TRANSGENICS TO GENOME ENGINEERING IN CROP IMPROVEMENT

Abstract

Crop development is crucial to fulfill food demand and increasethe nutrition of the rising population. Transgenic techniques in biotechnology and modern environmentally friendly novel breeding methods, including genome editing, provide practical methods for agricultural genetic improvement. The range of features that have been enhanced via transgenesis and genome editing technologies includes resistance to herbicides, climate change, pesticides, insects, and nutrition. Conventional breeding techniques have drawbacks including lots of resources and time along with biosafety issues. The recently developed genome editing technologies that alter DNA sequences using sequence-specific nucleaseshaveovercome these constraints. Modern genome editing methods accelerate crop development, offering precise, specific modifications to enhance yield and environmental resilience in contrast to conventional breeding procedures.This chapter provides an introduction to transgenic and gene editing technology and examines recent developments that have increased the effectiveness of genetic transformation and regeneration. Also tried to throw some light onhow related genetic engineering methods have been used to raise the yield and quality of different crops. The regulatory framework for crops that have had their genomes altered as well as high-throughput approaches for GE crop modification are also covered.

Keywords: Conventional breeding, Mutation breeding, Genetic engineering, genome editing

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I. INTRODUCTION

Why Transgenesis to Genome Engineering Technologies Are Needed?:

Malnutrition and food insecurity are presently among the most serious health problems, taking countless lives in countries that are developing. Our daily diet must contain a sufficient number of high-quality meals that are rich in all the necessary nutrients as well as foods that have additional health benefits. Even Because of the ongoing loss of arable lands and the presence of unfavorable circumstances like drought, salt, floods, diseases, and so on, maintaining the amount of food per capita that we currently receive will become an increasingly difficult task in the future. Modern breeding programs are required to find new genetic diversity sources in order to increase adaptability to abiotic or biotic challenges, produce performance and quality attributes, and deal with the growth of the human population and climate change. A decisive understanding of the genomes of plants has aided in achieving these objectives since the turn of the 20th century.Conventional breeding based on randomly induced mutations demands less work and technology used in the early stages of the program, but since the selection is primarily based on phenotype, it takes a consistent and prolonged effort to identify the genotypes that maintain the desired traits in a large progeny. Although MAS can greatly speed up the selection step, it requires a longer preparatory investigation to determine the genetic relationship between the phenotypic features and molecular markers. With the identification of the causative genes/alleles governing the trait, biotechnological strategies based on transgenesis need an even lengthier initial development of knowledge of the genetic basis of the many traits. In fact, in order to do transgenesis, the gene must be discovered, including in species that are sexually incompatible, cloned, and incorporated into the constructions that will be employed in the various techniques for genetic transformation.

In desired organisms, many metabolic and functional systems can work better due to genetic engineering. By using the concepts of genetic engineering, non-native genes can be expressed inside the host organism, resulting in the synthesis of protein products that were not previously available.Target genes' functions are ascertained through controlled expression techniques. Crops that have been genetically modified (GM) may prove to be effective complements to those grown using traditional techniques for supplying the world's demand for high-quality foods (Figure 1**)**. Genetically modified crops can be utilized to raise yields and nutritional value as well as their tolerance to a variety of biotic and abiotic challenges.

Additionally,GMcrops are the result of very targeted and precise genome change, with the end results, such as proteins, metabolites, or phenotypes, being extensively described. In conventional breeding, the genomes of the parents' respective offspring are combined and randomly rearranged. As a result, certain genes may be deleted in the offspring while other genes may be passed together with the favorable genes. Plant breeders perform repeated back-crossing to the desired parent to address these issues. This requires a lot of work and might not always be successful in separating a tightly linked unsafely coupled gene.In particular, for crops that have biosynthetic mechanisms known, gene editing is anticipated to be an efficient breeding method for changing the metabolism of nutritious functional components. With the use of a site-specific recombinase or site-specific nuclease system, a large range of tools are available for genome editing. Both systems demand the ability to recognize a familiar sequence. Single or double-strand breakage of DNA is produced by the SSN system, which also promotes endogenous DNA repair mechanisms. Depending on

the orientation of the particular sites (loxP, FLP, etc.) flanking the target site, SSR technology can knockdown or knockin genes in the entire genome of eukaryotes. Mega-nucleases (homing endonucleases), zinc finger nucleases (ZFNs), the CRISPR/Cas nuclease mechanism (clustered regularly interspaced short palindromic repeat/CRISPR-associated protein), and transcriptional activator-like effector nucleases (TALENs), are the four main classes of SSN developed to cleave genomic sequences.

