**Current Status and Future Prospects of Genetically Modified Crop Plants in India**

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**Abstract**

genetic engineering and plant transformation have dramatically enhanced harvests in crop plants, by introducing advantageous foreign genes or blocking the expression of endogenous gene(s) in agricultural plants. Herbicide tolerance, insect resistance, abiotic stress tolerance, disease resistance, and nutritional enhancement are all positive features that crops with genetic modification may have. 32 crops with more than 525 distinct transgenic events have been approved for production worldwide as of this writing. It has been demonstrated that transgenic technology increases agricultural yields, lowers pesticide and insecticide use, lowers CO2 emissions, and lowers the cost of food production. However, concerns about the toxicity and allergenicity of transgenic crops for humans as well as potential environmental issues like the likelihood of gene fling, negative effects on non-target animals, the rise of resistant weeds and insects, etc. prevent their widespread use. In response to these concerns, other techniques including cisgenesis, intragenesis, and most recently genome editing, have been used. Since some of these alternative methods may produce agricultural plants free of any foreign DNA, it is projected that these crops will likely be more popular with consumers than transgenic crops and will receive regulatory clearances more rapidly. The most recent approaches and instruments developed to address some of these issues and give a full update on the genetically modified (GM) crops that have been grown. The issues with the extensive usage of GM (genetically modified) crops are also briefly discussed.

Keywords: Genetically modified crops, Transgenic, Cisgenesis, Intragenesis, Genome editing

**Introduction**

Crops that have undergone genetic engineering have had their genomes modified to enhance current traits or introduce a new trait that does not naturally occur in the specific crop type. Transgenic plants are those that have undergone direct gene transfer or transformation mediated by an agrobacterium. Some foreign nucleic acid or gene sequence segments have been inserted into the genomes of these plant [1]. Transgenic genes are those that have been introduced into a cell from a different species of bacterium, virus, fungus, or plant. The ti plasmid was made accessible as a vector to introduce foreign genes into plant cells when it was originally produced in 1977. The Ti plasmid DNA (T-DNA) may spontaneously be inserted into the host plant cell's genome by the Agrobacterium tumefaciens [2]. This research paved the way for the development of transgenic plants. Then, for the first time, it was shown that a specific gene sequence had been transformed into a plant cell using recombinant DNA [3]. The first transgenic plants were created the same year that tobacco and petunia with antibiotic resistance were created [4]. According to Murai et al. (1983), the "phaseolin" gene from the bean was discovered to be expressed in the sunflower. Their research showed that a plant gene may continue function even after being transferred to a taxonomically different angiosperm family. The Food and Drug Administration (FDA) approved the transgenic tomato "*Flavr Savr*," developed by Calgene (Monsanto) in 1994, for sale in the USA. This tomato has a prolonged period of storage life or delayed ripening. A variety of transgenic plants, including glyphosate- and bromoxynil-resistant soybeans, Bt cotton, Bt potatoes, Bt maize, and Bt cotton, were later permitted for sale. The development of transgenic plants has greatly increased agricultural food output during the past 20 years. A worldwide meta-analysis of the adoption of these crops found that using transgenic crops increased farmer profitability by an estimated 68%. Agricultural yields have increased by an average of 22% thanks to technology [5].

Crops with foreign gene(s) continue to be a source of worry due to the possibility of gene flow between transgenic crops and their wild relatives, the potential for lateral transfer of antibiotic resistance genes to environmental microbes, and the possibility of adverse health effects like toxicity and allergenicity to humans. Due to a lack of widespread acceptability, these problems have impeded the widespread adoption of transgenic crops in many parts of the world. To address concerns regarding the introduction of foreign genes, two innovative techniques—cisgenesis and intragenesis—were developed as an alternative to transgenes. For crop improvement, both of these techniques involve genetic material from sexually compatible gene pools generated from related or the same species. Furthermore, crop genome modification is now possible with a level of simplicity, accuracy, and precision never previously possible because to the recent invention of the breakthrough genome editing technology. The Clustered Regularly Interspaced Short Palindromic Repeats (CRISPR)/Cas system, Transcription Activator-Like Effector Nucleases (TALENs), and Zinc Finger Nucleases (ZFNs) are a few of the new editing techniques that use various site-specific nucleases (SSNs) to address concerns about the unpredictability and inefficiency of conventional random mutagenesis and transgenesis.

Crops with foreign gene(s) continue to be a source of worry due to the possibility of gene flow between transgenic crops and their wild relatives, the potential for lateral transfer of antibiotic resistance genes to environmental microbes, and the possibility of adverse health effects like toxicity and allergenicity to humans. Since gene flow between transgenic crops and their wild counterparts is likely to happen, crops with foreign gene(s) continue to be a cause for concern. These gene editing tools have the potential to address many of the regulatory issues associated with transgenics and are thus intended to aid in the development of improved varieties. These interventions include targeted mutagenesis, precise editing of endogenous genes, and site-specific insertion of a trait gene. This article aims to give readers a thorough analysis of the current state of variously described commercially farmed transgenic crops, recent advancements in plant genetic engineering techniques, public concerns, and potential biosafety issues related to the use of transgenic crops (Fig 1).

