CURRENT STATUS AND FUTURE PROSPECTS OF GENETICALLY MODIFIED CROP PLANTS IN INDIA

Abstract

By introducing advantageous foreign genes or blocking the expression of endogenous gene(s) in agricultural plants, genetic engineering and plant transformation have dramatically enhanced harvests. 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 CO_2 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, and Genome editing.

Authors

Esha Kumari
Faculty of Agriculture and Allied Sciences
C. V. Raman Global University
Bhubaneshwar, India.
eshaprasad2412@gmail.com

Sneha Kumari
Faculty of Agriculture and Allied Sciences
C. V. Raman Global University
Bhubaneshwar, India.

Sai Santosh Das
Faculty of Agriculture and Allied Sciences
C. V. Raman Global University
Bhubaneshwar, India.

Laxmi Kanta Panigrahi
Faculty of Agriculture and Allied Sciences
C. V. Raman Global University
Bhubaneshwar, India.

I. 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 plants.[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.
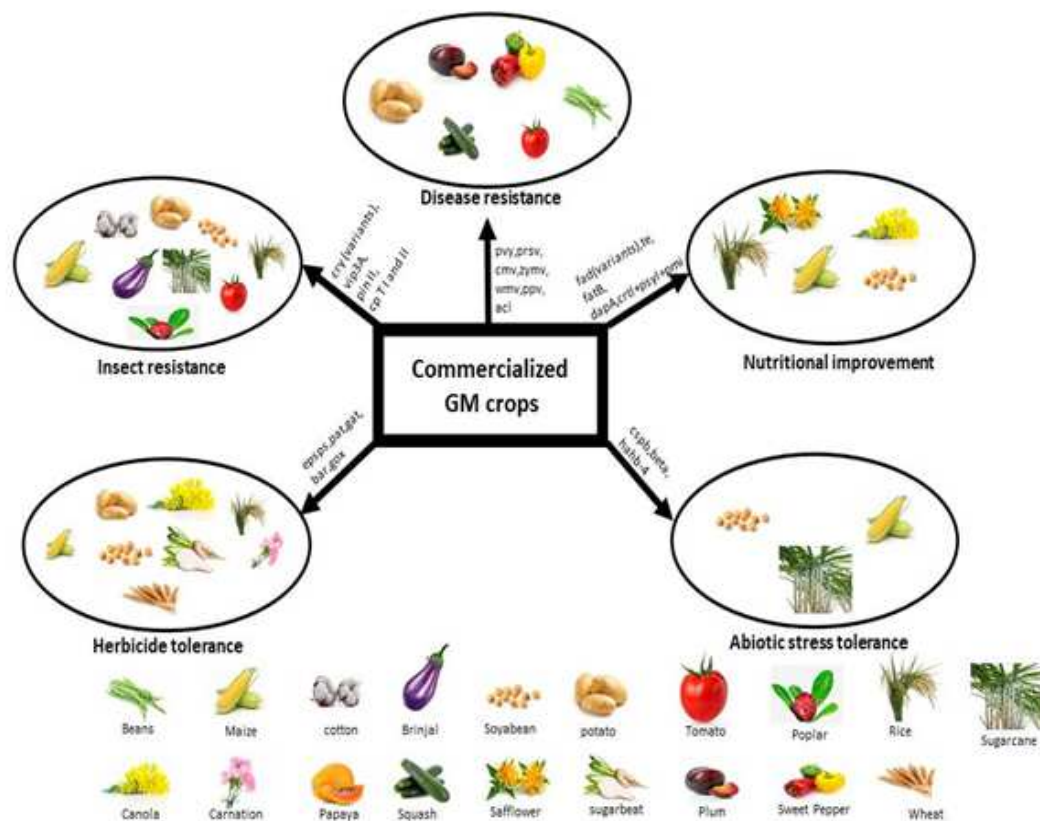
II. TRANSGENIC PLANTS THOSE ARE HERBICIDE-TOLERANT

By competing with agricultural plants for nutrients, water, sunshine, and space, weeds significantly reduce crop yields. It is crucial to aggressively control weeds using a number of techniques, including the use of pesticides, because they lower agricultural yield. Even while most weeds are herbaceous, this does not always make it simpler to get rid of them without hurting the crop plant. One potential option to allow flexible use of powerful non-selective and broad-spectrum herbicides is the introduction of herbicide tolerance traits in the primary crop.

