO 2008; FAO 2010). Examples of such oils include oils derived from fish, walnuts, flaxseeds, sunflower, safflower, soybean and corn. Dietary replacement of saturated fats with polyunsaturated or monounsaturated fats is believed to be beneficial for the heart as it reduces the levels of low-density lipoproteins (LDLs) also referred to as “bad” cholesterol and triglycerides in the blood. It has been shown that when long-chain triglycerides (LCTs) are substituted with medium-chain triglycerides (MCTs) in the diet, it results in increase of basal metabolic rate, storage of less adipose tissue (Geliebter et al. 1983; St-Onge and Jones 2003). Oils are also one of the major sources of essential fatty acids, viz., oleic acid, linoleic acid and alpha-linolenic acid. Besides, higher content of monounsaturated fatty acids (MUFAs) results in improved stability and flavour. To date, 18 transgenic events with modified lipid content from three oilseed crops, viz., Argentine canola (4 events), safflower (2 events) and soybean (12 events), have been commercialised (ISAAA database 2019) (Fig. 2; Supplementary Table 2).

The U.S. Department of Agriculture (USDA) has approved Vistive Gold® soybean developed by Monsanto which has <3% linolenic acid content in its seed oil, vis-a-vis 8% for traditional soybeans. A lower linolenic acid content oil is more stable and hence requires less hydrogenation leading to a concomitant reduction in trans-fatty acids. Recently, *Camelina sativa* has been engineered with genes from marine microbes to produce high levels of omega-3 long-chain polyunsaturated fatty acids (PUFAs) like eicosa-pentaenoic acid (EPA) and docosahexaenoic acid (DHA) similar to those found in fish oil (Ruiz-Lopez et al. 2014; Usher et al. 2017). The omega-3 fatty acids are considered good, for their well-known role in brain development and reducing risk for cardiovascular disease keeping the heart healthy. Similarly, Argentine Canola has been genetically transformed for higher production of the omega-3 fatty

acid—DHA. This transgenic canola was developed by introducing seven genes sourced from yeast and marine microalgae, which are involved in the metabolism of long-chain polyunsaturated fatty acids (with 20 or more carbons). In 2018, Nuseed Pvt Ltd has been granted regulatory approval to release this DHA-enriched canola for commercial cultivation in Australia (Supplementary Table 2).

Essential Amino Acids

Humans and animals cannot synthesise certain amino acids and should obtain them through diet. Amongst the essential amino acids, lysine (Lys), tryptophan (Trp) and methionine (Met) are particularly relevant for biofortification as they are not abundantly found in cereals (Lys and Trp) and legumes (Met). In the previous decade, a few transgenic approaches targeted altering the amino acid composition of plant proteins with an aim to engineer essential amino acid metabolic pathways and increasing the content of particular essential amino acid(s) for nutritional enhancement. Transgenic wheat and rice have been developed by heterologously expressing lysine-rich pea legumin protein in the endosperm (Sindhu et al. 1997; Stoger et al. 2001). Another success was achieved by engineering a seed storage protein from *Amaranthus hypochondriacus* in cereals. This protein is rich in all the essential amino acids needed by humans. In Rascón-Cruz et al. 2004 and others developed transgenic maize expressing AH protein in its seeds. These seeds were found to have up to 32% more protein content than the wild-type seeds and with larger amounts of the essential amino acids, such as lysine, tryptophan and isoleucine. Similarly, Bicar et al. (2008) developed transgenic maize by heterologously expressing lysine-rich animal proteins, i.e. porcine α -lactalbumin that increased the lysine content by 47%.

So far, two transgenic events with modified amino acid trait have been commercialised in maize (Fig. 2). These events employed the *cordapA* gene from *Corynebacterium glutamicum* to increase free lysine content in maize kernels via embryo-specific expression of the transgene. The *cordapA* gene encodes a dihydrodipicolinate synthase (DHDPS) enzyme which catalyses the first committed step of lysine biosynthesis pathway in plants and bacteria (Azevedo and Lea 2001). In plants, DHDPS is inhibited by lysine through feedback inhibition, and hence, it acts as the rate-limiting step in lysine production (Galili 1995). The DHDPS enzyme isolated from bacteria has > 50-fold lower sensitivity than the plant enzyme, i.e. it is insensitive to feedback inhibition, which allows the synthesis of lysine even in the presence of high lysine content (Cremer et al. 1988; Vauterin et al. 2000). Out of the two commercialised events in maize, one is stacked event having more lysine production and insect-resistance (*cry1Ab* gene) trait (ISAAA database 2019).

Concerns associated with the transgenic crops

The global adoption of transgenic crops in past two decades has delivered economic and environmental benefits in terms of increased crop yield, reduced chemical insecticide and herbicide applications, reduced CO₂ emission, increased farmer income and improved consumer health (Klumper and Qaim 2014; Zhang et al. 2016; ISAAA 2017; Brookes and Barfoot 2017 and 2018). However, some concerns have been raised about these crops being ecologically hazardous and potentially unsafe for human consumption. There are five principal concerns against transgenic crops, which are briefly discussed below.

Biosafety issues related to transgenic crops

Concerns have been expressed regarding the safety of transgenic food in terms of potential threat to human health (toxicity and allergenicity) and environment (possibility of transgene flow into environment and adverse effect on biodiversity) (Mertens, 2008; Suzie et al. 2008; Lovei and Bøhn 2010). The issue of potential health risk of toxicity and allergenicity associated with transgenic crops has always been controversial. For instance, *Cry9c* expressing ‘Starlink’ maize has been approved for animal feed and industrial use in the USA in 1998 but is not approved for human consumption owing to its possibility for being allergenic to humans due to high stability of the protein (Bucchini and Goldman 2002) and the potential to interact with immune system. Later, this Lepidopteron insect-resistant maize named ‘Starlink’ was recalled worldwide by Aventis in 2000 owing to detection of residues of the Cry protein in food products (EPA 2017). However, direct link could not be established between *Cry9c* and allergic reactions in consumers.

The results of one of the most controversial and criticised studies (Serralini et al. 2012) have claimed potential health hazards, viz., high tumour incidences, chronic kidney disease, increased liver congestion and necrosis in males, and increased female mortality in rats fed with transgenic NK-603 Roundup Ready maize. Since its publication, this study has been subjected to heavy criticism from the scientific community, popularly known as the ‘Seralini affair’, due to flawed experimental design, and inappropriate statistical analysis which led to eventual retraction of this article. In 2014, the same group republished a nearly similar work in an expanded form, and stressed the need for conducting long-term feeding trials to thoroughly evaluate the safety of transgenic crops (Sereralini et al. 2014). With only a few exceptions, most of the studies from the past decade pertaining to health hazards of transgenic crops, such as maize, rice, wheat and soybean on rodents, poultry, pigs, frogs, cows, and monkeys, have not reported adverse effects of a diet comprising transgenic food on animal health (as reviewed by

Domingo 2016; Tsatsakis et al. 2017; De Vos and Swanenburg 2018). Further, concern has also been raised about the possibility for horizontal transfer of antibiotic-resistant marker genes from transgenic food to animal and human gut microbes which may result in antibiotic resistance in the gut microflora (Netherwood et al. 2004; Heritage 2004; Keese 2008). However, the possibility of this type of gene transfer is extremely low. Moreover, to address this issue, recent efforts have focused on developing marker-free transgenic crop plants (Tuteja et al. 2012).