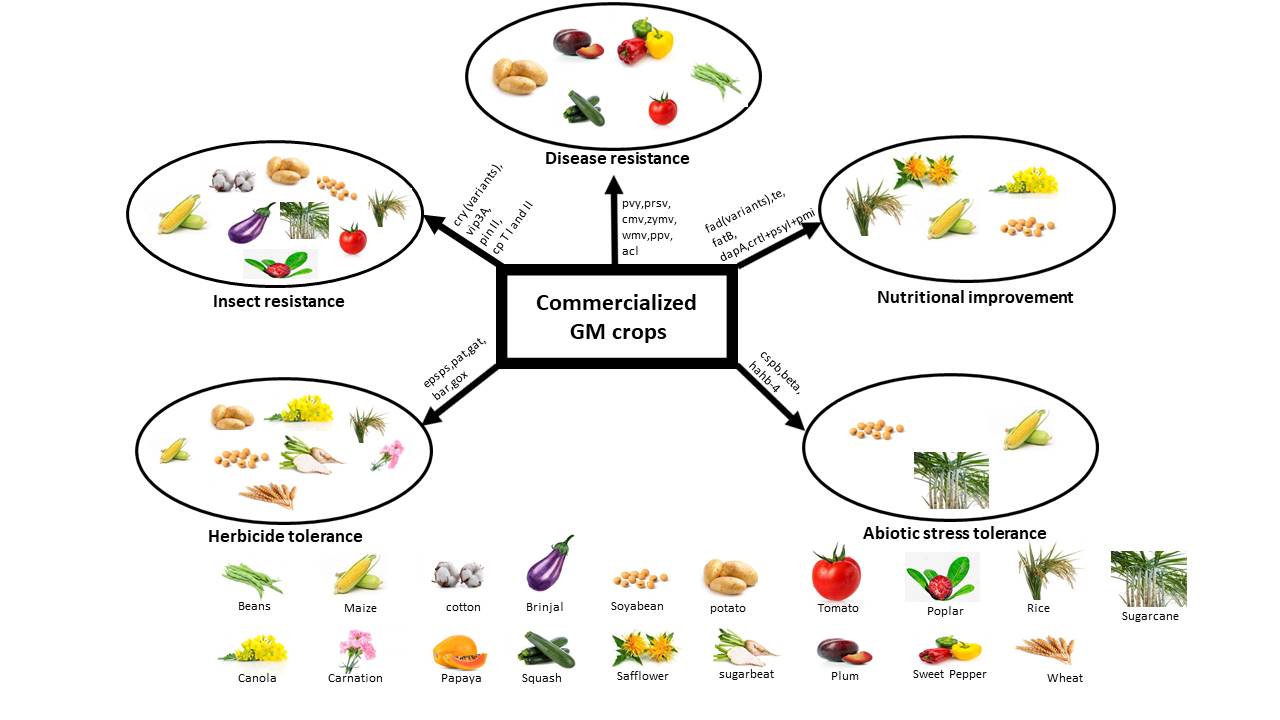


Fig 1. Commercialized genetically modified crops and their examples

**Transgenic plants those are herbicide-tolerant**

By competing with agricultural plants for nutrients, water, sunshine, and space, weeds significantly reduce crop yields. It is necessary to actively control weeds using a variety of techniques, including the application of pesticides, as they diminish agricultural productivity. Nevertheless, because most weeds are herbaceous, it is not always practical to get rid of the weeds without harming the crop plant. The introduction of herbicide tolerance characteristics in the main crop is one potential solution to enable flexible use of potent non-selective and broad-spectrum herbicides. The two primary modes of action for weed-killing herbicides are selective and non-selective. Glyphosate and glufosinate are the two non-selective herbicides that are used the most often. It should be noted that the majority of transgenic plants that can tolerate glufosinate and glyphosate are known as "herbicide-tolerant" (HT) plants.

The herbicide glyphosate particularly inhibits 5-enolpyruvyl shikimate3-phosphate synthase (EPSPS), a key enzyme in the shikimate pathway of aromatic amino acid biosynthesis. Because they lack the shikimate pathway, humans, birds, insects, and other creatures are unaffected by glyphosate. It was produced using either a chemically synthesised gene that is identical to the epsps grg23 gene of *Arthrobacter globiformis* or the mutant form of maize epsps seen *in A. tumefaciens* strain CP4 [6]. Glyphosate-resistant genetically modified plants. As the first herbicide-tolerant transgenic crop, glyphosate-tolerant ("Roundup Ready") soybean carrying the cp4epsps gene was commercialised in 1996. This gene is present in the vast majority of glyphosate-resistant plants sold commercially [7]. The glyphosate oxidoreductase (GOX) or glyphosate acetyltransferase (GAT) genes, which were acquired from Ochrobactrum anthropi or Bacillus licheniformis, respectively, are also expressed in a small number of commercially available transgenic plants. Glyphosate is broken down by these two enzymes into inert byproducts.

The other non-selective herbicide is called glufosinate, sometimes called phosphinothricin, and it works by competitively inhibiting glutamine synthetase [8]. This enzyme aids in the conversion of glutamate and ammonia into glutamine. This enzyme is inhibited by glufosinate, which results in an accumulation of ammonia and impaired performance of photosystems I and II [9]. Streptomyces spp. pat and bar, two different bacterial genes, were utilised to produce glufosinate-resistant plants. Both of these genes encode the phosphinothricin acetyl transferase (PAT) enzyme, which uses acetylation to detoxify this insecticide.Transgenic plants that resist insects

Diseases and insect infestations drastically lower agricultural output. More than 67,000 different bug species harm significant economic crops. They damage crops by devouring plant parts like leaves, stems, and roots or sucking plant sap. In addition, a variety of plant diseases are transported from one plant to another by insects that serve as their vectors [10]. To manage and control insects, farmers use pricey chemically synthesised insecticides. Farmers have to pay for and use this harmful crop protection method.