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. "Herbicide-tolerant" (HT) plants are the majority of transgenic plants that are glufosinate and glyphosate resistant.

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 produced gene that is identical to the epsps grg23 gene of Arthrobacter globiformis or the mutant form of maize epsps observed in A. tumefaciens strain CP4 [6]. Glyphosate-resistant genetically modified plants. The cp4epsps gene-carrying glyphosate-tolerant ("Roundup Ready") soybean was initially offered for sale as a transgenic plant in 1996. This gene is present in the majority of commercially available glyphosate-resistant plants [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. These two enzymes transform the pesticide glyphosate into harmless metabolites.

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 the accumulation of ammonia and diminished performance of photo systems I and II [9]. The development of glufosinate-resistant plants involved the utilization of the pat and bar genes from two different Streptomyces spp. bacteria. The PAT enzyme, which uses acetylation to detoxify the pesticide phosphinothricin, is produced by both of these genes. The production of agriculture is decreased by transgenic plants that are resistant to insects, diseases, and pests. Over 67,000 different bug species harm significant agricultural crops.

They damage crops by ingesting plant materials like as leaves, stems, and roots or by sucking plant sap. As their carriers, insects also carry a variety of plant diseases from one plant to another [10]. Costly chemically synthesized insecticides are used by farmers to manage and control insects. Farmers are required to pay for and use this destructive crop protection method.

It has become more and more usual to create alternatives, such genetically modifying crops to increase their insect resistance, to address these problems with pesticide use. There are now ten transgenic crops that can be produced commercially that are pest-resistant. Insecticidal genes (often different cry gene mutations and occasionally VIP genes) [11] have been put into the majority of these commercially developed crops to protect them from damaging insects. Transgenic crops that are resistant to insects make up the second-largest production area today, which is estimated to be 23.3 million hectares by ISAAA 2017. In all, 304 approvals have been given for cultivation. There are 208 distinct types of maize with varying IR genes that have been approved for planting, depending on how frequently insect pests are present. There are 208 distinct types of maize with varying IR genes that have been approved for planting, depending on how frequently insect pests are present. Other commonly produced crops with a variety of IR genes include cotton (49 occurrences), potato

(30 events), soybean (6), rice (3), sugarcane (3), poplar (2), brinjal (1), and tomato (1). One of the few genes that is frequently used for producing transgenic crops with insect resistance is the cry gene from the soil bacteria Bacillus thuringiensis (Bt)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 commercialized crops with a range of IR genes. The cry gene from the soil bacterium Bacillus thuringiensis (Bt) is one of the few widely utilized genes for creating transgenic crops with insect resistance.

Cry, a gene from the soil bacterium Bacillus thuringiensis (Bt), is one of the few genes that is regularly utilized to create transgenic crops with insect resistance. Crystalline inclusions are a result of the cry protein, which is created by the cry genes, appearing in bacterial spores. B. thuringiensis' insecticidal properties are due to the Cry protein. Three domains make to the Cry toxin fragment.

The third assists in binding to receptors and protease defense against the toxin. The toxin penetrates the cell membrane of the epithelial cells lining the insect midgut after binding to certain receptors. When domain I connects to the receptor, a membrane hole forms that ultimately results in the insect losing its ability to move and dying. Only a few of the insect pests that are resistant to B include Lepidopterans, Coleopterans, and Dipterans. cry genes in thuringiensis [12].

In order to create long-lasting insect resistance in a variety of insect species, cry genes are utilized in gene stacking. The Cry protein's safety for animals makes the use of cry genes even more advantageous. The first economically viable crop, cotton, was successfully modified with cry genes to control the lepidopteron insect pest [13]. As a result of the success of transgenic cotton, the Cry genes have been put into a number of crops, including potato [14], rice [15], canola [16], soybean [17]; [18], tomato [19], and lucerne. In addition to cry, other insecticidal genes have also been utilized in commercially cultivated crops, such as vip genes, which encode vegetative insecticidal proteins. According to reports, the Bacillus species B. thuringiensis and B. cereus were used to retrieve the vip genes. VIP3A(a) and VIP3Aa20 genes exhibit heterologous expression in the insect is unable to get the amino acids needed for growth and development as a result. Protease inhibitors like trypsin inhibitor, which is encoded by the gene CpTI, and potato protease inhibitor II reduce the action of insect digestive enzymes [20]. The cptII and potato protease inhibitor II genes have been exploited to provide insect resistance in tobacco, rice, and cotton, respectively. The only three commercially approved examples 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. (ISAAA database 2019).