High levels of homologous recombination are induced by recombinase-mediated genome engineering, which is dependent on the recombinase (sub-) family and target site. Although targeted gene editing has been possible for 20 years using transcription activator-like effector nucleases (TALENs) and zinc-finger nucleases (ZFNs), it has only recently gained attention due to the creation of CRISPR/Cas systems [1]. These systems make targeted gene editing simple and straightforward. Using genome-editing techniques in a range of plants, numerous gene knockout mutants, some gene substitutes, and insertion mutants, and many of these kinds of mutants have been found to be valuable for crop improvement. To address the issue of nutritional deficiencies, a number of biofortified crops, including rice, maize, and wheat, has been developed. Golden rice is a well-known example of a food that has been genetically altered to produce a substantial amount of beta-carotene in order to benefit persons who are at risk of a vitamin A deficiency.

II. FOOD SECURITY: CONFRONTING ONGOING ISSUES AND NEW DANGERS

Food must offer a sufficient amount of nutrients as well as calories to support life. Malnutrition is a hazard to millions of people around the world due to food insecurity, which results from limited access to adequate food supplies. The population of the world is predicted to increase quickly and reach 8.3 billion people around 2030 [2], making the issue worse. As a result, there will be a rise in consumer demand for livestock feed, food, and fuel. Abiotic stress increases led by climate change, reductions in arable land owing to deforestation, salinity, agriculture, and developing diseases have joined population growth as a threat to food security. Despite the projected concerns, such as climate change, the globe must double the present crop production rate to improve food security for future generations. To combat food insecurity, plant breeders have used both natural and chemical mutations as well as crucial strategies like breeding for hybrid vigor. However, more effort will be needed to address ongoing and future difficulties. The use of genetic modification and genome editing techniques attempts to enhance the total amount of food supplied per planted area and mitigate crop failures in order to increase agricultural production.

Breedershave focused on features that improve the number of grains grown per plant, the number of plants thatcan be farmed per unit area, and the size of each grain in order to increase yield per area in grain crops like rice. Many of these features require modifying the structure of the plant by coordinating hormone signaling and meristem activity. Breeders have focused on features that assist crops to withstand stressors in order to reduce crop failures and hence increase yield stability. Researchers have focused on the tolerance to heat, cold, high salinity, light, heavy metals, and other challenges when it comes to abiotic stress. Researchers have discovered loci providing resistance to different viral, bacterial, and fungal pathogens, as well as loci affecting responses to animal and plant pathogens, including nematodes and parasitic plants like Striga. Biologic stresses are also becoming more of an issue as globalization and weather accelerate the spread of pathogens. Finding the crucial loci to insert and quickly introducing those loci into elite cultivars are the two challenges in

disease resistance. Furthermore, it is still challenging to strike a balance between the energy needs for growth and resistance while minimizing yield losses. Genetic modification and genome editing attempt to increase the nutritional value of crops by supplying a variety of balanced diets with suitable amounts of minerals and vitamins that improve human health.

