Chapter

Genetically Modified Crops and Their Impact on New Era of Agriculture

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Abstract

Genetically engineered crops are different from genetically modified crops. Changes in genetic make-up of crops by any conventional or any means technology fall under genetically modified crops category. In general, among different traits, herbicide and pest tolerance were more exploited in which herbicide tolerant crops occupy more than 90% of the total cultivated area of genetically engineered crops as the mode of actions of herbicides are well known and tolerant gene sources are readily available in wild weedy forms and various microorganisms. By knowing the pathway of mode of action of herbicides and pest tolerance, herbicide and pest tolerant crops were transformed by altering the structure and functions of rate limiting enzymes without affecting the normal functionalities of plants. Other than these two economically important characters, several characters were engineered in different crop plants such as disease resistant, increased yield and biomass production, male sterility and quality characters like anti-allergy factors, modified composition of fatty acid, protein, increased micronutrients and enzyme contents, reduced contents of anti-nutritional factors and toxic substances. Through genetic engineering, transformed plants are used for establishing pharmaceutical products. In terms of area coverage, soya-bean followed maize, cotton, canola, alfalfa. Apart from genetically engineered crops, genome edited crops are developed for nullifying the negative effects and up-regulating the target traits having positive effects thus ultimately assisting in achieving food and nutritional security.

Keywords: agriculture, GE/GM crops, RNAi, ZFN, TALEN, CRISPR/Cas9

1. Introduction

Plant genome engineering aims to modify crops by incorporating agronomically desirable traits, which could not be achieved through conventional plant breeding methods. Genetically modified crops vary considerably from genetically engineered crops in that the former recruits the modification through both natural and artificial means, whereas the latter is distinguished only by artificial means that would not

occur naturally. Genetically modified (GM) crops have proven to be great complement to conventional crops in meeting global demands for increased yields, increased food security, decreased pesticide use, and higher nutritional quality. The modified composition of fatty acid, protein and increased micronutrients, resistance to pests and disease, male sterility, quality characters like anti-allergy factors, reduced contents of anti-nutritional factors and toxic substances and medical benefits among which herbicide and pest tolerance are more remarkably exploited. The genetically modified herbicide tolerance crops occupy more than 90% of the total cultivated area of genetically engineered crops as the mode actions of herbicides are familiar and tolerant gene sources are more obtainable in wild weedy forms and microorganisms, which paved way for transformation by altering the structure and functions of rate-limiting enzymes without affecting the normal functionalities of plants. In terms of global acreage, genetically modified soya beans are widely cultivated followed by maize, cotton, canola, and alfalfa. However, apart from genetically modified crops, genome-edited crops are generated by nullifying the negative effects of traits of interest while up-regulating the positive effects of the target traits. Genetically modified crops benefit humanity by increasing the availability and quality of food and medical care, as well as contributing to a cleaner environment and alleviating hunger and disease around the world. In this chapter, a detailed discussion addressing the global need for resistance to insect pests, disease, herbicide tolerance, stress tolerance, quality improvement, male sterility and yield improvement through genetic modification in crop plants have been made.

2. Insect resistance

One of the most difficult issues in plant crop cultivation is dealing with insect pests. Insect pests are primarily managed using insecticides, but the rising occurrence of insect resistance genes in many organisms could be harnessed and introduced to crop plants through the effective use of transgenic technology. The cloning of genes codes for insecticidal δ-endotoxins dates back to the early 1980s [1]. Transgenic tomato and tobacco produce modified toxin genes which provide insect resistance res the first examples of genetically modified plants [2–4]. As insect-resistance genes transferred into plants predominantly act on the digestive system of insects, researchers are currently identifying genes with distinct modes of action to combat the development of resistance in the target insects, and to enhance potency. Few noteworthy insect control proteins (ICPs) such as protease inhibitors, different enzymes, ribosomal inactivating proteins, and lectins derived from various genus and domains that have an antimetabolic or toxic effect on insect digestion are being viewed as an alternative to control insect infestation or confer resistance to plants.

2.1 Source of transgenes

2.1.1 Resistance gene from microorganisms

2.1.1.1 Cry gene from Bacillus thuringiensis

The most important and successful example of a transgene derived from the bacterium *B. thuringiensis* is Bt gene toxin [5]. It is a significant soil borne spore-forming bacteria that produces insecticidal crystal (Cry) proteins encoded by *cry*

genes (*cry*-represents gene; Cry-represents protein) during sporulation [5, 6]. Proteinases in the insect gut proteolytically cleave the inactive protoxins to produce the active 60–65Kd toxin made up of high homology regions interspersed with (hyper)variable regions. Sequence analysis [7, 8] and X-ray crystallography were used to infer the structure and functional roles of the toxin's three domains [9] that bind to glycoprotein receptors in the brush border membrane of the midgut epithelium of susceptible insects. The nature of the receptors that explicitly play a significant role in establishing susceptibility/resistance to a specific Bt toxin, is under intensive investigation, with a number of midgut integral membrane glycoproteins, including aminopeptidase and a cadherin-like protein, being identified [10–13]. Following binding, the toxin rapidly and irreversibly inserts into the cell membrane resulting in the formation of a pore supposedly by Cry proteins leading to disruption of the electrical, K+ and pH gradients eventually causing irreversible damage to the midgut wall which gives rise to epithelial cell lysis [6, 14] paving way to gut paralysis, cessation of feeding and finally (typically after 1–3 days) death from starvation and/or septicaemia. These toxins are mainly targeted enzymes and lectins of digestive systems of Lepidopteron and coleopteran pests.

2.1.1.1.1 Codon optimization for higher expression

Specificity, efficiency and insecticidal activity of toxins are more in codon optimised proteins than native form of Bt toxin and hence the former was introduced into various plants for increased level of expression [14]. Plants harbouring modified codon with plant preference rather than bacterial preference in which G:C rich codons are preferred over A:T rich codons, and undesirable mRNA secondary structure and polyadenylation signals eliminated, produced 100X higher than plants were transformed with unmodified (native) Bt gens [15, 16]. Genes are frequently inserted with constitutive promoters such as maize ubiquitin, CaMV35S, or rice actin 1, promoted protein expression at all times and in all parts of the plant allowing broader-spectrum ICPs to be targeted at different components of the pest complex. The use of tissue specific (e.g., RsS1 promoter for phloem-specific expression or PHA-L promoter for seed-specific expression) or inducible (e.g., potato pin2 wound-induced promoter) promoters is recommended to conserve the space and time of expression of toxic proteins and thus avoid unfavourable interactions with the beneficial insect's ecosystem [17].

The highest risk of resistance development would most likely arise from prolonged exposure to ineffective levels of the transgene, a situation that farmers would not tolerate and would almost certainly necessitate additional (different) control measures (deployment of which would in fact reduce the risk of resistance development). CaMV35S has a notoriously low/no expression in pollen. Temporal and spatial promoters are used to target the pest at its most vulnerable stage and time of infestation.

2.1.1.1.2 Cry protein classification

Cry protein classification is based on the degree of homology of Cry proteins. Primary Cry protein group: Cry proteins with less than 45 percent amino acid similarity fall into this category. Cry 1 to Cry 78, for example (in 2018). Secondary Cry protein group: Cry proteins with less than 78 percent amino acid similarity fall into this category. Cry 1A and Cry 1B are two examples. Tertiary Cry protein group: Cry proteins with less than 95% amino acid similarity fall into this category. Cry 1Aa, Cry 1Ac, etc. Cry proteins that share more than 95% of their amino acid sequences are

Classification	Percentage of homology of amino acids (%)	examples
Primary group	< 45	Cry 1 to Cry 78
Secondary group	< 78	Cry 1A, Cry 1B
Tertiary group	< 95	Cry 1Aa, Cry 1Ac
Quandary group	> 95	Cry 1Aa1, Cry 1Aa2

Table 1.
Classification of cry proteins based on their amino acids classification.

classified as part of the Quandary Cry protein group. Cry 1Aa1, Cry 1Aa2 (Cry 1Aa25 was recently discovered in 2019). Each Cry protein of the Bt bacterium has a distinct host range cry protein (**Table 1**) [18].