To reduce these issues with pesticide use, it has become increasingly common to develop alternatives like genetically engineering crops to boost their insect resistance. Currently, ten transgenic crops with pest resistance are available for commercial cultivation. Most of these commercially produced crops have insecticidal genes inserted (often various cry gene mutations and occasionally VIP genes) to prevent harmful insects from hurting crops [11]. Insect-resistant transgenic crops make about 23.3 million hectares, the second-largest area currently in cultivation, according to ISAAA 2017. 304 times have been authorised for cultivation worldwide. 208 events in maize having different IR genes have been cleared for planting according on the frequency of insect pests. Cotton (49 occurrences), potato (30 events), soybean (6), rice (3), sugarcane (3), poplar (2), brinjal (1), and tomato (1) are other commercialised crops with a range of IR genes. The cry gene from the soil bacterium *Bacillus thuringiensis* (Bt) is one of the few widely utilised genes for creating transgenic crops with insect resistance.

The cry gene from the soil bacterium *Bacillus thuringiensis* (Bt) is one of the few widely utilised genes for creating transgenic crops with insect resistance. Crystalline inclusions appear in bacterial spores as a result of the cry protein, which is made by the cry genes. The insecticidal properties of B. thuringiensis are due to the Cry protein. There are three domains in the Cry toxin fragment.

The first one encourages the formation of holes, the second one encourages receptor binding, and the third one aids in the protease defence of the poison. Following attaching to certain receptors, the toxin passes through the cell membrane of the epithelial cells lining the insect midgut. A hole in the membrane opens when domain I binds to the receptor, gradually paralysing and killing the insect. Only a few insect pests are B-resistant, including Lepidopterans, Coleopterans, and Dipterans. thuringiensis Cry genes [12]. Cry genes are used in gene stacking to produce persistent insect resistance in a range of insect species. The adoption of cry genes is made even more favourable by the Cry protein's safety for mammals. Cry genes were originally successfully introduced into cotton, the first economically viable crop, to effectively control the lepidopteron insect pest [13].

The Cry genes have been included into a variety of crops as a result of the success of transgenic cotton, including potato [14], rice [15], canola [16], soybean [17]; [18], tomato [19], and lucerne. Other insecticidal genes, such as vip genes, which encode vegetative insecticidal proteins, have also been used in commercially grown crops in addition to cry. The vip genes, according to scientists, were retrieved using the *Bacillus* Sp, *B. thuringiensis* and *B. cereus*. VIP3A(a) and VIP3Aa20 genes express themselves heterologously inAs a result, the insect is unable to get the amino acids required for growth and development. Insect digestive enzyme activity is decreased by protease inhibitors like trypsin inhibitor (encoded by the gene CpTI) and potato protease inhibitor II [20]. In tobacco, rice, and cotton, respectively, the cptII and potato protease inhibitor II genes have been used to give insect resistance. Only three commercially approved instances of the use of genes encoding protease inhibitors to confer resistance against a variety of insect pests have been documented: the introduction of the cptI gene from *Vigna unguiculata* into cotton, the api gene (encoding the Arrowhead Protease Inhibitor) from *Sagittaria sagittifolia* into poplar, and the pinII gene from Solanum tuberosum into maize.

**Transgenic plants that can withstand abiotic stress**

Abiotic stressors, which can include a variety of environmental factors including heat, cold, floods, salt, etc., negatively affect the growth and development of agricultural plants, resulting in decreased grain yield [21]. The continuing changes in the environment are assumed to be the cause of the increased influence of these abiotic stresses [22]. As part of their response to abiotic stresses, plants alter their antioxidant defence system to maintain cellular homeostasis. They do this by energising signalling pathways and regulatory proteins (such as transcription factors and heat shock proteins), and they synthesise and store compatible solutes that support osmotic correction (such as polyamines, sugars, betains, proline, etc.). By preserving the almost ideal conditions for plant growth and development, these plant adaptations in response to abiotic stresses seek to prevent negative effects on plants. Abiotic stressors have an effect on the molecular expression of a variety of genes. Therefore, several gene networks need to interact for abiotic stress adaptation to take place. Due of the intricacy of the trait, abiotic stress tolerance has been commercialised less frequently than characteristics like herbicide, insect, and disease resistance. According to some scientists [24], seven, three, and two abiotic stress tolerance events in maize, sugarcane, and soybean, respectively, have so far been commercialised. Rice and maize are sensitive to the effects of heat, cold, and water shortages, much as how bacterial cold shock proteins (csp) may be used to lessen the effects of abiotic stresses on Arabidopsis [24]. Both the cspB gene from the soil bacterium B. subtilis and the cspA gene from *E. coli* were utilised in this experiment.

Additionally, it was discovered that using cold shock proteins had no pleiotropic effects on transgenic plants. In well-watered environments, the transgenic maize had a typical phenotype, but it shown improved adaptation in environments with limited water supply. A subset of bacterial RNA chaperones makes up the commonly utilised cold shock proteins. RNA chaperones transform misfolded RNA structures into stable forms, just like protein chaperones do. They thereby help the maintenance of cellular processes under dehydration stress by promoting protein translation and RNA stability [25]. Wheat *(Triticum aestivum*) has been shown to contain an E. coli CSPA homolog [26]. The aforementioned homolog, WCSP1, was shown to have two RNA-binding domains, and it was shown that the protein content increased when exposed to cold. The RNA-binding protein GRP2 from the plant Arabidopsis (Arabidopsis thaliana) has also been found to have a dual purpose in the adaptation to salt and cold stress. 2007; [27]. The cold shock protein Csp3 has been shown to increase salt and drought tolerance by scientists [28].