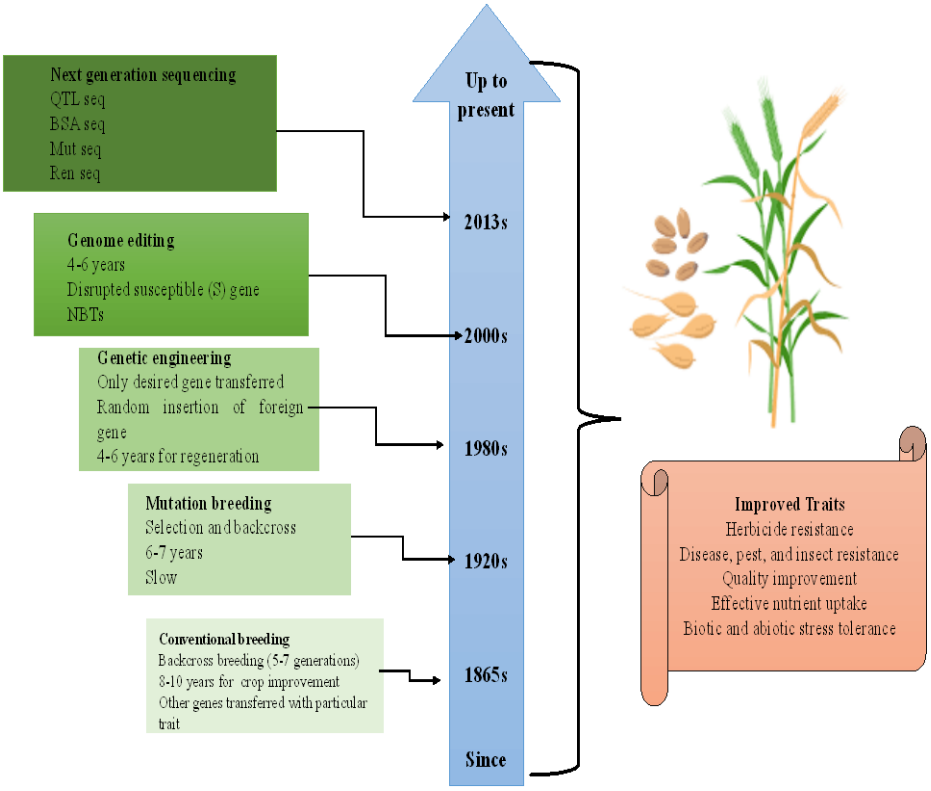

Figure 1: Transgenesis to genome editing technologies for crop improvement

III. GENETIC ENGINEERING TECHNIQUES FOR CROP IMPROVEMENT

Depending on the plant species, several approaches including *Agrobacterium tumefaciens*mediated gene transfer or direct gene transfer have been widely used to facilitate genetic improvement of the crops. Even though the recombinant DNA technique was invented in 1983, the first genetically modified crop was commercially launched in the middle of the 1990s, and at the present day, numerous transgenic crops have been created with tolerance to diverse biotic and abiotic challenges. The genetic modification of plants can take a variety of forms, from using *Agrobacterium*'s natural gene transfer mechanism to isolated protoplasts' chemical treatment with polyethylene glycol (Figure 2). A strong DNA delivery mechanism, advantageous target tissues suitable for efficient regeneration, and a highly repeatable and direct regeneration system to avoid somaclonal variations are essential for producing more productive transgenic plants. Alternatives to *Agrobacterium*-mediated gene transfer have been created, including protoplast and intact cell electroporation, polyethylene glycol-mediated (PEG) transfer, microinjection, and gene gun technology. These methods target specific plant cells and regenerate them into complete GM plants.