The issue related to adverse effects on the environment includes the pollen-mediated transgene flow, which has been reported from transgenic crops to conventional cultivars as well as to wild relatives of many crops, like rice, maize, cotton, barley, beans, creeping bent grass and rapeseed (Chen et al. 2004; Watrud et al. 2004; Ford et al. 2006; Han et al. 2015; Yan et al. 2015). The potential introgression of a transgene from transgenic crops to their wild relatives would pose the risk related to loss of biodiversity whilst gene flow to weedy relatives or weeds might have detrimental effects, such as advent of new weed species and appearance of herbicide-resistant weeds referred as 'superweeds'. For instance, out of 24 glyphosate-resistant weed species, so far, 16 have been reported from transgenic cropping systems (Heap 2014). Amongst them *Conyza canadensis* is the most widespread weed plant whilst *Amaranthus palmeri* and *Amaranthus tuberculatus* are the two most economically damaging glyphosate-resistant weeds globally (Heap and Duke 2018).

Resistance breakdown

Extensive cultivation of transgenic IR and HT crops may increase chances of resistance development in the targeted insect population and weeds, respectively due to high selection pressure. The high selection pressure may potentially lead to evolution of new insect biotypes and can potentially result in the emergence of a superweed having resistance against the transgenic technology (Bawa and Anilakumar 2013; Gilbert 2013). For instance, field-evolved pest resistance to *Bt* maize has been reported for three major pest species, viz., *Busseola fusca* (African stem borer) in South Africa to *cry1Ab* expressing corn (Rensburg 2007), *Spodoptera frugiperda* (fall armyworm) in Puerto Rico to *cry1F* expressing corn (Storer et al. 2010) and *D. virgifera virgifera* (western corn rootworm) in USA to *cry3Bb* expressing corn (Gassmann et al. 2011). Similarly, field-evolved pest resistance to *Bt* cotton has been reported for two pest species; *Pectinophora gossypiella* (pink bollworm) in India to *cry1Ac* expressing cotton (Bagla 2010) and *Helicoverpa zea* (cotton bollworm) in USA to *cry1Ac* and *cry2Ab* (Luttrell et al. 2004; Tabashnik et al. 2008; Tabashnik and Carrière 2010). Further, an analysis of 77 studies confirmed field-evolved

pest resistance to *Bt* crops for these 5 out of 13 major pest species (Tabashnik et al. 2013). The fall armyworm resistance to *Bt* maize in Puerto Rico was reported in the shortest time, i.e. just after 3 years which prompted withdrawal of the crop. Nonetheless, these few examples notwithstanding, experience with *Bt* crops have demonstrated that resistance in crops can remain effective against most pests even after a decade (Tabashnik et al. 2008; Carrière et al. 2010). Moreover, pyramiding of multiple insect-resistant genes is a strategy which has been successfully utilized to delay the resistance breakdown.

Adverse effects on non-target organisms

Potential unintended effects of transgenic crops on non-target organisms have also been explored in the literature. For example, Losey et al. (1999) reported the higher mortality of monarch butterfly (*Danaus plexippus*) larvae fed on milkweed leaves dusted with the genetically modified *Bt* maize as compared to control under laboratory condition. This report sparked off controversy due to flaw in experimental design and extrapolation of lab assays to fields. Subsequent studies concluded negligible or no adverse effect of *Bt* maize on monarch larvae (Dively et al. 2004; Sears et al. 2001). Besides, reduction in monarch butterfly population has also been reported in Mexico and USA upon adoption of glyphosate-resistant transgenic crops. The decline in breeding habitat due to killing of milkweed plant by increased use of glyphosate was found to be the main reason for reduction in monarch butterfly population (Brower et al. 2012). Furthermore, shift in weed population, such as shatter cane, common water hemp, hemp sesbania, velvetleaf, nut edge and night shade, has also been reported due to continuous usage of glyphosate (Mertens 2008). It is possible that killing of the major pests may allow secondary pests to take over as major pest. For instance, it has been reported that wide-scale adoption of *Bt* cotton in China led to increase in population size of a previous minor pest, mirid bug, which in turn, acquired major pest status in past decade (Lu et al. 2010).

Cost for commercialization

One of the major limiting factors for development and deployment of transgenic crop products is the high cost of safety assessment and lengthy as well as complex regulatory approval process required for commercialization (Davison 2010; Miller and Bradford 2010). McDougall (2011) estimated that the average cost for regulatory safety assessment, securing global registration and authorizations was US\$ 35.01 million. It has further been estimated that the complete process from the initiation of a transgenic crop development project to its commercial launch was about 13 years. The

safety evaluations and obtaining regulatory approval account for the longest phase in transgenic product development and commercialization process (McDougall 2011). Smart et al. (2017) estimated that the mean approval time for these crops to pass through the regulatory pipelines of the European Union (EU) and the United States (US) was about 5 years (1763 days) and 7 years (2467 days), respectively. Owing to high resource intensive and time-consuming regulatory approval process, transgenic crop development and commercialization are not affordable by small companies and public institutions, which in turn, acts as a bottleneck for entry of small biotechnology firms into this sector.

Oligopoly of multinational companies

The genes used for development of crop with improved traits are patentable thus limiting the access of useful genetic elements to others. Most of the commercialised GM crop events are developed and patented by a few multinational companies (MNCs). This owes, in part, due to the tremendous cost associated with research, development and regulatory process. The big five MNCs namely, Monsanto, Syngenta, Bayer CropScience, Dupont and Groupe Limagrain account for about 70% of GM seed market (Business Wire 2016). Further, these five MNCs are acquiring, merging and forming joint ventures with competing smaller firms which might reduce the competition in this industry. Therefore, farmers may have fewer choices due to dominance of a handful of companies in the seed market which, in turn, raise the concern about possible exploitation of farmers by charging higher price for GM seeds.