The drought-tolerant transgenic maize hybrids known as Genuity® DroughtGuardTM (MON 87460 event) were first made available in the United States by Monsanto in 2013. These hybrids include the CspB protein. The stacking of insect and/or herbicide resistance events with drought stress tolerance has resulted in further six events in maize (ISAAA database 2019). The plant needs less water as a result of the drought-tolerant maize's considerable reduction in water loss through transpiration in challenging conditions. By combining insect- and drought-resistant characteristics in a single grain of maize, this variety aims to address two of the most critical issues in the Sub-Saharan Africa region, namely drought and insect pest. A small number of smallholder farmers in 2017 planted transgenic maize with stacking insect and drought resistance (Bt) for demonstration purposes, and successful outcomes were seen (ISAAA 2017). Transcription factors (TFs) have been successfully employed to improve abiotic stress tolerance alongside chaperones. The homeodomain-leucine zipper (HD-Zip) class of transcription factors (TFs) is one such class that is exclusive to plants.

Leucine zipper (Zip) and homeodomain (HD) motifs are seen in HD-Zip TFs [29]. It has been demonstrated that these transcription factors interact with developmental networks that are abscisic acid-regulated, enhancing the relationship between environmental dynamics and gene expression. As an example, the homeobox-leucine zipper gene Hahb-4 from Helianthus annuus (sunflower) attaches to cis-elements of genes that are affected by dehydration and is dramatically and irreversibly activated by water scarcity circumstances [30]. It has been demonstrated that a constitutive or its own promoter may increase this TF's overexpression and increase both yield under stress- and control-free circumstances [31, 32].

It has been authorised for production in Argentina since 2015 and in the US and Brazil beginning in 2019 of the Verdeca HB4 soybean, a transgenic soybean that heterologously expresses the sunflower gene Hahb4 (https://www.isaaa.org/). The transgenic HB4 soybean showed up to a 14% yield boost during multi-location field testing over six seasons in Argentina and the USA under drought and low water conditions. In 2013, Indonesia also approved the commercial production of sugarcane, a transgenic plant with drought tolerance. Three transgenic events using the betA gene *from E. coli* and *Rhizobium meliloti* have been authorised [33]. The choline dehydrogenase protein, which is encoded by the betA gene, aids in the body's adaptation to water stress by catalysing the synthesis of the osmoprotective compound glycinebetaine [33].

The buildup of osmoprotectant or suitable solutes, such as proline and glycinebetaine, as well as non-reducing sugars (such as fructan, trehalose, mannitol, and sorbitol), helps plants survive under osmotic stress, claims a scientific study [34]. These osmosis Protectants can aid to safeguard and maintain the cell membrane's osmotic potential. It is believed that glycinebetaine (N,N,N-trimethyl glycine) is the optimum solute. An increase in its level preserves the integrity of the cell membrane and aids in stabilising enzyme and protein structures under environmental stress [35]. These transgenic sugarcane plants were able to endure water stress conditions for up to 36 days [36] in a field study done during a drought and produced 10–30% more sugar than non–transgenic plants.

**Disease resistance transgenic crop**

In order to combat the threats posed by plant diseases, it is essential for agricultural plants to establish an intrinsic disease resistance. It is crucial to locate the genes causing disease resistance and transfer those genes to plants via biotechnological or breeding methods in order to accomplish this. The majority of virus-resistant transgenic crops have been created utilizing gene silencing techniques, such as antisense RNA directed against viral genes and co-suppression/RNAi. [37]. The expression of the viral coat protein (cp) gene to confer resistance through a "pathogen-derived resistance" mechanism, the expression of the viral replication protein (Rep) sense and antisense RNA strands to confer resistance through a "gene silencing mechanism," and the use of antisense RNA to degrade mRNA coding are all successful transgenic methods for the development of virus resistance. One study developed virus-resistant plants using the PRSV replicase gene (rep). These plants were subsequently sold under the name Huanong No. 1 papaya [38]. 18 of the 19 marketable disease-resistant events that the potato industry has identified combine either insect resistance (IR) or a changed product quality trait. The disease-resistance and IR properties were improved by co-expressing the cry3A gene with either the potato

virus Y (PVY) coat protein (cp) gene or the gene encoding the replicase (plrv\_orf1) and helicase (plrv\_orf1) domain of the potato leaf roll virus (PLRV) [39].

**Nutritionally improved transgenic crop**

**Provitamin A biofortified rice**

According to projections from 2005, 15% of pregnant women and close to one third of preschool-aged children worldwide will suffer from vitamin A deficiency (VAD), a serious public health problem (WHO 2009). Rice and other common foods typically lack beta-carotene, a crucial precursor molecule for the synthesis of vitamin A. In order to address vitamin A deficiency, transgenic rice with provitamin A increased endosperm was created by altering the -carotene production pathway. [40]. Due to its golden hue, this genetically modified rice is referred to as "Golden rice". Later, Syngenta inserted the two transgenes—psy and crtI—into the American rice variety using an endosperm-specific promoter to create Golden Rice 1 (GR1), which may accumulate up to 6 g/g of carotenoids in the endosperm Cocodrie. The last obstacle to be overcome before any significant carotenoid accumulation was the *psy* transgene. Using the maize psy1 and *Pantoea ananatis* bacterium *crtI* genes, the International Rice Research Institute in the Philippines developed Golden Rice 2E (GR2E). This transgenic rice additionally contains the phosphomannose isomerase (pmi) gene from Escherichia coli strain K-12, which serves as a selection signal to encourage the transformed rice cells to thrive on mannose as a carbon source. The use of one event of the provitamin A biofortified rice line GR2E as food was authorized in 2017–18 by Australia, New Zealand, Canada, and the United States [41].