III. TRANSGENIC PLANTS THAT CAN WITHSTAND ABIOTIC STRESS

The growth and development of agricultural plants are harmed by abiotic stressors, which might include a range of environmental conditions including heat, cold, floods, salt, etc., lowering grain yield [21]. The enhanced impact of these abiotic pressures is thought to be caused by the environment's ongoing changes [22]. Plants modify their antioxidant

defense system in response to abiotic stressors in order to preserve cellular homeostasis. They do this by creating and storing osmotic-correcting solutes including polyamines, sugars, betains, proline, and other suitable solutes in addition to activating signaling cascades and regulatory proteins (including transcription factors and heat shock proteins). These plant responses to abiotic stresses work to prevent negative effects on plants by preserving the almost ideal conditions for their growth and development. Abiotic stressors affect a variety of genes' molecular expression. Multiple gene networks therefore need to interact for abiotic stress adaption to take place.

Due of the intricacy of the trait, abiotic stress tolerance has been commercialized less frequently than characteristics like herbicide, insect, and disease resistance. The ISAAA database (2019) reports that seven, three, and two abiotic stress tolerance events in maize, sugarcane, and soybean, respectively, have so far been marketed. Rice and maize are sensitive to the effects of heat, cold, and water shortages, similar to how bacterial cold shock proteins (csp) may be used to lessen the effects of abiotic stresses on Arabidopsis. Both the cspA gene from E. coli and the cspB gene from the soil bacterium B. subtilis were utilized in this investigation.

Additionally, it was discovered that transgenic plants had no pleiotropic effects from the application of cold shock proteins. In places with abundance of water supplies, the transgenic maize had a typical phenotype, but it demonstrated improved adaptation in areas with less water sources. A subclass of bacterial RNA chaperones includes commonly utilized cold shock proteins. RNA chaperones transform RNA structures from unstable to more stable, similar to protein chaperones. Therefore, they support cellular function when under the stress of dehydration by promoting protein translation and RNA stability [25]. According to reports, wheat (Triticum aestivum) has an E. coli CSPA homolog [26].

The aforementioned homolog, WCSP1, was shown to possess two RNA-binding domains, and it was also shown that exposure to cold increased the protein concentration. The RNA-binding protein GRP2 from the plant Arabidopsis (Arabidopsis thaliana) has also been shown to have two purposes in the adaptation to salt and cold stress. 2007; [27]. The cold shock protein Csp3 has been shown to improve salt and drought tolerance [28]. The drought-tolerant transgenic Genuity® DroughtGuardTM (MON 87460 event) maize hybrids were introduced by Monsanto in the US in 2013.These hybrids include the CspB protein. The stacking of insect and/or herbicide resistance events with drought stress tolerance led to an extra six occurrences in maize (ISAAA database 2019). The plant needs less water because drought-tolerant maize significantly reduces water loss through transpiration in challenging situations. This maize variety aims to combine insect and drought resistant features in a single grain of maize to address two of the region's most urgent issues, namely drought and insect pest. For demonstration purposes, a small number of smallholder farmers in 2017 planted transgenic maize with stacking insect and drought resistance (Bt), and positive results were seen (ISAAA 2017). Along with chaperones, transcription factors (TFs) have been successfully used to increase abiotic stress tolerance. One such class of transcription factors (TFs) that is unique to plants is the homeodomain-leucine zipper (HD-Zip) class.