Even within the Cry protein subfamily, the toxic spectrum varies depending on the host. Cry1A and Cry1C proteins, for example, are toxic to larvae of lepidopteran pests such as the codling moth (*Cydia pomonella*), the European corn borer (*Ostrinia nubilalis*) [19], and heliothine bollworms, respectively. The Cry3A protein, on the other hand, is toxic to coleopteran pests such as the Colorado potato beetle (*Leptinotarsa decemlineata*) [20]. So far, 26 plant species have been genetically engineered and expressed for the Bt toxin [18].

2.1.1.2 Ipt gene from Agrobacterium tumefaciens

The introduction of *Isopentenyl transferase gene* (*ipt*) isolated from *Agrobacterium tumefacien* encoding a key rate limiting enzyme in the cytokinin biosynthetic pathway into the tobacco with wound inducible promoter recorded reduced consumption of leaves by the tobacco hornworm (*Manduca sexta*) and reduced survival of the peach potato aphid (*Myzus persicae*) leaving negative effects on plant development, such as an underdeveloped root system and a reduced total chlorophyll content [21].

2.1.1.3 Cholesterol-oxidase gene from streptomycete fungus

A *cholesterol-oxidase* gene from a streptomycete fungus has also been engineered into tobacco that was highly toxic to larvae of the boll weevil (*Anthonomus grandis*) and retarded the growth of the tobacco budworm (*Heliothis virescens*) by damaging the membranes of the insect-midgut epithelium [21–23].

2.1.1.4 Chitinase gene from Serratia marcesens

A bacterial *endochitinase* (from *Serratia marcesens*) has been shown to work in tandem with Bt toxin against *S. littoralis* larvae [24], but not (yet) in transgenic plants.

2.1.2 Resistance gene from higher plants

Plants have co-evolved with insects for millions of years, and have developed many adaptations in terms of antifeeding and anti-shelter, among which digestive enzyme inhibitors (proteinase and amylase inhibitors) and lectins have shown significant effect on insects, which have been transferred into crop plants without major alteration, and expression has been at a similar level to codon-optimised Bt toxins [25].

2.1.2.1 Proteinase inhibitors (PI)

Serine-like proteinases (trypsin-, chymotrypsin- and elastase-like endoproteases) dominant in lepidopteran larvae [26], a wider range of dominant gut proteinases in coleopteran species [27] and thiol proteases observed in corn rootworms (*Diabrotica spp*.) are some of the proteinases in insect which catalyse the release of amino acids from dietary protein, thereby providing nutrients essential for normal growth and development and thus the proteinase inhibitors plays an important part of the plant's natural defence system against herbivory by inhibiting the protein metabolism. Although the mode of action of PIs are not fully understood, it may be claimed that hypersecretion of digestive enzymes caused by the presence of the inhibitors, would result in depletion of essential amino acids [28, 29].

Serine and cysteine-proteinase inhibitors have been shown to inhibit the growth and development of a variety of insects, primarily lepidopteran and coleopteran species [29, 30]. The expression of a gene encoding a sweet potato trypsin protease inhibitor (TPI) in transgenic tobacco (at a relatively low level for a plant-derived ICP – 0.2%) results in severe growth retardation of *Spodoptera litura* caterpillars fed on it besides the presence of high levels of the same naturally in it [31].

The first instance of a plant-derived ICP gene being used in transgenic plants was the constitutive expression (through the CaMV35S gene promoter) of a trypsin inhibitor gene taken from cowpea (*Vigna unguiculata*) and in tobacco [32]. Proteinase inhibitors do not just alter gut digestive enzymes; they can also affect insect water balance, moulting, and enzyme regulation [33]. The majority of research has focused on serine-proteinase inhibitors derived from the plant families Fabaceae, Solanaceae, and Poaceae, which are mostly aimed against not only lepidopteran pests but also some coleopteran and orthopteran pests. The cowpea trypsin inhibitor (CpTI), which has been introduced into at least 10 different plant species, is the most active inhibitor discovered to date the protection provided by CpTI was less pronounced and consistent than that of tobacco containing a truncated Bt-toxin gene [34].

2.1.2.2 α-Amylase inhibitors

To block carbohydrate metabolism, a-amylase inhibitors from wheat (WAAI) and common bean (*Phaseolus vulgaris*) (BAAI) are utilised. When introduced into transgenic tobacco, the former increased mortality of lepidopteran larvae fed on it by 30–40% [35], while the latter, when expressed in transgenic pea seeds and driven by the pha1 gene promoter, elevated resistance to bean weevils [36, 37].

2.1.2.3 Lectins

Lectins are a diverse group of carbohydrate-binding proteins, that are toxic to insects of the orders Homoptera, Coleoptera, Lepidoptera, and Diptera. The very first demonstration of enhanced resistance of transgenic plants expressing a foreign lectin used is the gene encoding the glucose/mannose-binding lectin from pea (*Pisum sativum*) [38]. The mode of action of lectins against insects is unknown, but it has been shown that some bind to midgut-epithelial cells [39], and some insecticidal lectins also show significant mammalian toxicity, including lectins from *P. vulgaris* (phaeton haemagluttinin, PHA), and the greater insecticidal activity is shown by chitin-binding lectins from wheatgerm (WGA) expression in transgenic maize [40].

Recent interest has primarily focused on the mannose specific lectin from snowdrop (GNA), which has shown activity against peach potato aphids, potato tuber moths [41] and the rice brown planthopper (*Nilaparvata lugens*) [42]. GN expressed in potato and tomato significantly reduced fecundity and enhanced resistance, respectively, in laboratory experiments [42]. When overexpressed in different species of tobacco, tomato, and sweet gum, the tobacco anionic peroxidase, which is involved in crosslinking and polymerisation, inhibition of digestive enzymes, and the generation of highly reactive, toxic species, led to significant levels of resistance to several lepidopteran, coleopteran, and peach potato aphid [43]. The expression of tryptamine and tryptamine-derived alkaloids in plants may serve as anti-oviposition and anti-feedant agents, or as inhibitors of larval and pupal development, and when expressed in tobacco, inhibition of reproduction of the whitefly *Bemisia tabaci* by observed up to 97 percent [44].

2.1.3 Resistance genes from animals

Based on in vitro testing of proteolysis inhibition by several lepidopteran larvae midgut extracts, as potential insect-resistance proteins like bovine pancreatic trypsin inhibitor (BPTI), a1-antitrypsin (a1AT), and spleen inhibitor (SI) were identified and introduced into a variety of crop. Proteinase inhibitors derived from *M. sexta* and expressed in cotton and tobacco were reported to impede *B. tabaci* reproduction [45, 46]. Despite the introduction of chitinase (from the tobacco hornworm) into tobacco, these plants only exhibited a limited level of resistance to lepidopteran larvae and peach potato aphids [47].

2.1.4 Microbial proteins

The bulk screening of microbial culture supernatants against specific pests has been one strategy to the discovery of novel insecticidal proteins. Two proteins, Vip1 and Vip2, were isolated from vegetative *Bacillus cereus* culture supernatants and have been shown to be acutely poisonous to maize rootworms [47]. Some *B. thuringiensis* vegetative culture supernatants include a protein (Vip3A) that is acutely poisonous to Agrotis and Spodoptera caterpillars [48]. These proteins activity is extremely similar to that of Bt-endotoxins, yet they are distinctly separate from them.