**Modified oil/fatty acid**

In order to improve the nutritional value of seed oil, such as by altering the endogenous fatty acid composition to make it trans-fat free for health advantages and to increase the shelf life of oils, the transgenic approach to metabolic engineering of oilseed crops has been widely adopted. Oils having a higher proportion of polyunsaturated fatty acids (PUFAs) and a lower proportion of saturated fatty acids are preferred for human consumption, according to WHO (2008) and FAO (2010). This group includes oils from sources including fish, walnuts, flaxseeds, sunflower, safflower, soybean, and corn, to name a few. It is thought that replacing saturated fats in the diet with polyunsaturated or monounsaturated fats is good for the heart because it lowers blood levels of low-density lipoproteins (LDLs), commonly known as "bad" cholesterol and triglycerides. Medium-chain triglycerides (MCTs) have been proven to raise basal metabolic rate and decrease the accumulation of adipose tissue when long-chain triglycerides (LCTs) are substituted for them in the diet. [41]

**Essential amino acid**

Certain amino acids cannot be synthesised by humans or animals, therefore they can only be received from diet. Three essential amino acids—lysine (Lys), tryptophan (Trp), and methionine (Met)—are particularly crucial for biofortification due to their scarcity in grains (lysine and tryptophan) and legumes (methionine). Transgenic wheat and rice have been produced by heterologous expression of the lysine-rich pea legumin protein in the endosperm [42]. The addition of a protein from *Amaranthus hypochondriacus*, which is used to preserve the seeds, is another innovation. All nine of the necessary amino acids that humans require are present in significant concentrations in this protein. Lysine biosynthesis and insect resistance are both increased by a stacking feature (cry1Ab gene) in one of the two commercialised maize events [42].

**Beyond traditional transgenic technology**

***Genome editing***

Genome editing technology allows for the permanent replacement, removal, or alteration of some genes and/or other genetic components. For accurate gene knockdown and knockin alterations, this method uses synthetic oligonucleotides. Sequence-specific nuclease (SSN) is used to induce precise point mutations in the target DNA region.[43]. Recently, novel single nucleotide polymorphisms (SNPs) have been created utilising synthetic oligonucleotides for targeted editing, including RNA/DNA chimeric oligonucleotides and single-stranded DNA oligonucleotide molecules of 20–100 nucleotides. This method is known as ODM, or oligonucleotide-directed mutation. Additionally, three additional SSN variations than ODM are used in genome editing. Some examples of SSNs include Clustered Regularly Interspaced Short Palindromic Repeat-associated Endonucleases (CRISPR/Cas), Transcription Activator-Like Effector Nucleases (TALENs), and Zinc-Finger Nucleases (ZFNs).

The CRISPR method's amazing technological simplicity, which encompasses ease of execution, flexibility, efficiency, accuracy, cost-effectiveness, and easy multiplexing, has led to its wide adoption and significantly altered the field of genome editing. It has so been dubbed "the biggest biotechnology discovery of the century." The sulfonylurea herbicide-tolerant canola variety (SU CanolaTM), which was created using an ODM-based point mutation in the acetohydroxyacid synthase (AHAS), also known as the acetolactate synthase (ALS) expressing gene, is the first crop to be commercially sold as having been genome edited (GEd) [44] Additionally, rice resistant to bacterial blight with small base deletions in the promoter regions of two sugar transporter genes, OsSWEET14 and OsSWEET11, as well as nutritionally enhanced lucerne and wheat (created by Calyxt) developed using the TALEN approach have all been de-regulated in the USA (USDA APHIS 2020).

The University of Florida's j2 (JOINTLESS2) loss-of-function mutant tomato and the *Camelina sativa* with enhanced omega-3 oil have both been generated by Yield10 Bioscience using NHEJ-mediated deletion of three loci/copies of one gene (USDA APHIS 2020). These examples suggest that gene/genome editing approaches provide substantial possibilities for agricultural development targets that were previously considered to be difficult.

**Genetic engineering in rice**

***Herbicide-resistant rice***

Herbicide-resistant rice may tolerate one or more specific herbicides since the only plants that are killed by herbicides in rice fields are weeds. To reduce weeds in regions where rice is directly sown, this herbicide-resistant rice variety was created. There are already several nations that sell transgenic rice that is herbicide-resistant, including Malaysia, the United States, and maybe more Asian nations in the upcoming years. This comes after transgenic soybean and maize were accepted.

Before being made accessible in Malaysia in 2010, Clearfield rice was initially grown in the USA in 2002. In the upcoming years, it could be made available in more Asian nations. According to BASF Malaysia [45], the yields with Clearûeldrice increased by two times, from 3.5 to 7 metric tons per hectare. In Arkansas, where the majority of the rice planted in the United States is cultivated, it is being used more frequently than it was 12 years ago to control weedy rice [46].

**Disease resistance**

Approximately 70 diseases can be caused by nematodes, bacteria, viruses, or fungi that affect rice [47]. Despite

the widespread use of resistant cultivars and chemical pesticides, the discovery of transgenic rice resistant to

diseases via genetic engineering techniques is more significant for permanent resistance, providing protection

for a long time and over a big geographic range.

***Viral diseases***

The three most significant viral pathogens of rice are Rice Stripe Virus (RSV), Rice Hoja Blanca Virus (RHBV), and Rice Yellow Mottle Virus (RYMV). Both protein-mediated and RNA-mediated viral resistance have been effectively produced in transgenic rice [48].

***Bacterial and fungal diseases***

*Magnaporthe grisea*, *Rhizoctonia solani*, and *Xanthomonas oryzae pv*. *oryzae* are the three most significant pests preventing rice from generating high yields. R genes that offer broad spectrum resistance could accelerate the development of superior rice varieties with high levels of disease resistance. In the last ten years, more than 100 disease resistance (R) genes have been discovered at the genetic and molecular levels, including Pi-b, Pi-ta, Pi2, Pi9, Pid2, Pi36, Pi37, and Piz-t for blast resistance and Xa1, Xa3/Xa26, Xa5, Xa21, Xa27, etc. for bacterial leaf blight resistance [49]. These cloned R genes offer a high level of resistance for enhancing blast and leaf blight resistance and are promising innovative genetic engineering resources.