In HD-Zip TFs, leucine zipper (Zip) and homeodomain (HD) motifs are present [29]. It has been shown that these transcription factors interact with abscisic acid-regulated developmental networks, strengthening the link between environmental dynamics and gene

expression. For instance, the Helianthus annuus (sunflower) homeobox-leucine zipper gene Hahb-4 interacts with cis-elements of genes that are impacted by dehydration and is noticeably and persistently active in conditions of water shortage [30]. Under stress- and control-free conditions, it has been shown that a constitutive or its own promoter may increase this TF's over expression and improve both yield [31] [32]. The Verdeca HB4 soybean, a transgenic soybean that heterologously expresses the sunflower gene Hahb4, has been approved for production in Argentina since 2015 and in the US and Brazil starting in 2019 (https://www.isaaa.org/). During multi-location field testing over six seasons in Argentina and the USA in drought and low water circumstances, the transgenic HB4 soybean demonstrated up to a 14% yield improvement. The commercial cultivation of sugarcane, a transgenic plant with drought tolerance, was also permitted by Indonesia in 2013. According to https://www.isaaa.org, three transgenic events involving the betA gene from E. coli and Rhizobium meliloti have been approved.

The choline dehydrogenase protein, which is encoded by the betA gene and assists in the body's response to water stress, makes the osmoprotective chemical glycinebetaine more readily [33].

According to a scientific research [34], the accumulation of osmoprotectant or appropriate solutes, such as proline and glycinebetaine, as well as non-reducing sugars (such as fructan, trehalose, mannitol, and sorbitol), aids plants in surviving under osmotic stress. The osmotic potential of the cell membrane can be protected and maintained with the help of these osmosis Protectants. The ideal solute is thought to be glycinebetaine (N,N,N-trimethyl glycine). An increase in its concentration aids in protecting the cell membrane's integrity and stabilizes the structures of enzymes and proteins in the face of environmental stress [35].In a field trial conducted during a drought, these transgenic sugarcane plants were able to withstand water stress conditions for up to 36 days [36] and generated 10–30% more sugar than non–transgenic plants.

IV. DISEASE RESISTANCE TRANSGENIC CROP

Agricultural plants must have a built-in disease resistance to lessen the risks posed by plant diseases. To do this, it is essential to identify the genes encoding disease resistance and transfer those genes to plants through biotechnological or breeding techniques. The majority of transgenic plants that are virus-resistant have been developed via gene-silencing methods, such as co-suppression/RNAi and antisense RNA directed towards viral genes [37]. Successful transgenic methods for the development of virus resistance include the expression of the viral replication protein (Rep) sense and antisense RNA strands to confer resistance through a "gene silencing mechanism," the expression of the viral coat protein (cp) gene to confer resistance through a "pathogen-derived resistance" mechanism, and the use of antisense RNA to degrade mRNA coding. One study used the PRSV replicase gene (rep), which is an example of a strategy that utilizes damaged replicase genes to establish viral resistance, to create virus-resistant plants. Following that, these plants were sold and promoted as Huanong No. 1 papaya[38].

In the potato business, 19 commercially viable disease-resistant events have been identified, of which 18 either feature insect resistance (IR) or a modified product quality characteristic.

The co-expression of 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 improved the replication and IR characteristics of the potato leaf roll virus (PLRV) [39].

V. NUTRITIONALLY IMPROVED TRANSGENIC CROP

1. **Provitamin A Biofortified Rice:** Vitamin A deficiency (VAD), a severe public health issue, may affect up to one third of preschool-aged children worldwide and 15% of pregnant women, according to estimations from 2005 (WHO 2009). For the formation of vitamin A, beta-carotene, an essential precursor molecule, is commonly absent from rice and other everyday meals. Transgenic rice with provitamin A enhanced endosperm was developed to treat vitamin A insufficiency by changing the -carotene synthesis pathway. [40]. Due to its golden hue, this genetically modified rice is referred to as "Golden rice". The two transgenes, psy and crtI, were then integrated into the American rice variety by Syngenta using an endosperm-specific promoter to create Golden Rice 1 (GR1), which has the capacity to accumulate up to 6 g/g of carotenoids in the endosperm Concordia. Before any significant carotenoid build up, the psy transgene had to be eliminated.