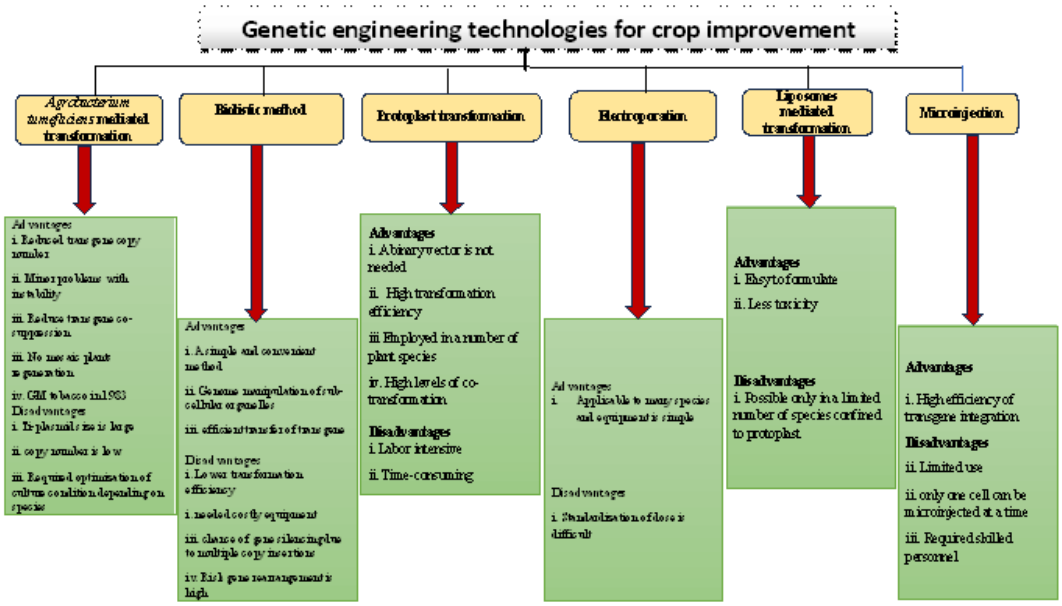

Figure 2: Advantages and disadvantages of genetic engineering techniques

IV. GENETIC ENGINEERING IN CROPS

The creation of pesticide and insecticideresistance in agricultural crops has been the earliest and most profitable application of GE (Figure 3). Tolerance to environmental shocks is currently being included in crop cultivars with a lot of interest in order to stabilize the yield under changing environmental conditions.Additionally, various transgenic genotypes with fortified nutritional contents have been made available to fulfill the eating preferences of naturalists and the growing interest in enhanced nutritive benefits of crops to fight malnutrition in poor nations. The development of crops with chemical components useful for industry and the use of plants as hosts for pharmaceutical items have both met with some success (Fig 3).The development of commercially available transgenic variants of several cropsis listed below (Table: 1).

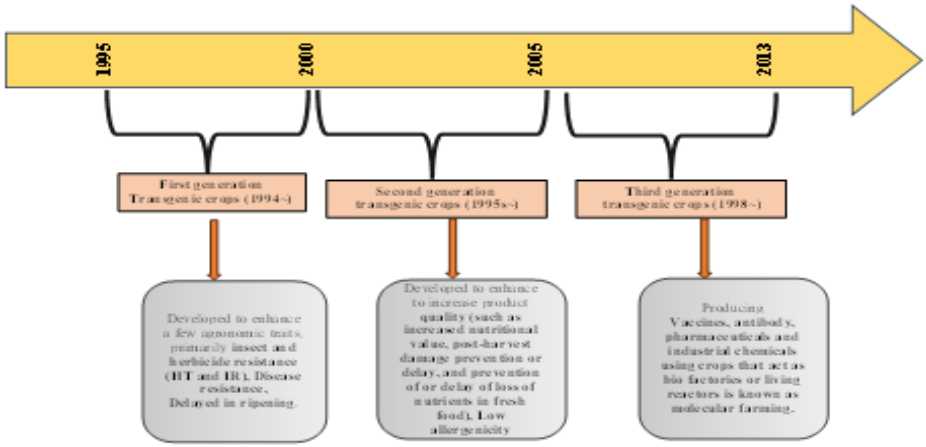

Figure 3: Generations and Trait Characteristics of Genetically Modified Crop Plants.

Table 1: GM Plants are currently being tested in Fields or in Clinics

Generations of transgenic crops	Introduced traits	Purpose	GM crops	Institution/company	References
First generation	Insect/Pest Resistance	*Bt*-(Cotton Bollworm, tobacco budworm and pink bollworm)	Cotton	Monsanto	[3]
		Bt gene (Colorado potato beetle)	Potato	Monsanto	
		Bt gene (Corn borer)	Maize	Northrup King (Sandoz)	
	Virus resistance		Tobacco	China	
			Tomato	China	
Second generation	High nutrient	High vitamin A content	Rice	SFIT*/UF*/Syngenta	[4]
		Ferritin-rich	Lettuce	CRIEPI*/NIAES*/STAFF*	
	Amino acid-rich	Lysine rich	Maize	Monsanto	
		Tryptophan rich	Rice	NICS*	
	Premium quality	High laurate content	Canola	Monsanto/Calgene	
		The high oleic acid content	Canola	Pioneer Hi-Bred	
		The high oleic acid content	Soybean	Dupont	
		Pectin rich	Tomato	Zeneca/ Kagome, Japan	
	Low protein	Low gluterin (rice		Orynova (Japan Tobacco), Japan	
	Low allergen	Low albumin	Rice	Mitsui Toatsu, Japan	
Third generation	Vaccine	Against E. coli labile enterotoxin	Potato	Arizona State University, USA	
		Against hepatitis B	Potato	Arizona State University, USA	
		Against Norwalk virus	Potato tobacco	Arizona State University, USA	