2.1.5 Predator toxins

Genes producing neurotoxins from predatory mites [49] and scorpions [50] have been inserted into recombinant baculoviruses, where they effectively boost the rate of killing.

3. Disease resistance

Since the identification of the chemicals and genes involved in disease resistance in plants, attempts have been made to develop permanent disease resistance in commercially significant crop plants. Unfortunately, many of these efforts have failed because to the complexities of disease-resistance signalling and the wide range of infection routes employed by various pathogens. Although disease-resistant transgenic plants

or seeds are not currently commercially accessible, future product development looks to be feasible as our understanding of pathophysiology and plant defence deepens.

In general, plants are protected by structural defence (plant cell wall, thick and waxy epidermis, trichomes, thorns) and chemical defence (production of secondary metabolites, proteins, and digestive enzymes) [51]. Plant defence response genes are classified into susceptibility genes (S), resistance genes (R), and non-host resistance genes (NHR). Pathogen-associated molecular pattern (PAMP)-triggered immunity (PTI) is broad-spectrum resistance, effective against non-host pathogens that fail to establish virulence [52, 53], and ETI is regarded to be the basis of R-gene resistance (host resistance). NHR is regarded as a quantitative trait encompassing numerous genes and varied pathways, whereas R gene resistance is regarded as a qualitative trait that adheres to Flor's gene-for-gene model and is dependent on a complex regulatory mechanism for pathogen detection and defence response [54, 55].

The successful transfer of R genes that express specific effector proteins in a gene-for-gene manner among plant species has resulted in long-term resistance and crop protection against a variety of pests and diseases. Polymorphisms in the coiled-coil (CC) and nucleotide-binding (NB) domains have been shown to be critical for recognition specificity, hence R genes could be edited [56]. In wheat, a two-amino acid mutation in the NB domain of the R gene (PM3F) protein was known to improve the resistance spectrum and HR response. S gene mutations can give both broad-spectrum and pathogen-specific resistance. Most S genes play functional roles in the plant, and mutations in them can cause serious pleiotropic defects. For example, CRISPR–Cas9-mediated disruption of the *OsSEC3A gene* in rice improved defence response against *Magnaporthe oryzae*, but the resistant plant also had dwarf stature and a lesion-mimic phenotype [57]. Similarly, leaf chlorosis was observed in wheat plants that had been MLO gene-edited for resistance to powdery mildew [58–60]. Rice *OsSWEET14* and 11 mutations resulted in shorter height and pollen abortion [61, 62].

Understanding the processes of NHR, which has a long lifespan and a broad spectrum, is essential for breeding disease-resistant cultivars. Type I NHR is asymptomatic [63, 64], whereas Type II NHR is similar to host resistance ("gene-for-gene") and is associated with the hypersensitive response triggered by pathogen penetration into plant tissue and activation of the resistance gene [65–68]. For conferring and developing disease resistance technologies are RNAi, TALENS, CRISPR/Cas, ZFN used transfer gene in various crops (**Table 2**) [76].

4. Herbicide resistance

Herbicides are essential components of today's integrated weed management strategy. To address the issue of repeated use of the same herbicide resulting in the evolution of resistant weeds, complicating their control, the development of diverse herbicide-tolerant crops is critical.

4.1 Photosystem based herbicides

Herbicides of various types disrupt various organelles and parts of plants. For example, now recognised by numerous names, including QB (Quinone), D1, encoded by the psbA gene,the 32-kDa weighted protein found in the thylakoid membrane acts

Crop	Disease	Gene and its function	GE Methods used	Ref.
Wheat	*Blumeria graminis*	*TaMLO* Repress immunity against powdery mildew	RNAi	[69]
Rice	*Xanthomonas oryzae pv. Oryzae*	EBS of *OsSWEET14* and *OsSWEET12*, Transcriptional induction	TALEN	[70–73]
Wheat	powdery mildew	Three homoallele *TaMLOof A1, B1, D1* homoeologs	TALEN	[59, 60, 74]
Rice	Rice tungro spherical virus (RTSV)	*eIF4G* Translation of viral RNA genomes	CRISPR/ Cas9	[75]
Rice	*Magnaphorthe oryzae*	*OsERF922* Negative regulator of blast resistance	RNAi CRISPR/Cas9	[59, 60]

Table 2.
RNAi, CRISPR/Cas 9 and TALEN mediated gene transfer for various diseases in different crops.

Amino acid position in Q_B protein	Wild type	Mutant
264	Serine	Alanine
264	Serine	Glycine
219	Valine	isoleucine
251	Alanine	Valine
255	Phenyl alanine	Tyrosine
275	Leucine	Phenyl alanine

Table 3.
Amino acid exchanges in wild and mutant plant proteins.

as a herbicide binding protein for a variety of proteins was identified using a photoaffinity marker (azidoatrazine) [73, 75]. This is the first gene found for herbicide tolerance research. Atrazine, the most commercially important PS II herbicide, is the most extensively used herbicide in maize and sorghum as these crops can extensively degrade atrazine by glutathione conjugation. Herbicide-tolerant weeds (*Senecio vulgaris*) discovered in maize for atrazine, simazine, and other s-traizine category herbicides, which were previously susceptible for these herbicides, demonstrated maternal inheritance and conferred 100 times less binding affinity for herbicide in thylakoid membrane [77–79]. There have been six recorded amino acid exchanges (**Table 3**) [80–83]. The 3D arrangement of the amino acids revealed that different herbicide classes had different binding sites on the QB protein.

4.1.1 Glyphosate

Glyphosate N-(phosphonomethyl) glycine is a post emergence herbicide and is a potent competitive inhibitor of the enzyme 5-enol-pyruvyl shikimic acid 3-phosphate (EPSP) synthase and it is rate limiting enzyme for aromatic amino acids synthesis pathway [84]. A gene (*aroA*) from gram negative bacteria *Salmonella typhinutrium* (now the same found in *Aerobacter aerogenes* [85], possessing mutation on 101th

position of amino acid wherein proline is changed to serine a gene (*aroA*) encoding resistant EPSP enzyme against glyphosate was transformed to tobacco with octopine synthase promoter through agrobacterium transformation which led to insensitive response to herbicide [86]. The mutated gene was fused to an octopine synthetase promotor to enable expression in plants leading to regeneration of transgenic plants with glyphosate tolerance and impaired growth due to compartmentation of aromatic amino acid biosynthesis and disruption of EPSP synthase and other shikimic acid pathway enzymes located in chloroplasts. The precursor protein is directed into the chloroplast by amino-terminal regions known as transit peptides, which are eliminated during the absorption process. EPSP synthase is also a precursor protein that is enzymatically active and binds glyphosate. A fusion gene encoding the sequence of a plastidic transit peptide before the aroA sequence, which exhibits a 40-fold increase in EPSP-synthase activity in petunia plants, is likely to provide a greater level of resistance [87, 88].

4.1.2 Sulfonylurea and Imidazolinone herbicides

The herbicides imidazolinones and sulfonylureas operate by limiting acetolactate synthase (ALS), the first enzyme in the biosynthetic chain that results in the production of branched chain amino acids [89–91]. Thus, chlorsulfuron is readily detoxified by wheat, barley, or oats by phenyl ring hydroxylation and consequent conjugation with glucose. Sulfonylurea-resistant mutant strains have been isolated from species as varied as bacteria, fungi, and plant cell cultures. Using cell biology techniques, maize plants resistant to imidazolinone and tobacco plants resistant to sulfonylurea herbicides have been successfully established [90, 91].