***Insect resistant rice***

Stem borers (*Chilo suppressalis*), which decreased rice crop productivity by 5–10%, were the main culprit. Planthoppers and leaf folders (*Cnalhalocrocis medinalis*), two more harmful insect pests, significantly lower. Yearly production across the country. There are still considerable financial, environmental, and health hazards. While managing rice pest insects, despite the regular application of numerous synthetic pesticides. Although Bt Genes have been successfully expressed in a wide range of rice cultivars in this instance we wish to focus on a few more recent alterations.

**Abiotic stress tolerance in genetically modified rice**

The primary abiotic stressors that affect plants are water shortage, excessive heat or cold, and ion toxicity or deficiency, which inhibit development and result in significant output losses. Salt and drought in particular are abiotic stresses that are responsible for 70% of the decline in agricultural production. Increasing tolerance to abiotic stresses has long been one of the key goals in agriculture. The most popular technique for increasing abiotic stress tolerance in plants is to modify individual genes that have a specific target (metabolites or proteins) as a target [50]. Drought is a significant biotic stress that has a significant influence on typical plant growth and development but minimal effect on production. Numerous genes that code for different proteins involved in signal transduction and transcription control include Heat Shock Factor (HSF), C-Repeat-Binding Factor (CBF), Dehydration Responsive Element Binding Protein (DREB), ABA-responsive element binding factor/ABA responsive element (A) [51], Salt Oversensitive Kinases [52], and Phospholipases. In order to produce transgenic rice plants that are resistant to a range of abiotic stressors, these proteins are completely used.

Heat shock proteins, molecular chaperones, late embryogenesis, and abundant proteins all have a significant impact on how plants react to abiotic stresses. The overexpression of HSP101 in transgenic Basmati rice plants significantly sped up the recovery of plant development after heat stress [53]. Numerous studies [54] have demonstrated that amino acid proline increase in particular improves plant function under salt stress. Transgenic plants that overexpressed the rice enzyme 1-pyrroline-5-carboxylate synthase (P5CS) were able to tolerate salt. One of the primary concerns is the introduction of the C4 photosynthetic pathway into C3 crops, which will increase photosynthetic activity, enhance growth, and raise production. Rice's photosynthetic rate and production were increased through the expression of genes for enzymes such phosphoenolpyruvate carboxylase (PEPC), chloroplast pyruvate orthophosphate dikinase (PPDK), and NADP-malic enzyme (NADP-ME) [55].

**Bio-fortified cereal crops**

A crop's nutritional content can be increased in an eco-friendly and perhaps economical method by bio-fortification. According to the research thus far, bio-fortification of crops has been found to drastically reduce malnutrition in nations all over the world. Genetically modified features that are advantageous to customers have not yet reached the commercialization stage, despite the fact that it first seems like this attempt will be less expensive than strategies for boosting or fortifying food.

***Golden rice***

A significant issue with rice is vitamin A deficiency, which affects 124 million children worldwide and can result in blindness and death. To create vitamin A, mammals use β -carotene, a distinctive carotenoid pigment present in plant photosynthetic membranes. Golden rice was invented in the 1990s as a result of the concept to add carotenes to rice. The golden rice, first developed in 2000 by Professor Ingo Potrykus, Dr. Peter Beyer, and other European researchers, was genetically altered to produce pro-vitamin A [56]. The pro-vitamin A concentration of golden rice 2, which was launched in 2005, was significantly raised by a more than 20 factor.

***Engineering higher folate levels in rice endosperm***

The role of folate, also known as vitamin B9, is to promote and repair cellular formation, as well as to speed up metabolism. To boost folate synthesis in seeds, [57] scientist had modified rice by employing targeted expression of Arabidopsis GTP-cyclohydrolase I (GTPCHI) and aminodeoxychorismate synthase (ADCS). The technique was most successful when GTPCHI and ADCS were generated from a single locus, resulting in increases in folate levels of 15 to 100 times in several separate transgenic strains.

***Iron accumulation in transgenic rice with ferritin gene***

One of the most common micronutrient deficiencies, iron deficiency, is known to cause anemia, cardiac issues, and cognitive issues. Whole grains, vegetables, and fruits all contain iron, but because the metal is bound to phytic acid, it is challenging to absorb it from these foods. More iron in rice may help the fight against iron deficiency, especially in developing nations where more than 3 billion people use rice as their main source of nutrition. Using the soybean ferritin gene and increasing the production of nicotianamine synthase (NAS), researchers have increased the amount of iron that is readily accessible in rice seeds [58].

***Developing allergen-free rice***

It is recognised that rice seed proteins are a causal antigen in certain people with food allergies, especially cereal allergies, who have eczema and dermatitis as clinical symptoms. Based on specific identification by serum IgE from allergy sufferers, the amylase/trypsin inhibitors (14–16 kDa), globulin (26 kDa), and glyoxalase I (33 kDa) are regarded as important potential allergens of rice seed [59]. By utilizing a null mutant in conjunction with an RNA silencing strategy, Japanese researchers were able to lower the concentrations of all three allergens in a mutant with the 'Koshihikari' background lacking the 26 kDa allergen (GbN-1).