Beyond traditional transgenic technology: alternative technologies for generating improved crop plants

Owing to public concern and lesser consumer acceptance of transgenic crops in many parts of the world, a set of alternative technologies have been utilised for developing improved crops in the recent years. These new alternative methods, such as cisgenesis, intragenesis and the most recent one, genome editing, aim to eliminate various public concerns and uncertainties associated with the transgenic technology in the past. These techniques make use of a genetic modification step, but the end products (modified crop genome) can be so designed that they do not contain any foreign gene (transgene). Therefore, crop plants developed using such techniques are genetically similar to plants developed through breeding. Importantly, these techniques can also be used to develop improved crop plants which are otherwise difficult to obtain through the traditional breeding methods. A comprehensive updated picture on the current status of

the crops developed by employing such alternative methods is given below.

Cisgenesis and intragenesis

Cisgenesis technique implies genetic modification by introducing a copy of complete natural gene sequence including intron(s) as well as its own regulatory elements (native promoter and terminator) in the sense orientation. The source of the desired cisgene is the crop species itself or a sexually compatible plant species (Fig. 1) as used in conventional breeding (Schouten et al. 2006). However, unlike conventional plant breeding, cisgenic crops contain the desired gene(s) only and no undesired genetic elements. Successful deployment of cisgenesis has been reported for imparting improved trait in crop plants, like late blight resistance in potato (Haverkort et al. 2009), scab resistance in apple (Vanblaere et al. 2011), and high phytase activity in barley (Holme et al. 2012). To the best of our knowledge, no cisgenic event has been commercialised so far.

Intragenesis involves introducing desired gene(s) originated from the same crop species or sexually compatible plant species in the sense or antisense orientation. However, the regulatory elements, such as promoter and terminator, could be from other genes, i.e. not from the same gene thus giving novel genetic combination (Fig. 1) (Rommens et al. 2007). This can lead to change in expression pattern of desired gene which may be driven by regulatory elements from other genes. Intragenesis has been used successfully for tuber-specific silencing of asparagine synthase-1 (*StAs1*) gene in potatoes with the aim to reduce acrylamide (potential carcinogen) levels after processing. The field trial results reported up to 70% reduction in acrylamide forming potential of potatoes without affecting the tuber shape and yield (Chawla et al. 2012). Recently, J.R. Simplot Co. has developed intragenic potato exhibiting multiple traits, such as low acrylamide, resistance to bruising and discoloration (Waltz 2015). This potato was created by tuber-specific silencing of four genes, i.e. *asn1* (asparagine synthetase 1), *ppo5* (polyphenol oxidase 5), *PhL* (α-glucan phosphorylase), and *R1* (starch-related R1 protein). Silencing of *asn1* lowers asparagine formation which results in reduced potential for acrylamide formation whilst cooking at high temperature. Silencing of *ppo5* imparts reduced black spot bruise development characters whilst silencing of *PhL* and *R1* leads to less reducing sugar accumulation which further contributes to reduced acrylamide formation. Various events of the improved potato developed using intragenesis have been commercialised under different trade names, like Innate® Cultivate, Innate® Generate, Innate® Accelerate and Innate® Invigorate (ISAAA database 2019).

In 2012, the European Food Safety Authority (EFSA) GM organism panel assessed the risk associated with cisgenic

and intragenic plants *vis-à-vis* transgenic plants and conventionally bred plants. The panel concluded that potential risk associated with cisgenic plants is similar to traditionally bred plants, whilst for intragenic and transgenic plants, novel potential hazards are possible (EFSA 2012). The US Department of Agriculture (USDA) considers the cisgenic and intragenic plants as non-regulated (exempted from Biotechnology/GM organism regulation) provided that the introduced genetic elements are not derived from “plant pests”. Recently, intragenic tomato lines resistant to tomato spotted wilt virus (TSWV) and cauliflower mosaic virus (CMV) developed by Nexgen Plants Pty Ltd using RNAi-mediated silencing approach have been de-regulated in the USA (USDA APHIS 2020).

Genome editing

Amongst the genetic modification technologies, genome editing technology is the latest one. Genome editing technologies can be used so that specific gene(s) and/or other genetic elements can be stably mutated, knocked out, or replaced. This technique utilises either a sequence-specific nuclease (SSN) to produce precise gene knockout and knock-in edits, or synthetic oligonucleotides to introduce specific point mutations in the target DNA region (Songstad et al. 2017) (Fig. 1). Synthetic oligonucleotides (RNA/DNA chimeric oligonucleotides or single-stranded DNA oligonucleotide molecule of 20–100 nucleotides) have also been utilised recently for targeted editing such as producing custom single nucleotide polymorphisms (SNPs). This technique is known as Oligonucleotide-Directed Mutagenesis (ODM). In ODM, an oligonucleotide homologous to the target DNA sequence but having desired mismatch(es) is introduced into the plant cells. During cell division, the mismatch(es) between the oligonucleotide and target DNA sequence are recognised by the native repair system of the plant which repair cell's own DNA using the oligonucleotide as a template through homology-directed pairing thus resulting in incorporation of site-specific point mutations/SNPs, typically 1–3 nucleotides, directly into a gene of interest (Sauer et al. 2016). The plant cell having desired mutation can then be selected and regenerated into a full plant through tissue-culture methods.

Apart from ODM, three different variants of SSN are being utilised to carry out genome editing. These SSNs are Zinc-Finger Nucleases (ZFNs), Transcription Activator-Like Effector Nucleases (TALENs), and Clustered Regularly Interspaced Short Palindromic Repeat-associated endonucleases (CRISPR/Cas). SSNs function via binding to a pre-defined specific target DNA sequence in the genome and induce double-stranded breaks (DSBs) at a desired genomic location (Fig. 3). The DSBs are usually repaired either by error-prone endogenous repair mechanism (Non-homologous end joining/NHEJ) or by utilising an externally added

homologous DNA repair template (Homology-directed repair/HDR). This can be utilised to create mutation at the target site, such as gene knockout (insertion/deletion leading to frame shift mutations) via NHEJ or precise targeted sequence replacement/substitution (point mutations or targeted gene replacement or site-specific gene insertion) via HDR (Kim and Kim 2014). These multiple outcomes of DSB repair can be categorised into three types (Fig. 4).