		Against rabies	Spinach	DBI*	
	Antibodies	Non-Hodgkin's lymphoma antibodies	Tobacco	Large Scale Biology Corp	
		Dental caries antibodies	Tobacco	Planet Biotechnology	
	Others	Lactoferrin production	Maize	Meristem therapeutics	
		Lactoferrin production	Rice	Japan AgriculturalCooperatives, Japan	
		Gastric lipase production	Maize	Meristem therapeutics	
		Human intrinsic factor production	Arabidopsis	Cobento	

V. APPLICATIONS OF GENETIC ENGINEERING IN CROP IMPROVEMENT

1. **Herbicides Resistance:** Numerous studies have shown that genetic material that has been introduced into specific crop species will combine with associated weed varieties. The majority of GE crops with herbicide resistance have been designed to be tolerant to glyphosate, sometimes known as RoundUp. Because certain herbicides can be utilized in conjunction with herbicide-resistant crops, studies show that these crops contribute to higher yields when weed control is enhanced. Weed species that are inherently less vulnerable to that herbicides can invade a field in areas where glyphosate is widely used. Some weeds have developed glyphosate resistance in numerous regions. Particularly in farming systems that are yet to be exposed to constant glyphosate treatments, integrated weed-management strategies can be employed to postpone resistance. Herbicide resistance currently dominates farmed GM crops and will do so for the foreseeable future. Commercial cultivation of GM crops resistant to glyphosate and glufosinate broad-spectrum herbicides began during the 1990s.

   Recently, evaluated biotechnological plants that had been commercialized and goahead for cultivation, including transgenic cotton, cowpea, maize, soybean, potato, and rice. Even though it is not now sold, perhaps due to potential market loss due to consumer resistance, the story of the glyphosate-resistant wheat created by Monsanto and permitted for testing in the field in sixteen different states in the US from 1998 to 2005 is well-known [5]. The glyphosate-resistant soybean, which is currently planted in many other nations, including Brazil and Argentina has established itself as a leading biotech crop since it was the first herbicide-resistant crop to be commercialized in the USA in 1996. With the help of this soybean variety, growers can use herbicides to eradicate any weeds in the cultivation area without harming the crop of soybeans. Pineapple has been noted as yet another excellent example of herbicide resistance. The tolerance of transgenic plants

to the herbicide "Basta" has been produced and tested using a bar gene for bialaphos resistance to herbicides.

2. **Abiotic Stresses Tolerance:** Abiotic and biotic stressors always present challenges to plants. Furthermore, the majority of features that are crucial for agronomy are under complex multigenic regulation and can be altered by the interactions between genes and the environment So, it is clear that pyramiding GM characteristics has benefits. To reduce biotic and abiotic yield losses, GM techniques are being used. Abiotic and biotic stressors constantly present to plants face them with challenges. Furthermore, the majority of features that are crucial for agronomy are under complex multigenic regulation and can be altered by the interactions between genes and the environment. So, it is clear that pyramiding GM characteristics has benefits. To combat heat stress in tomatoes, it has been suggested to overexpress ROS-scavenging enzymes. In order to increase their tolerance to heat (40℃), [6] created transgenic tomato plants that overexpressed the (cAPX) cytosolic ascorbate peroxidase gene. In comparison to fruits from wild-type plants, fruits from the field-grown genetically modified tomato plants displayed increased tolerance to exposure to direct sunshine. This suggests that the genetic engineering of such genes might be able to improve plants' resistance to oxidative damage and their performance under stressful field circumstances.

   Through the overexpression in the *Arabidopsis* genes HTT2 (Heat-Induced TAS1 target2), Chinese cabbage has also developed an improved resilience to severe temperatures in the field. Exogenous HTT2, which promotes thermotolerance by lowering electrical conductivity and lengthening the hypocotyl, enhanced the ability to survive the rate of heat-shocked headed Chinese cabbage. Furthermore, semi-dwarfism and a dense plant architecture linked to drought resistance as well as enhanced yield were seen in stable dehydration-inducible GmMYB14 overexpressing recombinant soybean plants in field settings.

   Studies on the overexpression of biosynthetic enzymes for scavengers of reactive oxygen species (ROS), stress-induced protein, osmoprotectants, and stress-related transcriptional factors have shown promising results in the improvement of stress tolerance in plants. In rice, tobacco, and potato, genetic engineering has been used to raise the amounts of proline, mannitol, glycine-betaine trehalose,and other osmoprotectants.