The ultimate goal of breeding is to create cultivars that are completely and permanently immune to disease and insect pests. The most likely approach will be to genetically modify the regulatory and signalling mechanisms. New genes involved in the metabolic pathways that follow the defensive signalling pathways can be found using genomics and proteomics approaches. The utilization of these genes will significantly help in the development of new rice types with high degrees of resistance—possibly long-lasting resistance—to various ailments and insect pests. Additionally, allergy-free bio-fortified rice will soon be made available for purchase on a worldwide scale. The relatively low-key transgenic approach to teaching abiotic stress tolerance is slowly gaining traction.

The RNAi approach is becoming increasingly popular for the removal of function in addition to the insertion of function. Many nations have not embraced or allowed the commercialization of the GM crops that have been created and authorized in a number of nations. For instance, the herbicide-resistant rice Clearfield® was solely sold and distributed in the US. Similar to this, insect-resistant Bt crops like Bt cotton and Bt brinjal were first introduced and even approved before being again forbidden due to their risk to humans and other living things.

Crop plants may be effectively managed to withstand abiotic or environmental effects by altering a number of pathways or functions of a system in an organism. The future and sustainability of genetically modified rice are quite promising. Despite the fact that they provide a unique, varied, and unexplored region for the majority of individuals, challenges may occur due to their pervasive fear of negative repercussions. Politics is another obstacle to the marketing of GM rice. So, assuming no flaws, we may draw the conclusion that GM rice has a bright future. As genetic engineering is utilized to achieve breeding goals, GM rice is presently finding its way to fields in a number of countries.

***Bio-fortified Wheat***

Over the next 40 years, there will be a rise in the world's need for food due to sustained population and consumption increases [60]. Plant biotechnology is necessary to meet this demand. Soy, maize, cotton, and rapeseed now have higher levels of insect resistance and herbicide tolerance because to genetic engineering. For instance, according to USDA data, 93% of the soybean plants planted in the US in 2010 was transgenic, herbicide-resistant soybean plants. One of the most significant grains used as a staple in the world, wheat has a significant impact on economic growth, the availability and security of food, as well as on human health and nutrition. Transgenic wheat hasn't yet been made available for purchase on the market, despite the fact that genetic alteration has been thought to improve wheat's resistance to stress, yield, and quality. Wheat is a hexaploid plant with a large genome, many DNA repeat sequences, little capacity for regeneration, and challenging transformational features. Even though it takes time and there are times when it is difficult to obtain suitable donors or effectively hybridize the crop plant with the donor species, these procedures have historically been the principal method utilized to create new varieties of wheat.

***Analysis of several transformation techniques in wheat***

Priority should be given to the biolistic transformation and Agrobacterium-mediated transformation methods, which employ immature wheat embryos as explants and have a large, enough number of successful occurrences. In these two methods that employ immature embryos as explants, genotype has a major impact on the efficiency of the transformation. The spring bread wheat cultivar Bobwhite has apparently been used to produce many transgenic wheat plants [61]. Cell death occurs as a result of browning in growing wheat embryos caused by agrobacterium infections [62]. As a result, finding many transgenic plants is difficult. Even though this field has achieved great advancements, more work is still required to combat browning, the mortality of developing embryos, enhance the effectiveness of transformation, and boost the regeneration rates of resistant plants[63].

***Strategies for producing marker-free transgenic wheat plants***

Even though the incorporation of marker genes in the transgenic process has considerably increased transgenic efficiency, the existence and expression of these genes in plant genomes after selection creates health and environmental issues. Only a few methods, such as co-transformation [64], site-specific recombination (Srivastava and Ow, 2004), and transposon-mediated eradication, have been suggested by researchers to get rid of selection markers. The elimination of flag genes to produce transgenic wheat plants has been accomplished by blasting the linear plant expression cassette [65]. Another option is to use an agrobacterium to transform two T-DNA vectors. Since the selection marker and the target gene are separated by their respective T-DNA borders, this method results in unlinked integration. This method has been used to produce marker-free tobacco, rice, Brassica napus, and soybean plants [66]. Meanwhile, nothing has been said about wheat. It's feasible that this method will be used to wheat someday to create transgenic plants completely free of markers.

***Engineered mini chromosomes***

It may occasionally be necessary to insert several genes into a single agricultural plant in order to meet the demand for sustainable agriculture. In this context, the insertion of synthetic mini chromosomes is a very promising tactic. A type of short chromosome known as a mini chromosome has some or all of the components necessary for their replication and independent survival within a cell. Mini chromosomes separate from the host chromosomes on their own. In contrast to conventional techniques of gene transformation, mini chromosomes allow the simultaneous transfer and constant expression of many genes. Due to the unlimited quantities of DNA that could be incrementally added to these platforms using various site-specific recombination cassettes, introduced target genes could be generated at a level that was more predictable than through random integration [67]. This provides a significant opportunity to raise crop performance [68]. According to the positioning of the reporter genes on the maize B chromosome terminal, a context-specific faithful expression may take place [69].

Synthetic mini chromosomes may soon offer a unique and useful approach for persistently expressing a number of genes in wheat, based on the benefits mentioned above. In order to meet the demands of a growing population and its consumption, mankind must produce more food on the same amount of land or less. Because conventional breeding methods alone are unable to achieve these objectives, genetic engineering is crucial in raising crop tolerance to biotic and abiotic challenges, introducing desirable features, and increasing plant productivity. Both biolistic transformation methods and agrobacterium-mediated in vitro transformation of wheat have been employed extensively up to this point. To increase the number of wheat genotypes that may be transformed and the likelihood of enhancing desired features, researchers are also examining innovative wheat transformation procedures. Wheat genetic modification won't be a challenging process in the near future.