2. **Modified Oil/Fatty Acid:** The transgenic approach to metabolic engineering of oilseed crops has been widely used to enhance the nutritional content of seed oil, such as by changing the endogenous fatty acid composition to make it trans-fat free for health benefits and to lengthen the shelf life of oils. According to WHO (2008) and FAO (2010), oils with a greater proportion of polyunsaturated fatty acids (PUFAs) and a lower proportion of saturated fatty acids are favored for human consumption. Oils from fish, walnuts, flaxseeds, sunflower, safflower, soybean, and corn are just a few examples of this group. It is believed that substituting polyunsaturated or monounsaturated fats for saturated fats in the diet is beneficial for the heart because it decreases blood levels of low-density lipoproteins (LDLs), also referred to as "bad" cholesterol and triglycerides. The basal metabolic rate is shown to increase and adipose tissue formation to decrease when medium-chain triglycerides (MCTs) are substituted for long-chain triglycerides (LCTs) in the diet. [41]

3. **Essential Amino Acid:** Only food can provide certain amino acids, which neither humans nor animals can produce on their own. Three essential amino acids—lysine (Lys), tryptophan (Trp), and methionine (Met)—are particularly crucial for biofortification due to their deficiencies in grains (lysine and tryptophan) and legumes (methionine). Transgenic wheat and rice have been produced via heterologous synthesis of the lysine-rich pea legumin protein in the endosperm [42]. The use of a seed-protecting protein derived from the plant Amaranthus hypochondriacus is another novelty. Each of the nine essential amino acids that humans require in significant amounts is present in this protein. Lysine biosynthesis and insect resistance are both increased by a stacking feature (the cry1Ab gene) in one of the two marketed maize events (ISAAA database 2019).

4. **Beyond Traditional Transgenic Technology:**

   - **Genome Editing:** Using genome editing technology, certain genes and/or other genetic components can be changed, deleted, or replaced permanently. This technique

modifies knock in proteins and accurately knocks down genes using synthetic oligonucleotides. It is possible to produce precise point mutations to the target DNA area by using sequence-specific nuclease (SSN).[43]. Recent advances in targeted editing using synthetic oligonucleotides have produced new single nucleotide polymorphisms (SNPs). These oligonucleotides include single-stranded DNA oligonucleotide molecules of 20–100 nucleotides and RNA/DNA chimeric oligonucleotides. ODM, or oligonucleotide-directed mutation, is the name of this process. Three other SSN variants are employed in genome editing in addition to ODM. Zinc-Finger Nucleases (ZFNs), Transcription Activator-Like Effector Nucleases (TALENs), and Clustered Regularly Interspaced Short Palindromic Repeat-associated Endonucleases (CRISPR/Cas) are a few examples of SSNs.

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

5. Genetic Engineering in Rice:

- **Herbicide-Resistant Rice:** Since only weeds are destroyed by herbicides in rice fields, herbicide-resistant rice may tolerate one or more particular herbicides. This herbicide-resistant rice type was developed to lessen weeds where rice is directly seeded. Malaysia, the US, and maybe other Asian countries in the coming years will be current markets for transgenic rice that is herbicide-resistant. This occurs following the use of transgenic soybean and maize.

  Clearfield rice was first produced in the USA in 2002 before being made available in Malaysia in 2010. It could be made available in other Asian countries during the coming years. The yields with Clearûeldrice rose by two times, from 3.5 to 7 metric tons per hectare, according to BASF Malaysia [45]. It is utilized more often than it was 12 years ago in Arkansas, where the bulk of the rice cultivated in the United States is grown [46].

6. **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.

7. **Abiotic Stress Tolerance in Genetically Modified Rice:** The main abiotic factors that have an impact on plants include ion toxicity or deficiency, extreme heat or cold, and water scarcity. These conditions limit growth and cause large output losses. Abiotic stressors, particularly salt and drought, are to blame for 70% of the decline in agricultural production. One of agriculture's main objectives has always been to increase resistance to abiotic stressors. Modifying particular genes with a target (metabolites or proteins) as a goal is the most common strategy for increasing abiotic stress tolerance in plants. [50]. A severe biotic stress called drought has a considerable impact on typical plant growth and development but minimal effect on crop yield. 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 are just a few of the numerous genes that code for various proteins involved in signal transduction and transcription control. These proteins are fully used to produce transgenic rice plants that are resistant to a variety of abiotic stresses.