The three classes of SSNs differ in their structure, enzymatic mechanism, mode of action, specificity and efficiency of editing (Fig. 3; Table 1). Amongst the three designer nuclease systems, ZFNs and TALENs were the first-generation targeted genome editing tools (Joung and Sander 2013). Both, ZFN and TALEN are generated by fusing two independent protein domains, i.e. a series of DNA-binding domains [either zinc-finger domains (for ZFN) or TALE domains (for TALEN)] fused with a synthetic *FokI* endonuclease domain. The *FokI* is non-specific/sequence independent endonuclease and functions only as a dimer, thus at least a pair of ZFN or TALEN is required to target any specific locus which, in turn, introduce DSB at a specific target site of the DNA. The DSB induced by a pair of ZFN or TALEN activates endogenous DNA repair pathways, such as HDR or the error-prone NHEJ and hence enable custom alterations at the cleavage site (Miller et al. 2011). One zinc finger domain can recognise a nucleotide triplet (Kim et al. 1996) and ZFN, which is mostly composed of 3–4 zinc finger domains, hence bind sequence of 3–4 nucleotide triplets. The ZFN pair can target 18–24 base pair (bp) and this nucleotide length is enough to target-specific sites in genomes. Theoretically, the target site can be of any length, however the context-dependent assembly of ZFN modules limits the target site size (Sander et al. 2011). Unlike zinc fingers (ZF), each TALE effector (TALE) domain binds a single nucleotide specifically (Christian et al. 2010). TALE is originally derived from the *Xanthomonas* species and composed of 33–35 amino acid repeat. In a TALEN pair, engineered TALE domains are designed to recognise 30–60 nucleotides (15–30 nucleotides per half site). Due to longer target length TALENs are generally considered to bind with greater specificity than ZFNs (Guilinger et al. 2014a, b). The target half-sites (‘left’ and ‘right’) recognised by ZF or TALE dimers are usually separated by 5–7 and 12–21 bp spacer, respectively, which is recognised and cleaved by *FokI* endonuclease (Miller et al. 2007; 2011). The target sites in the genome can be chosen by customising ZFNs (or TALENs) design via selecting appropriate combinations of different zinc fingers (or TALE effector) domains, respectively. ZFN- or TALEN-mediated gene editing techniques have some disadvantages, such as cumbersome protein engineering steps, high costs and are difficult to multiplex.

CRISPR/Cas is the most recently developed SSN-based genome editing technique. CRISPR is evolved from bacterial

Table 1 Comparison between different genome editing methods

	ODM	ZFN	TALEN	CRISPR/Cas
Core component	Chemically synthesised oligonucleotides	Rely on chimeric proteins; Zinc finger (ZF) DNA-binding domains fused with <i>FokI</i> endonuclease domain	Rely on chimeric proteins; TALE DNA-binding domains fused with <i>FokI</i> endonuclease domain	Single guide RNA (sgRNA) and Cas endonuclease; sgRNA is used for target recognition
Binding principle and mode of action	Oligonucleotide binds to the target DNA sequences; target region sequence is modified by mismatch repair system	Protein-DNA; double-strand breaks (DSBs) in target DNA induced by <i>FokI</i>	Protein-DNA; DSBs in target DNA induced by <i>FokI</i>	RNA-DNA complementarity; DSBs (more common) induced by Cas9 or Cas12a, or Single-strand break induced by nCas9 in the target DNA
Recognition module	Watson-Crick base pairing between oligonucleotide and the target DNA with minor mismatches	Each ZF domain recognises 3 base pairs	Each module recognise 1 base pairs	sgRNA and PAM sequence (3–6 bp) upstream (for Cas9) or downstream (for Cas12a) to the target sequence
Functional Unit	Single-stranded oligonucleotide	Dimer	Dimer	Monomer
Design	Simple	Difficult	Less difficult	Simple
Multiplexing to edit several targets	Not easy	Difficult; because multiplexing requires re-engineering of different dimeric proteins for every desired target sequence	Difficult because multiplexing requires re-engineering of different dimeric proteins for every desired target sequence	Easy: sgRNA can be easily adapted for different targets No re-engineering of Cas enzyme required for different targets
Mutation structure	Point mutations (SNPs); typically 1–3 nucleotides	SNPs, short indels (1–10 bp), and gene replacement and insertion	SNPs, short indels (1–10 bp), and gene replacement and insertion	SNPs, short indels, and gene replacement and insertion
Cost	Cheap	Very expensive	Expensive	Cheap
Efficiency of editing	Low	Variable	High	Higher
Length of target	~20–100 bp	~18–24 bp	~30–60 bp	~20 bp + PAM sequence (3–6 bp)

ODM oligonucleotide-directed mutagenesis, *ZFN* zinc-finger nuclease, *TALEN* transcription activator-like effector nuclease, *CRISPR* clustered regularly interspaced short palindromic repeats, *Cas* CRISPR-associated endonuclease, *DSB* Double-strand Break