Between 1996 and the present, 510 applications have been submitted (the most recent was on April 22, 2013). The following traits will be tested in the 13 applications for 2013: herbicide tolerance (Monsanto); increased carbohydrate and protein content; drought/heat tolerance; nitrogen metabolism; yield increase; modified flowering time; altered oil content; fungal tolerance; insect resistance; and fungal tolerance.

***Barley***

Between 1994 and 2013 (the most current date), 109 applications were totaled. Starch quality (USDA), nitrogen utilization effectiveness (Arcadia), fusarium resistance (USDA), and rhizoctonia resistance (Washington State University) are some of the traits that will be put to the test in the six applications for 2012 applications.

***Millets***

***In vitro cultivation of millets***

The development of a successful in vitro regeneration system is required for the transformation and recovery of transgenic millet crops. Millets have been the topic of various in vitro culture techniques and multiple papers on millets' in vitro culture. Somatic embryogenesis appears to be more advantageous for the advancement of in vitro regeneration strategies for the efficient transformation and recovery of transgenic plants. Somatic embryogenesis and plant regeneration methods for pearl millet, finger millet, kodo millet, and foxtail millet have been described. For finger millet, we recently developed a method that is extremely similar to this. Genetic engineering of millets

***Genetic engineering of millets***

**Pearl millet:** In all currently available papers, the pearl millet (Pennisetum glaucum), which has drawn the most interest in transformation research, has been altered utilizing the biolistic method of gene transfer. Scientists used early embryos as the target explants for the first pearl millet transformation employing microprojectile bombardment (biolistic)[70]. The plasmid pMON 8678, which included the b-glucuronidase (GUS or uidA) gene under the control of the maize alcohol dehydrogenase gene (adh1) promoter, was used for the transformation. The validity of the transformation was assessed using the GUS histochemical assay.When the pearl millet was transformed in the same lab using those plasmids, it was subsequently determined that pAHC25 had stronger uidA gene expression than pBARGUS [71]. Then, using two plasmids (p35SGUS and pROB5), researchers used the biolistic approach to convert pearl millet. The hygromycin phosphotransferase gene (hpt), which provides hygromycin resistance, and the GUS gene were both present in p35SGUS and pROB5, respectively. There were numerous CaMV35S promoters that regulated their expression. The target material (embryonic calli or embryonic cell suspension) was blasted with 1-2 lm tungsten particles coated with plasmids at a distance of 8 cm from the macroprojectile's stopping plate. Southern blot examination [72] further supported the presence of the transgene.

The transformation frequency of pearl millet was dramatically increased using three distinct explants (embryogenic tissue, inflorescences, and apical meristems); the frequency varied from 5 to 85%[73]. They used the plasmids pAHC25 and p524EGFP, each of which contains the reporter gene uidA and the selectable bar gene, both of which are driven by various uq1 promoters. The enhanced green fluorescent protein-encoding gene (gfp) was produced in the plasmid p524EGFP under the control of the alfalfa mosaic virus enhancer sequences and the double cauliflower mosaic virus 35S promoter.1. They maintained a 6 cm gap between the target and stopping screen while coating the DNA on 0.6 or 0.75 lm gold particles**.**

**Bahiagrass:** The significant subtropical fodder plant known as bahiagrass (Paspalum notatum) is grown extensively in the Southeast of the United States, from Central Mexico to Argentina. Scientists used the biolistic technique to first describe the transformation process for this significant fodder grass [74]. After choosing the phosphinothricin-transformed plants, confirmation was done using PCR and Southern blot analysis. The use of particle influx guns to modify diploid bahiagrass was first studied [75]. Later, the same researchers changed diploid bahiagrass utilizing an effective plant recovery mechanism; out of 360 attacked explants, 22 transgenic plants were restored [76]. Additionally, they modified the processes for plant regeneration and callus induction.

Altpeter and James (2005) developed a biolistic transformation technique for bahiagrass using the nptll (neomycin phosphotransferase) marker gene. Later, in the same lab, transgenic bahiagrass with improved lawn quality was developed [77]. When the Arabidopsis ATHB16 transcription factor was overexpressed, bahiagrass produced more vegetative and reproductive tillers [78]. The Arabidopsis homeobox transcription factor gene ATH1 was expressed in ryegrass, resulting in late-heading or non-flowering plants with higher vegetative components [79]. They delivered the gene encoding the ATHB16 transcription factor using the same method as Altpeter and James (2005).

**Foxtail millet:** In India, China, and Japan, foxtail millet (Setaria italica), an essential food crop, is cultivated in salt-prone areas and in trying conditions like protracted drought. It is grown for hay and silage in Australia, North Africa, and South America [80]. Agrobacterium-mediated transformation was employed in both of the two published publications on foxtail millet transformation so far. developed the first agrobacterium-based foxtail millet transformation technique [81]. When the transformed explants were selected on 50 mg kanamycin l-1, assessed for GUS gene expression, and then validated by Southern blot analysis, this technique produced a transformation frequency of 6.6%.

**Conclusion**

Other important millet crops must be included in the usage of *Agrobacterium*-mediated methods in order to transfer crucial genes and generate improved traits. This is due to the fact that in these two foxtail millet studies, the viability of Agrobacterium-mediated millet transformation has been proven. In summary, millet transformation mostly employs physical gene transfer techniques (biolistic; electroporation). The evaluation of marker or reporter gene expression is another issue with many millet transformation claims. Recently, transgenic millets expressing advantageous foreign genes have been sold. To finally create transgenic millets expressing vital foreign genes for agronomy, it will be important to expand the research done on the Agrobacterium-mediated transformation of other cereals to millets. This will enable tolerance to biotic and abiotic stressors, greatly increasing millet output.

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**Author contribution:** All authors contributed equally for this book chapter.

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