   The enzymes for glycine betaine production are 6WA and cd* (encoding betaine aldehyde dehydrogenase and choline dehydrogenase, respectively). Bacteria like *Escherichia coli* and *Halomonaselongata* are the source of the genes otsA (encoding TPS, trehalose-6-phosphate synthase), otsB (encoding TPP, trehalose-6-phosphate phosphatase), and ectA, B, and C (encoding enzymes for ectoine synthesis). A few genes from plants were also employed, including 7MT7 (which codes for the enzyme Myo-inositol O-methyl transferase), f J (which codes for the biosynthetic enzyme pyrroline-5-carboxylate synthase), and PJC (which codes for the enzyme pyrroline-5-carboxylate reductase) from the ice plant and moth bean. Results indicate that the expression of one or more foreign genes that encode biochemical pathways or signal pathway endpoints in plants has resulted in a slight accumulation of an Osmo protectant, which has risen the resistance to stress of the crop by adjusting the osmotic environment. For instance, mannitol accumulated in the leaves and roots of transgenic tobacco cultivars that overexpressed the mannitol-1-phosphate dehydrogenase (mtlD) gene from *E. coli*. The

modified tobacco plants were able to thrive in conditions of high salinity (250 mM NaCl) [7]. *Arabidopsis* showed comparable outcomes.

3. **Resistance to Biotic Stresses:** Transgenic plants created either by traditional gene transfer or RNAi gene silencing have enhanced tolerance to a variety of biotic stressors, including certain bacteria, nematodes, fungal infections, insects, and viral diseases. In comparison to studies aiming to generate crops enhanced for abiotic challenges, a wider range of investigations that have been published resulting in crop improvements for biotic stresses is accessible in the literature. Crops can now be genetically engineered to withstand disease, which is advantageous in terms of both cost and effectiveness. Numerous genes, including glucanase, chitinase, defensin, and osmotin, are being inserted into horticulture crops all over the world to confer tolerance towards fungal and bacterial diseases. The ability of several glycolytic enzymes to break down cell walls within plant cells, such as those produced by the genes chitinase, glucanase, PR proteins, etc., makes them attractive for application in the development of transgenic plants that incorporate resistance to fungi infections [8]. With hp-PTGS mechanisms targeting P1 and CP, a transgenic maize resistance to (MDMV) maize dwarf mosaic virus was created. All three generations of SRGE were used to develop transgenic wheat resistant to the Wheat streak mosaic virus, and newer versions appeared to offer a stronger defense. The Waikaviruses, Phyto reoviruses, Tungroviruses, and Tenuiviruses including the Rice Yellow Mottle Virus, Rice Stripe Virus, Rice *Tungro Bacilliform* (RTBV), and *Rice Tungro Spherical* (RTSV), posed the greatest viral danger to rice production. In Asia, these viral diseases significantly reduced rice output, leading to the creation of numerous resistant genetically improved rice lines employing the hp-PTGS process.

   A few of the resistant traits had been ingested into cultivars of rice. Another virus connected to TMV by genetic engineering is the ToMV virus, which increases plant tolerance to biotic stress. In tomatoes, the Tm-22 locus confers resistance to ToMV. Tm-22 gene transformation made susceptible crops tolerant to ToMV[9]. The locus Rx in potatoes is one that is known to give resistance to potato virus X (PVX) [10]. The Rx gene product detects a virus coat protein and stops the virus' initial development by a mechanism unrelated to cell death from hypersensitive reaction. Later, severe resistance was attained in both crops when the potato Rx was cloned and produced in *Nicotiana* and potato.

   Transgenic wheat plants that have a *Fusarium*-specific antibody linked with an antifungal peptide were developed in an additional effort to reduce *Fusarium* head blight. An antifungal peptide of *Aspergillus giganteus* was bonded to a chicken antibody. This technique offers a brand-new, efficient, and environmentally friendly potential for crop modification against diseases. Plant diseases can ruin agricultural output and jeopardize the safety of the world's food supply. Cloning of such genes would enable long-lasting R gene deployment tactics, which provide a cost-effective and environmentally responsible way to control plant disease. The majority of R genes produce proteins with leucine-rich repeats and nucleotide binding. Using biotinylated RNA oligonucleotides created to be analogous to the NLR expressing genes of a reference genome, R gene enrichment sequencing (RenSeq) of a given gene class requires collecting segments from a genomic or cDNA library.