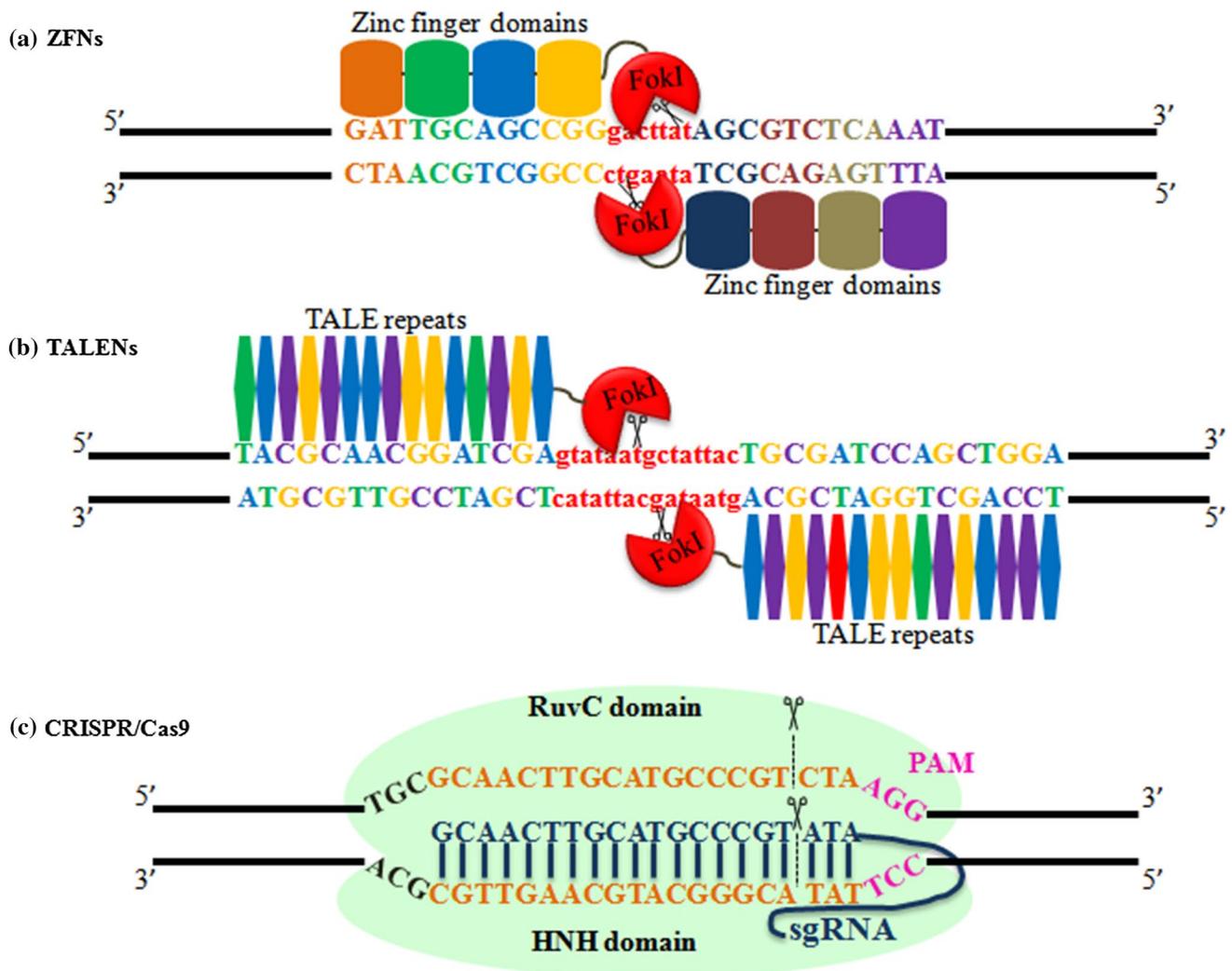


Fig. 3 Illustration of three classes of sequence-specific nucleases (SSNs) utilised for genome editing. **a** Zinc Finger Nucleases (ZFNs): the left and right ZFNs are constituted by fusing series of Zinc Finger (ZF) domains with a non-specific *FokI* endonuclease domain. Each ZF domain recognises specifically three nucleotides while the *FokI* domain cuts DNA strands at different positions thereby introducing sticky double-strand break (DSB) within the spacer sequence (represented by lowercase nucleotides; usually 5–7 bp) outside the left and right recognition sites. **b** Transcription Activator-Like Effector Nucleases (TALENs): the left and right TALENs comprise a series of TALE repeats with a non-specific *FokI* endonuclease domain. Each TALE repeat recog-

nises a single nucleotide whilst the *FokI* domain introduces sticky DSB within the spacer sequence (represented by lowercase nucleotides; usually 12–21 bp) outside the left and right recognition sites. **c** CRISPR/Cas9: this system consists of a single guide RNA (sgRNA) and Cas9 endonuclease. The sgRNA pairs with 20 nucleotide target sequences present in the genomic DNA upstream to the NGG sequence (Protospacer Adjacent Motif, PAM; N means any nucleotide). The Cas9 enzyme contains two catalytic nuclease domains: RuvC and HNH. It generates a blunt-end DSB, three nucleotides upstream of the PAM motif at the target site having complementarity to sgRNA

adaptive immune system that includes clustered palindromic repeats with the Cas endonuclease which protect the bacteria from invading plasmid and viral infection (Jinek et al. 2012). The CRISPR/Cas gene editing system consists of a Cas endonuclease protein (mostly Cas9 from *Streptococcus pyogenes* or Cas12a/Cpf1 from *Francisella novicida* or their variants) and a single guide RNA (sgRNA). The non-coding sgRNA contains 20 nucleotides at the 5' end which directs Cas9 endonuclease to the complementary target site

in the genome. The Cas9 endonuclease has two catalytic domains—RuvC and HNH. The HNH and RuvC nuclease domains cut the strand of genomic DNA complementary to the sgRNA (target strand) and non-complementary to sgRNA (non-target strand), respectively, thereby inducing a DSB in the 20-bp targeted genomic locus specified by the sgRNA (Jinek et al. 2012). To introduce a DSB, a short sequence called the protospacer-adjacent motif (PAM) has to be present. Examples of the PAM include the NGG sequence

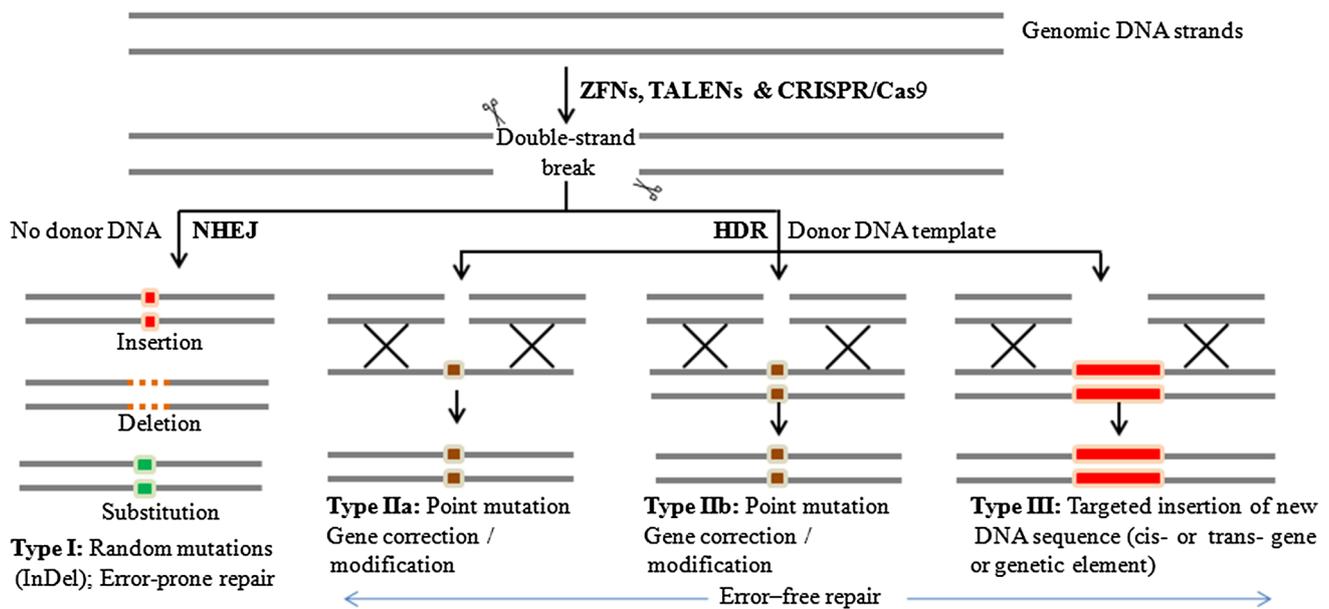


Fig. 4 Potential outcomes of genome editing involving double-stranded breaks (DSBs): the DSBs generated by genome editing tools can be repaired by either error-prone Non Homologous End Joining (NHEJ) or error-free Homology Directed Repair (HDR) mechanisms. Three possible outcomes of editing involving DSBs are: Type I—spontaneous repair of DSB via NHEJ leading to small insertion, deletion and substitution which would result into gene knockout or

change in activity; Type II—single-stranded (Type IIa) or double-stranded (Type IIb) template-guided repair of a targeted DSB via HDR leading to point mutations (precise alteration of the target sequence); Type III—double-stranded template (containing an entire gene or an genetic element)-guided repair of a targeted DSB via HDR leading to targeted insertion of a *cis*- or *trans*-gene or a genetic element

which should be present immediately after the gRNA target sequence on the DNA for Cas9 or the TTTN sequence which should be present upstream to the target sequence for Cas12a (Swartz and Jinek 2018). The small size makes sgRNA design and construction easy and amenable to multiplexing (Ma et al. 2015).