4.1.3 Phosphinothricin

L-Phosphinothricinis (an analogue of glutamate), a component of the tripeptide "bialaphos" produced by several *Streptomyces viridochromogenes*, is a potent irreversible inhibitor of glutamine synthetase with herbicidal activity causing a rapid increase of ammonia concentration in plants which leads to the inhibition in photosynthesis, which derives the plants to death of the plant cell [92, 93]. Resistance to L-phosphinothricin in alfalfa was established via overproduction of a glutamine synthetase gene connected to the promoter of the 35S transcript of cauliflower mosaic virus, and the construct was incorporated into the genome of N. Tobaccum var. W38 by *A. tumefaciens* [94, 95]. Transgenic plants demonstrated superior resistance to high doses of commercial formulations of phosphinothricin and bialophos after integrating the resistance gene from *Streptomyces hygroscopicus* to tobacco and regulating it with the 35S promotor of the cauliflower mosaic virus.The treated plants showed no elevation in ammonia concentration, signifying the total shielding of the plant glutamine synthetase from the herbicide's activity [94]. Herbicide resistance is acquired via TALENs-mediated gene mutation of OsALS in rice and ALS (SurA and SurB) in tobacco [96, 97] (**Table 4**).

5. Stress

Abiotic stress is a highly complicated phenomena that involves biochemical and physiological changes in plant cells causing increased amounts of ROS (reactive oxygen species), that are extremely reactive, toxic and impact chlorophyll

S. No	Herbicide	Gene	Source of gene	Mutation
1.	Glyphosate	*aroA*	*Salmonella spp.*	P101 → S
2.	Sulphonylurea	*ilvGM*	*Escherichia coli*	A26 → V
		ILV2	*Saccharomyces cerevisiae*	P192 → S
		SURBHra	*Nicotiana tabacum*	P196 → A W573 → L
		SURBc3		P196 → Q
		Csr1	*A. thaliana*	P197 → S
3.	Phosphinothricin	*GS*	*M. sativa*	G245 → S,C,R R264 → K
4.	Triazines	*psbA*	*Amaranthus*	S264 → G
			S. nigrum	S264 → G
			Chlamydomonas	S264 → A F255 → Y V219 → I

Table 4.
Genes from various sources responsible for mutation in herbicide tolerance.

production, photosynthetic capability, and carbohydrate, protein, lipid, and antioxidant enzyme activities.

Genes that code for synthesis of osmolytes (proline, betain, etc.), water uptake and transport (ion transporters and aquaporin), transcriptional regulation and signal transduction mechanisms (MAPK) are identified from a variety of organisms and transformed into sensitive genotypes for generation of stress-protecting chemicals [98, 99]. The most efficient candidates for genetic transformation are transcription factors (TFs) that regulate the expression of a number of genes involved in conferring abiotic stress tolerance in plants [100].

5.1 Drought tolerance

Stress-related ring finger protein 1 (*OsSRFP1*), drought-induced SINA protein 1 (*OsDIS1*), and dry- and salt-tolerant protein 1 (*OsDST*) are negative regulators of drought tolerance, whose silencing increased antioxidant enzyme levels, reduced H_2O_2 concentrations, and enhanced drought tolerance in rice.

The CRISPR–Cas9 system was used to introduce novel alleles in Arabidopsis *OPENSTOMATA 2 (OST2)*-encoding gene—a key plasma membrane H^+ ATPase causing two significant mutations at the *ost2* locus that led to constitutive functioning of proton pumps, induction of necrotic lesions and exhibiting a substantially higher rate of stomatal closure coupled with a lower rate of transcriptional water loss which resulted in enhanced drought tolerance (**Table 5**).

6. Quality

Over two billion people worldwide are malnourished as a result of nutritional stress. Genetically modified (GM) crops have the potential to fulfil the worldwide

Crop	Gene	Stress	Reference
Rice	*AtDREB1A* from *Arabidopsis thaliana*	Resistance to drought	[101]
	BrCIPK1 gene from *Brassica rapa*	Enhanced abiotic stress tolerance	[102]
	A. thaliana transcriptional regulator DNA polymerase II subunit B3-1 *(DPB3-1)* gene	Induced heat tolerance	[103]
	Rice LSD1-like type ZFP gene *OsLOL5*	Tolerance against salt and oxidative	[104]
	JERF3 transcription factor (*Solanum lycopersicum*)	Drought tolerance	[105]
	OsDREB2A from *Oryza sativa*	Drought and salinity tolerance	[106]
	OsERF4a from *O. sativa*	Increased tolerance to drought stress	[107]
Wheat	*TaPIE1* from *Triticum aestivum*	Enhanced tolerance to Cold	[108]
A. thaliana	SPDS (Spermidine synthase) from *Cucurbita ficifolia*	Multiple environmental stress	[109]
	Tomato Monodehydroascorbate reductase (*LeMDAR*) gene	Increased tolerance to protoplast abiotic stresses	[110]
	Wheat *WRKY2* and *WRKY19* genes	Salt, drought and freezing stress tolerant	[111]
Maize	Rab28 LEA gene	Osmotic stress tolerance	[112]
Potato	Strawberry D-galacturonic acid reductase (*GalUR*) gene	Increased stress tolerance to methyl viologen, NaCl and mannitol	[113]
	Rat *GLOase*	Resistant to methyl viologen, NaCl and mannitol	[113]
	DHAR from *A. thaliana*	Increased tolerance to salt and drought	[114]
Soyabean	Salinity stress tolerance and fungal resistance	Overexpression of tobacco osmotin	[115]
	Soybean Osmotin-like protein isolated from *Solanum nigrum var. americanum* (*SnOLP*)	Increased drought tolerance	[116]
Chick pea	tolerance by expression of Vigna	Enhanced salinity	[117]
	Increased drought tolerance	overexpression of miR408	[118]
Red gram	Expressing *Vigna* pyrroline-5-carboxylate synthetase	Increased salt tolerance	[119]
Black gram	Overexpression of glyoxalase 1	Salt tolerance	[120]
	Expression of Arabidopsis alate transporter, *AtALMT1*	Increased aluminium tolerance	[121]
	ALDRXV4 gene	Tolerance to H_2O_2, drought, salt and methyl viologen induced stresses	[122]
Mung bean	Expression of *AtNHX1*	Increased salt tolerance	[123]
	Expressing *AtICE1*	Cold stress tolerance	[124]
Sugarcane	*EaDREB2* from Erianthusarundinaceus	Drought and salinity stress tolerance	[125]

Crop	Gene	Stress	Reference
Tobacco	SbpAPX from Salicornia brachiata	Resistance against salt, cold, drought, abscisic acid and salicylic acid stress	[126]
	GmERF7 transcription factor from *Glycine max*	Increased tolerance to salinity	[127]

Table 5.
Genes responsible for abiotic stress tolerance in various crops.

need for high-quality food through genetic engineering by doing more than merely boosting nutritional quality.

6.1 Golden rice

The carotenoid biosynthesis pathway in plants is a multistep process and is accomplished via four desaturation reactions to produce all-trans-lycopene from 15-cis-phytoene by phytoene desaturase (*PDS)*, ζ-carotene isomerase (*Z-ISO*), ζ-carotene desaturase (*ZDS*), and carotenoid isomerase (*CRTISO*) whereas, a single enzyme encoded by the crtI gene accomplishes all the four reaction steps to produce lycopene in bacteria [128, 129]. In the carotenoid pathway, lycopene is an important branch point because it functions as the substrate for two competing enzymes, lycopene β-cyclase (*LYCB*), and lycopene ε-cyclase (*LYCE)*. Lycopene is converted into γ-carotene, which is rapidly converted into β-carotene (has pro-vitamin A activity) by *LYCB* in a single pathway. The bacterial gene *crtY* encodes *LYCB* to produce β-carotene. Alternatively, with *LYCE* lycopene generates δ-carotene (which has no pro-vitamin A activity) [130–132].