4. **Insect-Resistant Crops:** Rapid outbreaks of these infections are becoming more frequent, and they place a significant cost on the environment, agricultural production, and human health. Insect-borne pathogen management tactics include population control as a key element. The use of genetically engineered insects to decrease or replace existing insect populations is a new technique that could be included in tactics for eradicating infections carried by insects. Planting insect-resistant seeds has a significant potential to strengthen crop protection from insect attack, regardless of the manner of agricultural genetic improvement. The main gene for the production of methyl salicylate, limonene, Ebf, a-bisabolene, (E)-b-caryophyllene, (E)- a-bergamotene and ahumulene from FPP is rice tps46 (Os08g0167800). Among these limonene, volatiles and Ebf constitutive emissions may result from tps46 constitutive expressions in rice grown under natural conditions.

   Furthermore, it is demonstrated that suppressing tps46 expression makes rice susceptible to attack by *R. padi*, an herbivore that often avoids wild-type rice. This result implies that TPS46 is crucial for rice's inherent defense against aphids[11]. In regards to crop productivity and financial gains for the farming community, the commercial use of crops resistant to insects harboring*Bt* genes has been exceptional. The majority of commercially available insect-resistant crops contain genes from *Bacillus thuringiensis*, it is crucial to emphasize here.

5. **Improvement of Quality-Related Traits**: Transgenic technology to insert the TmNAS3 gene within the wheat genome and utilized a ubiquitin promoter for driving the expression using Agrobacterium. The iron concentration of wheat grains increased to 68.75 µg/g, over double the amount of the wild type when the metal response-related genes were activated. Further research revealed that expression of TmNAS3 also resulted in larger wheat grains, which in turn boosted production [12]. The biosynthetic process of anthocyanins within rice endosperm and created rice which is rich in anthocyanins within the endosperm using eight genes relevant to anthocyanin synthesis has been created. It is important to note that anthocyanins have potent antioxidant effects and have several potential applications in the treatment of some malignancies and cardiovascular illnesses. RNA-mediated gene silencing approaches have been developed as a result of the discovery that certain parts of RNA can suppress transcription or translation activities. By using RNA interference to silence the Se1 gene in maize, were able to increase the soluble sugar content while also altering the endosperm's starch metabolism, making the mutant maize better suited for intestinal digestion. YetRNA-mediated gene silencing method typically cannot alter the target nucleotide in the genome; it can reduce geneexpression.Thus, the utilization of technology for gene editing broadens the range of genetic engineering's potential applications.Thus, the utilizationof technology for gene editing broadens the range of genetic engineering's potential applications. Thewell-known transgenic soybean example, which has higher oil quality (oleic acid content) and is good for human health, was made possible by RNA interference (RNAi)-mediated knockdown of Glycine max fatty acid desaturase2-1B (GmFAD2-1B), an essential enzyme that converts oleic acid (18:1) precursors to linoleic acid (18:2) in the lipid biosynthetic pathway [13].

   Unfortunately, agronomic features and the total protein and oil levels of the transgenic lines tested in field experiments did not significantly differ from those of the wild-type plants. Another illustration is provided as the use of sweet potato orange (IbOr)

protein, which is used to create simultaneous carotenoids and anthocyanins in the store roots of purple flesh sweet potato plants. The transgenic plants had higher carotenoid concentrations and carotenoid biosynthesis pathway gene transcription levels. When the plants were tested in the field, there was likewise no difference found when examining the production of root storage and aerial parts between transgenic and wild-type (WT) plants.Branch chain amino acids like methionine (Met), lysine (Lys), and tryptophan (Trp) cannot be synthesized by humans or animals. As a result, certain amino acids must be added to diets. Crops utilized for human consumption and animal feed are low or entirely absent in a number of critical amino acids. For instance, soybean lacks Met while maize lacks Lys and Trp.The first committed enzyme in the methionine synthesis pathway, cystathionine -synthase (CGS), was overexpressed in recombinant tobacco plants, demonstrating that a high level of T-AtCGS expression might result in greater overall Met concentrations in transgenic tobacco plants [14]. PrLeg gene transformation was reported to increase Met concentration in the tubers of the potato, which has low levels of sulfur-rich amino acids [15]. Research has shown that M-16, an 11-kDa protein containing significant sulfur amino acids, could be employed to increase the number of proteins in soybean seed.