Owing to notable technological simplicity, such as ease of execution, flexibility, efficiency, precision, cost-effectiveness and easy multiplexing compared to the first-generation editing tools, CRISPR technique has been widely used and has virtually revolutionised the field of genome editing (Doudna and Charpentier 2014). Hence, it is often termed as “the biggest biotechnology discovery of the century”. However, the major concern related to CRISPR/Cas technology is the possibility of ‘off-target effects’—non-specific cleavage of untargeted regions in the genome, leading to undesired mutations. To improve specificity and minimise off-target effects, mutated variants of Cas9 endonuclease having higher editing specificity have been developed, such as the hyper-accurate Cas9 variant (HypaCas9) having high fidelity/proofreading (Chen et al. 2017), nCas9 (nickase which creates a nick, i.e. cut only single strand of DNA instead of creating a DSB), and dCas9 (catalytically inactive or nuclease deficient or ‘dead’ Cas9). nCas9 is generated by inactivating one of the catalytic nuclease domains, either RuvC or HNH. nCas9 could be utilised as paired nickase

and DNA modification may still take place via either the NHEJ or HDR mechanism. It has been shown that pairing of nCas9 with two different sgRNAs, each cleaving one strand of DNA, increases the number of specifically recognised nucleotides required for target cleavage, thereby reducing the off-target mutation drastically (~50- to 1500-fold) and improving specificity without compromising the cleavage efficiency (Ran et al. 2013; Mikami et al. 2016). The single nCas9 has been used for highly efficient and precise C to T and A to G base editing in crop plants by fusing it with cytidine deaminase (known as cytosine base editors/CBEs), and with adenosine deaminase (known as adenine base editors/ABEs), respectively, via base excision repair pathway without the generation of DSB (Zong et al. 2017; Li et al. 2018). dCas9 has been developed by mutating both the RuvC and HNH catalytic domains and hence making them non-functional. dCas9 has been used initially for targeted and precise transcription regulation, such as targeted gene activation and repression, by fusing it with transcription activator and repressor domains, respectively (known as CRISPR activator /CRISPRa and CRISPR interference/CRISPRi, respectively) and thereby recruiting them to the promoter (Li et al. 2017; Lowder et al. 2017). In recent years, the CRISPR/dCas9 system has also been utilised for precise epigenome editing, modulation of chromatin topology and DNA-free genetic modification via recruitment of

various chromatin-modifying enzymes (acetyltransferase, DNA methyltransferase) and reporter proteins to DNA target sites (Guo et al. 2015; Hilton et al. 2015; Liang et al. 2017; Veillet et al. 2019; Moradpour and Abdulah 2019). Further, a highly specific and precise genome editing strategy, the dimeric nucleases strategy, comprising dCas9–*FokI* fusion has also been employed as another elegant way to overcome off-targets. The dimeric nuclease strategy has been shown to reduce potential off-target mutations by thousands fold (Guilinger et al. 2014a, b; Tsai et al. 2014).

Recently, a highly accurate ‘search-and-replace’ genome editing system known as ‘Prime editing’ has been developed which enables a variety of highly precise and targeted DNA edits (insertions and deletions, all possible transition and transversion point mutations and combinations thereof) at a wide range of positions without DSB or donor DNA template (Anzalone et al. 2019). The prime editing (PE) complex consists of a PE protein containing an RNA-guided DNA-nicking domain, nCas9, fused to an engineered reverse transcriptase (RT) domain and complexed with an engineered prime editing guide RNA (pegRNA). The pegRNA has a binding region which binds to nicked DNA thereby specifying the target site, and an edited sequence region which serves as a template for the desired edit. The RT enzyme can generate new DNA by copying an RNA template, i.e. edited sequence region of pegRNA (Anzalone et al. 2019). This new versatile editing system has been demonstrated in mammalian cells and to the best of our knowledge, so far there is no report pertaining to use of prime editing in plants. However, considering its advantage, it is expected that the ‘Prime editing’ system would soon be utilised for genome editing in plants as well.

The genome editing methods can be utilised in agriculture for creating new mutations, correcting deleterious DNA mutations, regulating expression of desired gene, replacing faulty gene with the correct copy, swapping of regulatory element, editing regulatory elements, epigenome editing, and site-specific insertion of cis- or trans-genes in the plant genome. The targeted modification introduced in the genome using genome editing technology is often indistinguishable from those introduced via traditional plant breeding or chemical or random mutagenesis (except the Type III approach in Fig. 4). These genome editing methods are much faster than traditional breeding tools and can produce desired modifications either without incorporating any foreign DNA into the genome, or in a few cases; integrated foreign gene expressing the nuclease can be segregated through conventional breeding in the next generation(s) thus producing null segregant line. This approach can generate genetically edited (GED) plants with only the desired modification, and therefore, crop varieties generated through this technology may be similar to non-transgenic crops (Belhaj et al. 2013). However, a pre-requisite for developing non-transgenic GED

crops with new or improved traits is the presence of the target gene or genetic elements within the same crop species, i.e. endogenously, and understanding about the function of the endogenous gene intended to be edited. If the gene associated with the desired trait is not present endogenously in the same genome, then one has to utilise the transgenic approach for introducing the desired trait (for instance, *cry* gene from bacterium used for imparting IR trait in crop). Further, all the improved crops developed via genome editing tools may not be non-transgenic such as the HDR-mediated targeted insertion of a transgene or/and foreign genetic elements at a desired genomic locus by harnessing genome editing methods will be similar to transgenesis (GED plants derived from Type III approach in Fig. 4). Therefore, such type of genome edited crops might be subjected to the same stringent regulatory process that governs traditional transgenic GM crops.