About 60–80% by weight of total seed protein in rice is glutelin, and about 20–30% are prolamins which are the prime choice of promoter sequences responsible for expression of carotenoids in rice endosperm [133]. Six promoters of rice glutelin genes *(GluA-1, GluA-2, GluA-3, GluB-3, GluB-5, and GluC*) were isolated and examined in rice and listed in tables with their site of expression (**Table 6**) [134]. Newly reported promoters like *PROLAM26 RAL2 (LOC_Os07g11330), RAL4 (LOC_Os07g11380), and CAPIP (LOC_Os06g33640*) could be useful in the future [135].

A combination of Daffodils *phytoene synthase* (psy) gene, *lycopene-cyclase* (lcy) gene and *crtI* gene from bacteria *(Erwinia uredovora*) PDS (phytoene desaturase) are used to generate japonica rice with ß-carotene expression (mentioned here as GR1) [136, 137]. Gtu-1 promoter was used for psy, lcy and a constitutive CaMV35S promoter with a plastid-specific transit peptide (TP) used for the expression of *CrtI* gene [138, 139] in GR1, Under Gtu-1 promoter, an alliance of maize phytoene synthase (*Zmpsy*) gene with bacterial *crtI* has been expressed in rice to develop an improved golden rice variety (mentioned here as GR2) [140]. The synthetic gene constructions of two carotenoid biosynthetic genes, *psy* from Capsicum (*Capsicum annuum*) and *crtI* from Pantoea, were also reported for golden rice development. To create the PIC (Psy-IRES-CrtI) and PAC (Psy-2A-CrtI) constructions, coupling of two genes using either the synthetic codon-optimised 2A sequence (from foot-and-mouth disease virus) or the IRES sequence (the internal ribosome entry site) were utilised (**Figure 1**) [141, 142].

ZFN and TALENs mediated gene mutation of *IPK1* gene of the rice *OsBADH2* gene respectively encoded inositol1,3,4,5,6-penta-kisphosphate 2-kinase, resulted in both herbicide tolerance and reduction of phytate in developing seeds and production

S. No	Promoter	Expressing region
1.	*GluA1, GluA2 &GluA3*	Peripheral region of the endosperm
2.	*GluB5 and GluC*	The whole endosperm
3.	*GluB3*	Aleurone and subaleurone layers of rice grain

Table 6.
List of promoters and expressing region involved in Golden rice.

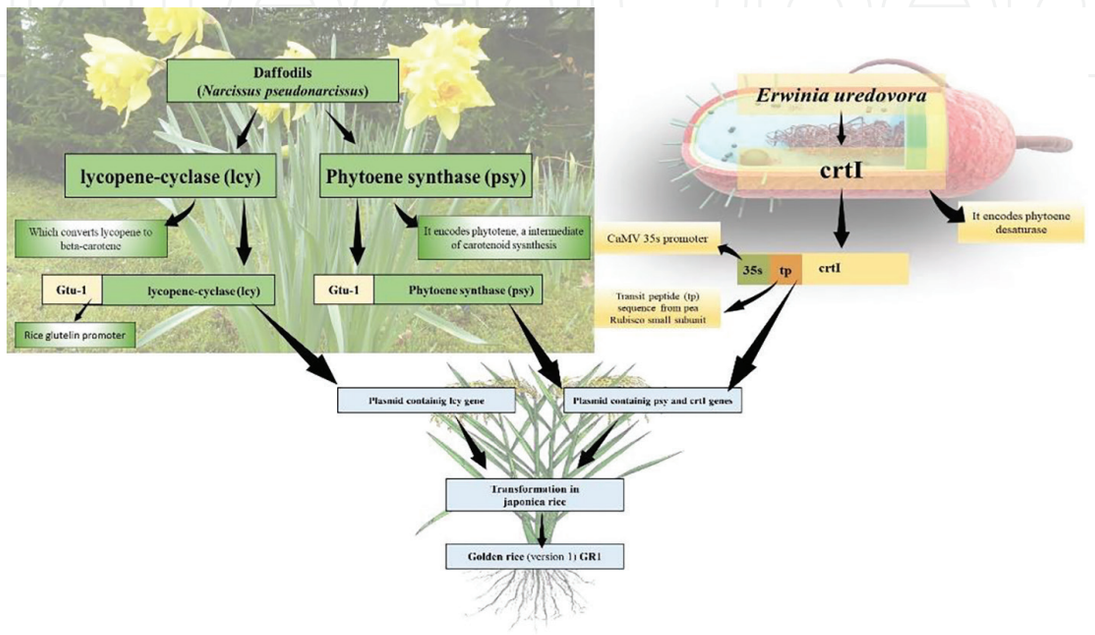

Figure 1.
A simplified version of ß-carotene expression in golden rice.

of fragrant rice by increased synthesis of fragrance compound 2-acetyl-1-pyrroline (2AP) [143]. In monocot plants, the *k1C* gene in sorghum plant was targeted using CRISPR for gene disruption at the N-terminal ER signal peptide region, resulting in greater Lysine content and improved protein digestibility [144]. Naim et al. deployed CRISPR-Cas to target the PDS, Phytoene desaturase expressing gene in Cavendish banana (*Musa acuminata*) for gene knockout to target the Albinism phenotype. CRISPR-Cas9 was used to disrupt rice genes critical for defining amylose concentration, fine structure of amylopectin, and physiochemical characteristics of starch, resulting in a larger proportion of long chains in amylopectin. The GBSS gene, which encodes Granule-bound starch synthase and is responsible for amylose production in the Potato plant, was targeted using CRISPR-Cas for Gene knockouts, resulting in a product with elevated amylopectin content [145].

In *Camelina sativa* the *FAD2* gene, which is important for fatty acid production, was targeted using CRISPR-Cas9 for gene deletion to enhance seed Oleic acid content. In tomato, CRISPR-Cas9 was used to target *SlAGL6*, a transcription factor that plays important roles in flower meristem and floral organ development, for gene deletion, resulting in a parthenocarpic phenotype [146]. The *CAO1* and *LAZY1* genes, which are responsible for synthesis of chlorophyll b from chlorophyll a and regulating shoot gravitropism, were disrupted using CRISPR-Cas to target the faulty synthesis of chlorophyll b and tiller spreading phenotypes, respectively (**Table 7**) [147].

Crop	Gene transformed	Quality character	Reference
Rice	Soybean ferritin gene	Increased iron content	[148]
	Phaseolus vulgaris ferritin gene	Enhancement in Fe content, cysteine and phytase level	[149]
	Lactoferrin gene from human	Increased iron content	[150]
	Ubi1-P-int (maize) & *GmFAD3 cDNA* (soyabean)	Enhanced α-linolenic acid content	[151]
	amino-deoxychorismate synthase (*ADCS 1*) & GTP cyclohydrolase I (*GTPCHI*) genes	100 times enhanced accumulation of vitamin B9	[152]
	BiP, lysine-rich binding protein	Increased lysine content	[153]
Maize	*SacB* gene *(Bacillus amyloliquefaciens)*	Stable accumulation of fructan	[154]
	Lysine feedback-insensitive *DHDPS*	Increased lysine content	[155]
	HGGT gene from barley	Tocotrienol content increase	[156]
	Wheat *DHAR* gene	Increased ascorbic acid	[157]
	Ferritin gene & phytase gene	Increased iron content	[158]
Red gram	Dihydrodipicolinate synthase overexpression	improve lysine content in seeds	[159]
soyabean	*DHDPS* and aspartokinase from *E. Coli*	Increased lysine content	[155]
	15-kDa Maize zein protein	Increased accumulation of C &M	[160]
Chick pea	Raffinose synthase 2 Silencing	Nutritional quality improvement	[161]

Table 7.
List of different genes transformed for quality character in different crops.