Abiotic stresses like as heat shock proteins, abundant proteins, late embryogenesis, and molecular chaperones have a substantial impact on how plants respond. The over expression of HSP101 in transgenic Basmati rice plants significantly sped up the recovery of plant development following heat stress [53]. Numerous studies [54] have demonstrated that when plants are subjected to salt stress, their functionality is particularly improved by an increase in the amino acid proline. Transgenic plants that

over expressed the rice enzyme 1-pyrroline-5-carboxylate synthase (P5CS) were able to tolerate salt.

The adaptation of the C4 photosynthetic pathway into C3 crops, which will boost growth and output while increasing photosynthetic activity, is one of the main issues. The expression of genes for enzymes including phosphoenolpyruvate carboxylase (PEPC), chloroplast pyruvate orthophosphate dikinase (PPDK), and NADP-malic enzyme (NADP-ME) boosted rice's photosynthetic rate and output. [55].

8. **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 factor of more than 20.

- **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 tactic will be to genetically modify the regulatory and signaling networks. New genes involved in the metabolic pathways that follow the defensive signaling pathways can be found using genomics and proteomics approaches. By using these genes, it will be much easier to develop new rice types that are extremely resistant to a range of ailments and insect pests, maybe with long-lasting resistance. Additionally, allergy-free bio-fortified rice will soon be offered on a worldwide scale. Abiotic stress tolerance training using transgenic technology is beginning to gain traction. The RNAi technique is becoming more and more common for both function insertion and function removal. Many other nations have not embraced or authorized the commercialization of the GM crops that have been produced and approved in a few of those nations. For instance, the herbicide-resistant rice Clearfield® was solely distributed and marketed in the US. In a similar manner, insect-resistant Bt crops like Bt cotton and Bt brinjal were first introduced and even approved before they were once again forbidden due to the harm they presented to people 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 approach and the Agrobacterium-mediated transformation technique, both of which employ immature wheat embryo explants as explants and have a sizable enough number of successful occurrences. The efficiency of the transformation in these two methods, which employ immature embryos as explants, is greatly influenced by genotype. According to reports, the spring bread wheat cultivar Bobwhite has been used to produce a large number of transgenic wheat seedlings. [61]. Cell death occurs as a result of browning in growing wheat embryos caused by agrobacterium infections [62]. It is therefore difficult to find many transgenic plants. Even while this technology has come a long way, more effort is still required to combat browning, prevent the mortality of developing embryos, boost transformation efficiency, and quicken the rate at which resistant plants regenerate. [63].

- **Strategies for Producing Marker-Free Transgenic Wheat Plants:** Despite the fact that the inclusion of marker genes into the transgenic process has considerably increased transgenic efficiency, the existence and expression of these genes in plant genomes after selection raises concerns for human health and the environment. Few methods, such as co-transformation [64], site-specific recombination (Srivastava and Ow, 2004), and transposon-mediated eradication, have been suggested by researchers to remove selection markers. Flag genes for transgenic wheat plants have been removed by blasting the linear plant expression cassette. [65]. Another strategy involves utilizing an agrobacterium to alter 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]. However, there has been no mention of wheat. This method could someday be used to wheat to create transgenic plants completely devoid 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 creation of a successful in vitro regeneration system is essential for the transformation and recovery of transgenic millet crops. Numerous papers on millets' in vitro culture and different in vitro culture methods have been written. Somatic embryogenesis appears to be more advantageous for the advancement of in vitro regeneration strategies for the effective transformation and recovery of transgenic plants. Somatic embryogenesis and plant regeneration methods for pearl millet, finger millet, kodo millet, and foxtail millet have been created. For finger millet, we recently developed a method that is very similar to this one. genetic engineering of grains.

  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.

➢ **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%.

VI. CONCLUSION

To transfer essential genes and produce enhanced qualities, it is necessary to use Agrobacterium-mediated techniques on other significant millet crops. This is because the viability of Agrobacterium-mediated millet transformation has been demonstrated in these two foxtail millet investigations. In conclusion, millet transformation mostly uses physical gene transfer methods like electroporation and biolistics. Another problem with many claims of millet transformation is the measurement of marker or reporter gene expression. Recently, millets that have been genetically altered to express useful foreign genes have been commercialized. It will be crucial to extend research on the Agrobacterium-mediated transformation of other cereals to millets in order to eventually produce transgenic millets expressing essential foreign genes for agronomy. This will considerably increase millet productivity by enabling resistance to biotic and abiotic stresses.

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