Site-specific nucleases and ODM have been used successfully to develop crop plants with improved traits (Supplementary Table 3). For example, using the TALEN technology, a gene functioning in aroma (*OsBADH2*) was knocked out and a fragrant rice variety was developed (Li et al. 2012). Another impressive example of multi-gene editing using TALEN and CRISPR/Cas9 is that in wheat. Three homologous alleles of the mildew resistance locus (MLO) genes have been edited to create wheat cultivar resistant to powdery mildew (Wang et al. 2014). Besides, gene editing has shown promising results in proof-of-concept studies in other plants, such as Arabidopsis (Sauer et al. 2016; Schiml et al. 2014; Christian et al. 2013; Mahfouz et al. 2012; Osakabe et al. 2010), potato (Clasen et al. 2016), Brassica (Lawrenson et al. 2015), soybean (Du et al. 2016; Haun et al. 2014), maize (as reviewed by Agarwal et al. 2018) and rice (Ma et al. 2017, 2015; Oliva et al. 2019; Osakabe et al. 2016; Shan et al. 2015). Various examples of successful use of gene-editing tools to generate improved crop plants have been enlisted in Supplementary Table 3.

Creation of genetic variation followed by targeted selection is the ultimate goal of any crop breeding programme. Genome editing techniques help in the rapid creation of loci-specific allelic diversity for crop breeding. Shen et al. (2017) successfully attempted the simultaneous editing of eight yield or quality-related genes in rice resulting in the generation of homozygous octuple mutants within a single generation. However, further studies of editing the same quantitative trait loci (QTLs) in different genetic backgrounds indicated the prevalence of strong background effect on target traits (Shen et al. 2018). Recently, Zhou et al. (2018) have successfully executed multiplex QTL editing in rice to produce triple mutants exhibiting improved yield-related traits like panicle length, flower number per panicle, grain length, grain width and grain weight. In addition to rice, three homeologous of the *CsFAD2* (*Fatty Acid Desaturase*

2) gene involved in fatty acid metabolism have been successfully altered in hexaploid oilseed crop, *Camelina sativa* to produce monounsaturated fatty acids enriched plants (Morineau et al. 2017). In soybean, the CRISPR/Cas9 system has been exploited to produce the late flowering phenotype and vigorous soybean plants via targeted mutation in *GmFT2a* (an integrator in the photoperiod flowering pathway) gene (Cai et al. 2017). Thus, these examples indicate that the CRISPR/Cas9 can help in harnessing the identified QTLs by precise editing of multiple QTLs to result into favourable allelic combinations for a particular trait or even combination of traits.

Beside generation of genome edited (GEd) crops, gene editing can also strengthen hybrid breeding which can contribute to boosting the crop productivity. Hybrid breeding relies upon the generation of pure inbred lines with desirable traits to achieve higher genetic gains. Doubled Haploid (DH) technology is one of the best approaches for the rapid development of new inbred lines. A reliable Haploid identification (HID) method or marker is the most important component of DH breeding programme (Geiger 2009). Considering the inefficiency of *R1-nj* marker [anthocyanin colour marker which has been used during in vivo haploid generation for rapid and easy identification of haploid kernels (purple pericarp) at seed stage] to express in certain germplasms, there is a need to look for an alternative HID marker (Chaikam et al. 2012). Recently, a gene called *MATRILINEAL (MTL)/ZmPLA1* has been cloned from maize maternal haploid inducer lines (Kelliher et al. 2017; Liu et al. 2017). Via targeting this gene, Dong et al. (2018) developed haploid inducer lines using the CRISPR/Cas9 system which were crossed with the haploid identification (HID) line (carrying double-fluorescence protein markers). The resulting haploid seeds exhibited enhanced green fluorescence and red fluorescence (DsRED), driven by an embryo-specific promoter (Liu et al. 2017) and an endosperm-specific promoter, respectively and hence can serve as effective markers for haploid identification (Kalla et al. 1994; Dong et al. 2018). Furthermore, *MTL (PLA1)* gene is conserved across cereal crops and hence is applicable to a wide range of crops. In addition, CRISPR/Cas9 directed targeting of the first exon of the phospholipase gene in haploid inducer lines has been found to enhance haploid induction rate by 2% (Liu et al. 2017). Similarly, TALEN-mediated deletions nearby the site of the 4-bp insertion in Stock 6-derived lines have been shown to enhance haploid induction up to 12% (Kelliher et al. 2017). Recently, a new approach known as Haploid-Inducer Mediated Genome Editing (IMGE) has been devised for developing genome-edited haploids using CAU5 HI line carrying the CRISPR/Cas9 cassette for *Zea mays liguleless 1 (ZmLG1)* or *unbranched 2 (UB2)* as pollinator and B73 as female (Wang et al. 2019). These studies demonstrate that the use of genome editing techniques in combination with

conventional breeding program such as DH can accelerate the breeding process.

The first commercialised genome edited (GEd) crop is Sulfonylurea herbicide-tolerant canola variety (SU Canola™), which was developed through ODM-based point mutation in the acetohydroxyacid synthase (AHAS) also known as acetolactate synthase (ALS) encoding gene (Gocal 2015; Gocal et al. 2015). The AHAS enzyme is involved in the biosynthesis of branched chain amino acids—valine, leucine and isoleucine. AHAS is the target of sulfonylurea herbicide which inhibits it. Specific point mutations leading to amino acid substitution, viz., S653N (AGT to AAT) and W574L (TGG to TTG) in the AHAS gene provide tolerance to sulfonylurea (Gocal et al. 2015; Sauer et al. 2016). The USDA considered this as non-genetically modified (non-GM) hence classified it as ‘non-regulated’ and it was commercially launched in the USA in 2015 (Wolt et al. 2016). The SU Canola™ also received Plant Novel Trait (PNT) approval from the Canadian government in 2014 and was permitted for commercial sale in Canada in 2017 (Jones 2015; Songstad et al. 2017; <https://www.cibus.com/press/press031814.php>). The SU canola offers unique opportunity to growers to integrate it into crop rotations with glyphosate-tolerant maize or soybean. Apart from SU canola, recently, a few more GEd crops have been de-regulated (exempted from GM organism regulation) in the USA as the final products are null segregant (non-transgenic) and are not likely to pose a plant pest risk (Table 2).