7. Male sterility

The use of genetically engineered male sterility has a variety of applications, ranging from hybrid seed production to transgenic bioconfinement in genetically modified crops. The influence of this technique has aided in dealing with global food security concerns. The production of transgenic male sterile plants through the expression of a ribonuclease gene under the direction of an anther- or pollen-specific promoter has shown to be an efficient method of producing pollen-free elite cultivars.

7.1 Male sterility due to mutation on nuclear genes

Mariani et al. [162] achieved the first success in developing genetically engineered male sterility in crop plants by transforming tobacco and rapeseed plants with a chimeric dominant gene *barnase* (bacterial RNase from *Bacillus amyloliquefaciense*) driven by a tapetum-specific promoter (TA29) from tobacco. The coding sequences of RNase T1 from *Aspergillus oryzae* and *barnase* from *B. amyloliquefaciens* used to manipulate the trait were fused with the tapetum-specific TA29 promoter which is responsible for the expression of the *barnase* gene specifically to anther tapetal cells, causing selective destruction of the tapetal cell layer that surrounds the pollen sac by hydrolysing the tapetal cells, causing abnormal pollen formation (**Figure 2**). Male sterile anther carries empty exine [162]. Mariani et al. [163] demonstrated fertility

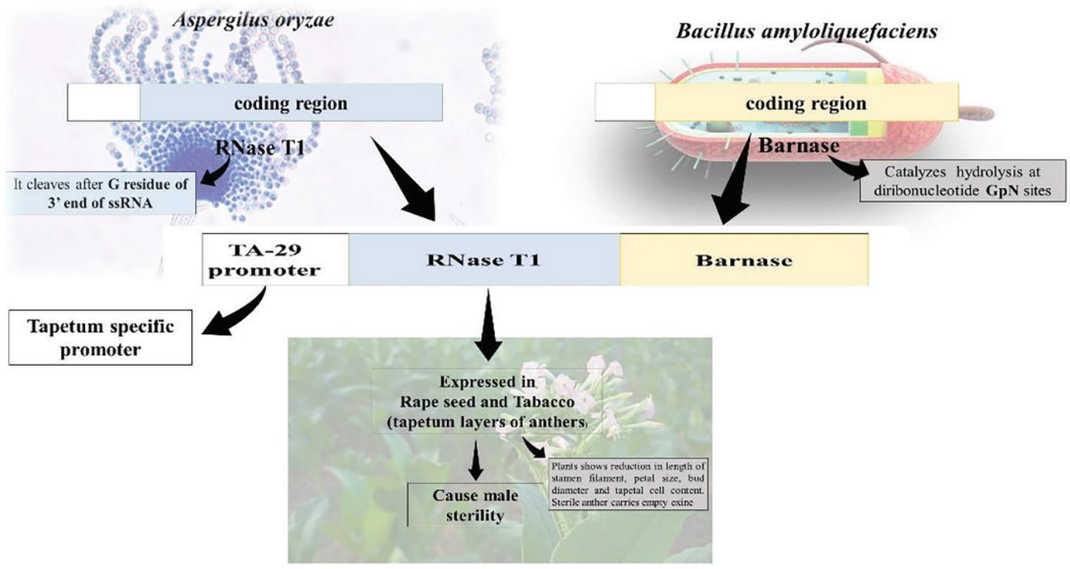

Figure 2.
Simplified version of expression of transgenic male sterile system.

restoration in TA29-barnase male sterile plants by gene encoding the barnase-specific RNase inhibitor called barstar which was isolated from same bacteria *B amyloliquefaciense*. When genetically engineered, male sterile plant is crossed with plant carrying TA29- barstar gene the F1 progeny shows co-expression of both genes in the anther of male fertile plants. In this system fertility restoration is due to the formation of tapetal cell-specific barnase/barstar protein complexes which completely inactivate the barnase enzyme [163]. This dominant nuclear genetic male sterility system faces same drawback as GMS system. During hybrid seed production the plants in female rows segregate in the ratio of 1:1 for male sterility and male fertility [164]. To counter the drawback of nuclear genetic male sterility system problem the *barnase* gene was linked to a dominant herbicide resistant gene (bar) under control of the constitutive promoter CaMV 35S which conferred resistance to broad-spectrum herbicide Basta (active ingredient is phosphinothricin or PPT). When seedlings are sprayed with Basta only the male sterile plants survive and the male fertile plants are killed as they lack bar gene.The use of bar gene allows elimination of male fertile segregants from female rows in the hybrid seed production plot thus assuring 100 per cent pure hybrid seed production [165].

7.2 Male sterility due to mutation on chloroplast genes

The genetic transformation of the plastid genome has various advantages, including high level transgenic expression, expression of multigene operons, transgene maternal inheritance, and expression of bacterial genes without codon optimization [166]. Ruiz and Daniell [167] elucidated that, with chloroplast transformation, hyper-expression of β-ketothiolase encoded by the phaA gene of Acinetobacter sp. in the leaves, flower, and anther of transgenic lines gets in the way of pollen development and results in male sterility. This was restored by exposing transgenic male sterile plants to continuous illumination, which allows acetyl CoA carboxylase (ACCase) to access acetyl CoA, restoring normal fatty acid synthesis and minimising PHB production through β-ketothiola.

7.3 Male sterility due to altering metabolic process

Callose is a plant polysaccharide comprised of β-1-3 glucan that is deposited around microspore tetrads during meiosis. Tight developmental regulation and the timing of callase activity are required for optimal microspore development. The expression of modified PR-b-1-3 glucanase in transgenic tobacco plants led in the premature disintegration of the microsporocyte callose wall, resulting in mild to total male sterility [168]. Chang et al. [169] created a rice hybrid breeding method employing the rice nuclear gene *Oryza sativa* No Pollen 1 (*OsNP1*), which encodes a putative glucose–methanol–choline–oxidoreductase with involvement in tapetum degeneration and pollen exine production. The ethyl methane sulfonate-induced rice mutant, *osnp1-1*, was completely male sterile.

7.4 Conditional male sterility

Conditional male sterility is a situation in which plants are typically fertile, but when a specific circumstance is applied, male sterility occurs. Hawkes et al. [170] revealed the use of inactive D-glufosinate as a male sterility inducer in transgenic plants expressing a modified (F58 K, M213S) version of *Rhodosporidium toruloides* Damino acid oxidase (DAAO) that converts oxidised D-glufosinate to its 2-oxo-derivative (2-oxo-4-methyl phosphiny to create transgenic plants, the modified DAAO encoding gene was coupled with the TAP1 promoter from *Antirrhinum majus* and transformed into tobacco plants. When D-glufosinate was sprayed on these transgenic plants, it caused full male sterility that lasted two or more weeks while having no effect on female fertility [170]. Guerineau et al. [171] expressed the temperature-sensitive diphtheria toxin A-chain polypeptide gene sequence under the tapetum-specific A9 promoter and generated transgenic Arabidopsis plants that were fully fertile at 26 C, but when the temperature was decreased to 18°C, male sterility was induced [171].