Besides the above examples, bacterial blight-resistant rice having few bases deletion in the promoter regions of two sugar transporter genes, *OsSWEET14* and *OsSWEET11* (developed by Iowa State University), and nutritionally improved alfalfa and wheat (developed by Calyxt) developed via the TALEN approach has also been de-regulated in USA (USDA APHIS 2020). Similarly, *Camelina sativa* with increased omega-3 oil (developed by Yield10 Bioscience via NHEJ-mediated knockout of three loci/copies of single gene) (Waltz 2018) and *j2 (JOINTLESS2)* loss-of-function mutant tomato (developed by University of Florida) developed by employing CRISPR/Cas system has also been de-regulated in the USA (USDA APHIS 2020). These examples suggest that gene/genome editing technologies have immense potential for crop improvement targets which were hitherto considered as difficult. However, the regulatory uncertainty in many countries for improved crop plants developed via genome editing techniques may be a bottleneck in its widespread adoption. This underscores the need for support of governments around the world for setting up an appropriate and updated regulatory framework. In the USA, and Argentina, which follow the product-based regulation procedure, GEd crops are generally not considered as GM subject to the condition that the final product/plant should be transgene-free and hence such GEd crops are

Table 2 Details of various genome edited (GE) crop products which are de-regulated and are near commercialization in the USA

Technique used	GEed crop	Target gene and modification	Function of the targeted gene	Trait developed	Developer	References
ZFN	Maize	<i>IPK1</i> : knockout via NHEJ	Encode inositol 1,3,4,5,6-pentakisphosphate 2-kinase enzyme which catalyses the final step in phytic acid biosynthesis	Low phytate	Dow AgroScience	Shukla et al. (2009)
TALEN	Soybean	<i>FAD3A</i> and <i>FAD3B</i> : knockout via NHEJ	Encode fatty acid desaturase 3 enzyme which is involved in oleic acid (monounsaturated) to linoleic acid (polyunsaturated) conversion	High oleic acid (increased shelf life and oil stability)	Collectis Plant Sciences	Haun et al. (2014); Waltz (2018)
		<i>FAD2-1A</i> and <i>FAD2-1B</i> : knockout via NHEJ	Encode fatty acid desaturase 2 enzyme (expressed specifically in seed) which is involved in oleic acid to linoleic and linolenic acids conversion	High oleic acid (increased shelf life and trans-fat free)	Collectis Plant Sciences	Waltz (2019)
	Wheat	<i>MLO</i> (mildew locus O): knockout via NHEJ	<i>MLO</i> protein is involved in negative regulation of vesicle-associated and actin-dependent defense pathways at the site of fungus penetration	Broad spectrum resistance to powdery mildew	Calyxt	Wang et al. (2014)
	Potato	<i>PPO</i> gene encoding polyphenol oxidase enzyme: knockout via NHEJ leads to reduction in enzyme activity	<i>PPO</i> causes the rapid polymerization of <i>o</i> -quinones to produce black, brown, or red pigments (polyphenols) that cause fruit browning	Non-browning (increased shelf life)	Simplot Plant Sciences and Calyxt	USDA APHIS ^a

Table 2 (continued)

Technique used	GEEd crop	Target gene and modification	Function of the targeted gene	Trait developed	Developer	References
CRISPR–Cas9	Waxy corn	<i>Wx1</i> : knockout	Waxy gene, <i>wx1</i> , encodes the endosperm's granule-bound starch synthase responsible for making amylose	High amylopectin starch content	Dupont Pioneer	Waltz (2016)
	Soybean	<i>Drb2a</i> and <i>Drb2b</i> genes: deactivation of the target genes due to frame shift mutations	Double-stranded RNA-binding2 (<i>GmDrb2a</i> and <i>GmDrb2b</i>) genes play functionally diverse roles in multiple small RNA-directed RNA silencing pathways in response to stress conditions	Drought and salt tolerant	USDA ARS, Plant Science Research Unit	Curtin et al. (2018); Waltz (2018)
	<i>Setaria viridis</i>	<i>ID1</i> (indeterminate1): deactivation via NHEJ	Key regulator of the transition to flowering	Delayed flowering	Donald Danforth Plant Science Centre	Waltz (2018)
	Northern Leaf Blight (NLB) resistant corn	<i>NLB18</i> : sensitive allele replaced with resistant allele via HDR	A wall-associated kinase (WAK) gene involved in pathogen recognition and cellular signalling	Improved Resistance to Northern Leaf Blight	Dupont Pioneer	USDA APHIS ^a

^a<https://www.aphis.usda.gov/aphis/ourfocus/biotechnology/am-i-regulated>

exempted from the regulatory process. This would ensure easy approval and faster commercialization in a case-by-case manner (Whelan and Lema 2015). Thus, non-regulation of non-transgenic GEd crops can potentially save many years and tens of millions of dollars off the cost of commercialization compared to the traditional transgenic crops. However, in the European Union (EU) which favours process-based regulation, as per a recent judgement of Court of Justice of the European Union (ECJ), these crops would be subject to the same stringent regulations that governs the GM crops (Callaway 2018). Hence, GEd crops would need to go through the approval process in the EU before they can be approved for commercialization.

The development of GEd crops using powerful and innovative genome editing techniques could be foreseen to further increase crop yields, advance development of climate resilient crops by combating biotic and abiotic stresses, and address consumer's concerns and nutritional needs. However, it is worth mentioning here that the Intellectual Property landscape related to genome editing technologies such as CRISPR/Cas should be considered when commercialization of the GEd crops developed using such technologies is contemplated. So far, the U.S. Patent and Trademark Office has granted hundreds of patents pertaining to the use of CRISPR in some form and many more are in the pipeline (<http://patft.uspto.gov>; Sherkow 2018). Developing GEd crop plants without infringement of patent rights is a major concern which needs to be addressed to facilitate the commercialization of such crops.

Conclusion

Considering the ever-increasing human population, shrinking arable land area and the rapid pace of climate change, there is a need to develop high-yielding crop varieties which are nutritionally enriched and tolerant to various environmental and biotic stresses. Transgenic technology has contributed towards development of crop varieties with enhanced yield, resistance to biotic and abiotic stresses, and enhanced food quality. Further, estimates also suggest that adoption of the transgenic technology has helped in reducing the use of pesticides and insecticides, decreasing environmental footprint and increasing farmer income. While these crops are granted regulatory approval only after strict food/feed safety assessment, such as allergenicity, toxicity and compositional analysis, etc., concerns have been raised regarding its safety to the environment and human health owing to potential risks related to gene flow, genetic drift, adverse effects on non-target organisms, evolution of resistant weeds and insects, and toxicity and allergenicity. Such potential environmental and human health implications of transgenic crops have contributed to their lesser

consumer acceptance in many countries. To address some of the major concerns associated with transgenic crops, new alternative techniques, such as cisgenesis, intragenesis and most recently, genome editing, are being used to develop improved crop plants. So far, intragenic potato exhibiting resistance to bruising and discoloration, and Sulfonylurea-tolerant canola developed using Oligonucleotide Directed Mutagenesis (genome editing) techniques have been granted commercial approval whilst many other crops are expected to be commercialised. Since a subset of the crops developed using genome editing techniques would be quite similar to the conventionally bred plants, it is hoped that such genome-edited crops might be granted faster regulatory approval which should lead to their widespread adoption in cultivation.

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Compliance with ethical standards

Conflict of interest The authors declare that they have no conflict of interest.

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