7.5 Male sterility through post transcriptional gene silencing

Jasmonic acid (JA), a plant hormone, is involved in several developmental signalling events in plants, including senescence, fruit ripening, anther dehiscence, and pollen maturation [172, 173]. Bae et al. [174] reported inducing male sterility by inhibiting OsAOS1 and OsAOS2 activity with the promoters of the anther-specific genes Osc4 and Osg6b, respectively. RNAi (pSK124) constructs were designed and converted into rice calli independently, concluding that the OsAOS2-RNAi vector driven by Osg6b promoter is potent enough for generating male sterility in rice.

7.6 Male sterility through modification of flavonoids

Any disruption in flavonoid production changes pigmentation and causes male sterility in plants. Fischer et al. [175] discovered the expression of a stilbene synthase (STS) gene from grape vine (*VstI*) driven by a 35S RNA promoter with duplicated enhancer region and a tapetum-specific promoter (Tap1) of *A. majus* produced male sterility strives for the substrates,4-coumaroyl CoA and malonyl CoA, which are required for sporopollenin and fatty acid biosynthesis, and hypothesised that there was a decrease in p-coumaroyl availability, resulting in impaired sporopollenin production and pollen wall formation, causing male sterility(**Figure 3**).

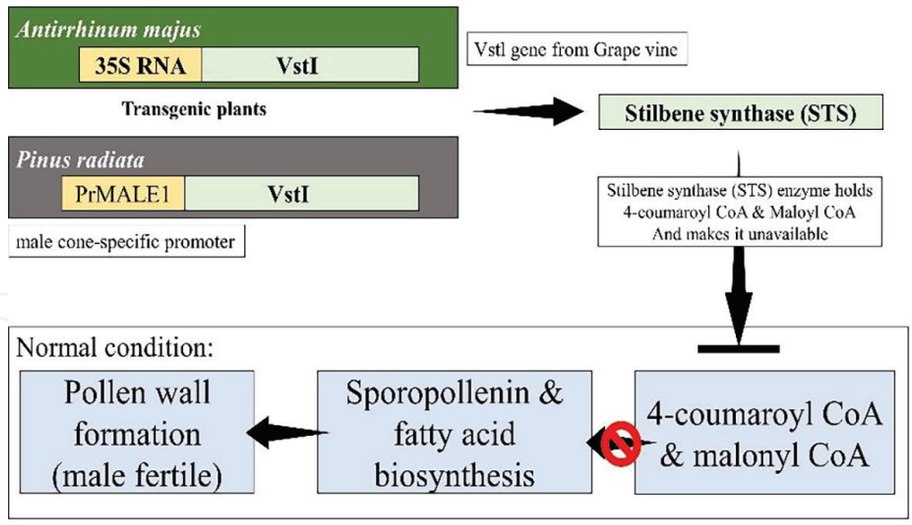

Figure 3.
Simplified version of male sterility through modification of flavonoids.

7.7 Male sterility through RNA editing

Nucleotide alterations or insertion of a nucleotide, leading to a change in the sequence of amino acid in polypeptide denotes RNA editing. Male sterility in CMS plants is connected to mitochondrial DNA rearrangement, causing the formation of novel chimeric open reading frames (ORFs), resulting in mitochondrial malfunction, such as the chimeric gene pcf-S of petunia, ORFB and ORF224 of polima in rapeseed [176]. The overexpression of unedited mitochondrial orfB gene in a transgenic strain of indica rice led to a decrease in activity of ATPase in F1F0-ATP synthase resulting in dose-dependent male sterility [177].

7.8 Heterologous male sterility

The association of CMS and new chimeric ORFs in mitochondrial DNA sequences, as well as mitochondrial dysfunction, is documented. Nizampatnam et al. [178] engineered transgenic tobacco plants to produce orfH522, a pet1-CMS-associated mitochondrial gene from sunflower that is driven by the TA29 promoter. Approximately 35% of the modified tobacco plants were completely sterile. Subsequently, by decreasing orfH522 transcripts using the RNAi approach, male fertility was restored [179].

8. GE for yield contributing characters

To address growing food demand as well as the challenges posed by climate change, major increases in yields of vital food crops employing transgenic technology are required. Using CRISPR-Cas9, researchers were able to increase grain number, dense erect panicles, and grain size in rice by disrupting the DEP1, Gn1a, IPA1, and GS3 genes, which are regulators of grain number, panicle architecture, grain size, and plant architecture [180]. CRISPR-Cas9 gene deletion targets the wheat genes TaGW2-B1, TaGW2-D1, and TaGW2-A1 that govern grain weight and protein content, leading to an increase in grain weight and protein content [181]. In maize, the gene ARGOS8 responded to water stress by increasing grain output [182].

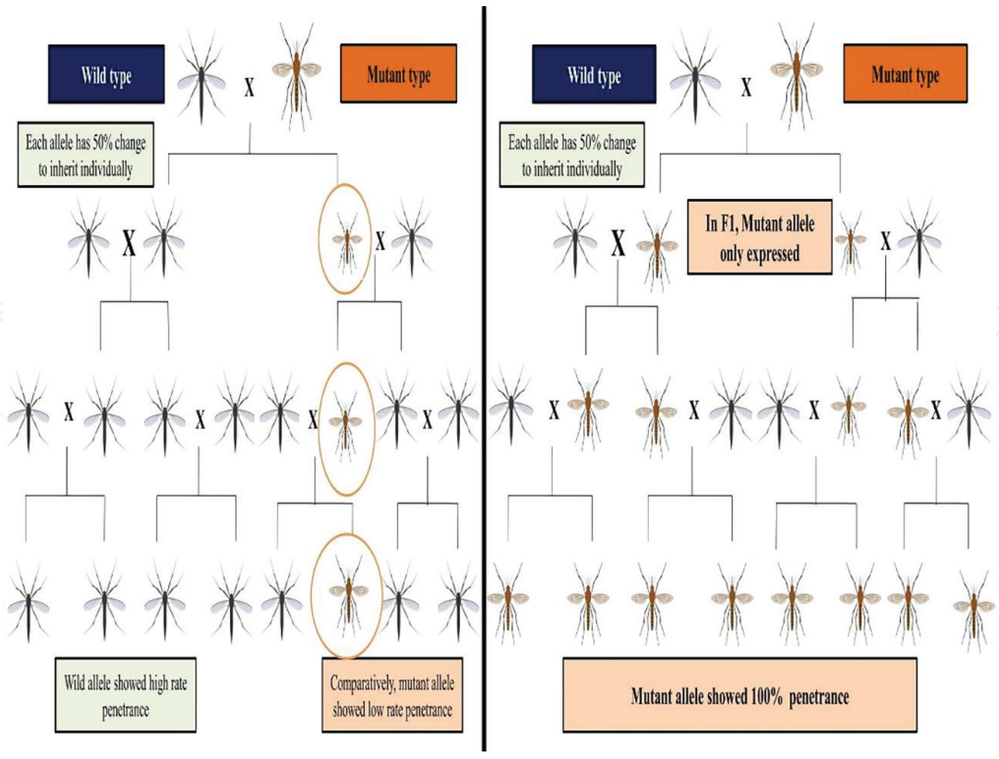

Figure 4.
Simplified version of comparison between normal mosquito population and genetic drive mosquitos' population. There is a huge discrepancy among the rapid adoption of GM crops for production, global markets, and consumer approval. However, the following is a list of transgenic crops that have been worldwide authorised and released for various characteristics (adopted from ISAAA database).

9. Gene drive

Gene drive is a genetic phenomenon of naturally occuring skewed inheritance mechanism that implies sexual reproduction and is essentially concerned with population suppression and population modification. Synthetic gene drives deploy genome editing technologies such as CRISPR to maximise the likelihood of a certain gene being inherited from 50% to almost 100% (**Figure 4**).

9.1 Working model of gene drive

When a gene drive is inserted into the genome of an animal, the progeny inherits the drive on one chromosome and a normal gene from the other parent. During early development, the CRISPR component of the drive shears the other copy. The cut is subsequently repaired using the drive as a template, resulting in two copies of the change being passed down to the progeny [183]. By suppressing the fertility gene termed doublesex on the usage of drive and thereby crashing a population of caged *Anopheles gambiae* mosquitos, Crisanti and his colleagues were able to prevent female mosquitos from biting or laying eggs while the drive was in place [184].

In *C. capitata* [185] and *Aedes aegypti*, a bisex RIDL system containing a tetracycline-repressible positive feedback transactivator (tTA) was successfully constructed, which does not require a specific promoter derived from the target species, but rather a minimal promoter used in conjunction with oligomerised tetO, the binding sequence. Under tight conditions, particularly in the absence of tetracycline, tTA (transactivator and lethal effector) accumulates to deadly levels in both sexes of the

transgenic insect. Females must be removed from a 'male-only' release programme using an independent approach based on an underlying molecular mechanism that is female-specific. Functional Tra protein is only produced by females as it is encoded by a splice variant exclusively produced in females leading to functional tTA protein only produced in females rendering the system female-specific. Fu et al. [185] integrated the first intron of the sex determining gene Cctra into the DNA sequence coding for tTA to provide a sex selective component to the positive feedback system. If this Cctra fragment is spliced in the same as it is in its native context, tTA production is only allowed when the intron is spliced in the female-specific form, as the continuous coding frame of tTA is only restored in this variant. As this intron's full splicing in its native gene is exclusive to females, tTA expression was only expected in transgenic females. The analysis of tTA transcription in transgenic *C. capitata* revealed a sex-specific pattern similar to the natural Cctra gene. The inserted intron spliced to produce three distinct tTA transcripts: one female-specific (F1) and two nonsex-specific (M1 and M2). The female-specific transcript was the only one that encoded a complete tTA. As a result, when grown under harsh conditions, all of the transgenic female progeny died as larvae or pupae [186]. Thus, the utilisation of gene drivers in agriculture may be primarily harnessed by implementing sterile insect techniques for successful insect pest management (**Table 8**).

10. Discussion and conclusion

The advent of advanced targeted, 'customizable,' and precise new technologies for insect resistant plants, in their various embodiments and combinations, symbolises a sustainable option countering the emergence of resistant weeds, lessening agrochemical use, and curtailing adverse effects on nontarget organisms. The direct application of chimeric ODNs or siRNAs to plant cells enables for the generation of technically non-GM organisms. Inevitably, the refinement of promising techniques that are not currently destined to assign insect resistance, such as RNA manipulation with pentatricopeptide repeat proteins, the use of polygalacturonase-inhibiting proteins (PGIPs), ribozymes, and riboswitches, will likely expand the defence mechanism against pests available to researchers and farmers. However, thanks to recent advancements like RenSeq and directed molecular evolution, which enable the rapid identification of novel immune receptor genes, the pool of deployable genes for enhanced resistance to other microorganisms has grown significantly. In the near future, developments in molecular stacking and targeted gene insertion by genome editing are projected to predominate in establishing broad-spectrum resistance against both viral and nonviral diseases. Moreover, increasingly diverse, accurate, and economical genome-editing techniques like CRISPR-Cas allow for precise change of endogenous genes for disease resistance, such as susceptibility and decoy genes. On the flip side, the persistent cultivation of herbicide crops is estimated to have resulted in the resurgence of herbicide resistance in numerous weeds. Nevertheless, by benefiting from the shortcomings of the issue, the adverse consequences of herbicide-based technologies might be substantially minimised by introducing variety in weed control using alternative approaches, with an emphasis on crop rotation, herbicide rotation, and herbicide formulations. Stress-resistant plants may be generated with or without tissue culture by using simple knock-in, knock-out, replacement, fine-tuning of gene regulation, and point mutations at any gene locus. Although genome editing technology is in in its beginning phases, disruptions in specific genes can have unintended

Crops	IR	HR	DR	Abiotic stress	Quality	Pollination control	Altered growth / yield
Maize	119	128	—	6	8	6	2
Cotton	49	44	—	—	1	—	—
Cowpea	1	—	—	—	—	—	—
Bean	—	—	1	—	—	—	—
Brinjal	1	—	—	—	—	—	—
Popular	2	—	—	—	—	—	—
Potato	30	4	19	—	18	—	—
Rice	3	3	—	—	1	—	—
rose	—	—	—	—	2	—	—
Soyabean	6	32	—	2	9	—	1
Sugar cane	3	—	—	3	—	—	—
Tomato	1	—	1	—	9	—	—
Papaya	—	—	4	—	—	—	—
Plum	—	—	1	—	—	—	—
Petunia	—	—	—	—	2	—	—
Squash	—	—	2		—	—	—
Cucumis melo	—	—	—	—	2	—	—
Sweet pepper	—	—	1	—	—	—	—
Alfalfa	—	4	—	—	2	—	—
apple	—	—	—	—	3	—	—
Canola	—	33	—	—	10	20	—
Carnation	—	4	—	—	19	—	—
Chicory	—	3	—	—	—	3	—
Creeping Bentgrass	—	1	—	—	—	—	—
Flax	—	1	—	—	—	—	—
Brassica	—	4	—	—	—	—	—
Sugar Beet	—	3	—	—	—	—	—
Pine apple	—	—	—	—	1	—	—
Tobacco	—	1	—	—	1	—	—
Wheat	—	1	—	—	—	—	—
Safflower	—	—	—	—	2	—	—
Eucalyptus	—	—	—	—	—	—	1

Table 8.
List of globally approved and released transgenic crops for various characters.

negative consequences for plant growth and development. More research is needed to fully exploit the ability of the CRISPR-Cas System in regulating abiotic stress. One rationale could be that the genes governing beneficial qualities in crops are largely

quantitative trait genes, which necessitates a deeper knowledge of gene regulatory networks. As a result, expanding our knowledge of gene regulatory networks is the foundation for greater crop development. Furthermore, the approach of knocking out target genes via NHEJ has attracted a strong interest for boosting agricultural output and quality. Gene targeted insertion or substitution enable the genes to integrate more easily. The HR repair pathway is ineffectual, constraining the use of site-specific insertion and substitution. It is also expected that eliminating the HR approach to editing would lead to more precise and effective crop improvement. If the seed is the most valuable portion of the plant and the crop is mostly self-fertilised, a good fertility restoration mechanism is necessary. It is likely that by coupling inducible male sterility with apomixis, fertile plants may be obtained once the trait was fixed. It is also necessary to guarantee that apomixis is inducible, encouraging apomixis to restore to sexual reproduction and enabling the breeder the opportunity of further enhancing the hybrids by establishing appropriate combiner lines.

The world has already experienced two technical revolutions and is currently undergoing a third revolution based on biotechnology and genomics, which is predicted to yield a plethora of transgenic crops for the benefit of humanity. Genetic engineering is a radical departure from traditional breeding since it allows scientists to transfer genetic material across organisms that could not be bred earlier. The degree of public knowledge regarding the benefits and drawbacks of transgenic plants should be strengthened, laying the groundwork for the effective dissemination of research findings to real time application. The central emphasis should be on the advancement of technologies competent of bridging the gaps in modern day technology. Nonetheless, developing countries are now required to assess genetically modified (GM) crops, and they will subsequently be expected to investigate the potential use of GM trees, cattle, and fish. These advancements may provide prospects for greater output, productivity, product quality, and adaptive fitness, but they will almost likely pose challenges to developing countries' research and regulatory capability.

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