VI. GENOME EDITING METHODS FOR THE IMPROVEMENTS OF AGRONOMIC TRAITS

Genome editing allows for the modification of an organism's DNA. At specific sites in the genome, these technologies enable the addition, removal, or modification of genetic material. There are several methods for genome editing that have been developed. CRISPR/Cas9 and TALEN are two good genetic modification techniques that have been shown to be effective instruments not only in the field of fundamental science but also in crop breeding. Genome editing technologies in general are also excellent genetic modification techniques. Recently, high GABA tomatoes and high oleic acid soybeans, two genome-edited crops intended to increase nutrition, were introduced to the market. Because of the mutation inducer, the modified foreign gene may be fully removed from all subsequent genome-edited hosts after inducing the mutation, genome-editing mutations are thought to be nearly comparable to random genetic mutations. The constraints of traditional breeding methods have been successfully overcome by genome editing, a revolutionary technology that uses sequence-specific nucleases (SSNs) for introducing targeted mutations in crops with great efficiency and precision [16]. SSNs that have been artificially created, such as transcriptional activator-like effector nucleases (TALENs), zinc finger nucleases (ZFNs), and clustered regularly interspaced short palindromic repeats (CRISPR/Cas9), have shown to be extremely effective in targeted mutagenesis in a variety of crops and model plants. In the target region of the DNA, the designed nucleases can cause double-stranded breaks (DSBs), which are then fixed by the cell's own inbuilt repair mechanisms of non-homologous end joining (NHEJ) or homologous recombination (HR).

Despite the fact that the first ten years of GE research were primarily focused on setting up the CRISPR/Cas editing system by making numerous technical advancements in its applications, the variety of academic publications explaining GE-edited plants for various traits of interest has recently been growing at a very rapid rate. Reports were presented on the general state of GE crops. The findings examined 231 studies conducted by governmental institutions and commercial businesses that involved 25 different nations, primarily the USA, China, Germany, Japan, the UK,and France. These studies involved twenty-five distinct

countries [17]. Up to this point, 41 crop plants and ornamentals, including two model plants (tobacco and *Arabidopsis*) and two crop plants (rice, tomato, and maize), as well as two model plants (*Arabidopsis* and tobacco), have been modified.Studies on kiwi, sugarcane, lettuce, peanut, poppy, lemon, salvia, banana, cacao, and manioc have also been conducted, albeit to a lesser level. Most relevant agronomic traits (storage performance, growth performance, yield increase, evaluated as grain size, weight, and number) concern GE applications with identified targeted trait improvements, followed by feed and food quality and nutritional traits (e.g., better digestibility, phytic acid, oil composition, increased vitamin content or reduced starch, and allergen content), traits for biotic stress tolerance (to virus, fungi, and bacteria), and traits for heritable resistance to biotic stress (to the virus, fungi Furthermore, several traits (growth performance, quality, herbicide tolerance, and yield improvement) have been modified at the same time [18].

VII. VAPPLICATIONS OF AGRONOMIC TRAITS THROUGH GENOME EDITING (GE)

1. **Yield and Yield-Related Traits:** Using GE to target many genes simultaneously to get a bigger impact on the intended phenotype is another technique to create mutants in various genes. To increase rice grain yield, a vector utilizing a CRISPR/Cas9 multiplex genome editing method that targets eight genes simultaneously was recently developed. Homozygous, sextuple septuple, and octuple mutants were discovered, and several of them increased yield-related parameters like grain length and width and thousand kernel weight when tested in the field. Similar to this, OsGS3, OsGW2, and OsGn1a, three yield-related genes that have been previously shown to adversely affect grain size, width, weight, and number, respectively, were simultaneously targeted in three elite rice varieties. The development of seven different single, double, and triple mutant combinations for the target genes had an impact on features such as grain width, number, length, and 1000-grain weight. Overall, the additive effects led to an increase in triple mutant panicle yield of up to 68% under field circumstances. These results demonstrate that GE is an extremely promising technology for simultaneously altering numerous genes and for producing a significant phenotypic effect under field conditions. By eliminating the waxy gene, CRISPR/Cas9 technology was used in maize to produce high amylopectin variants from improved cultivars.

   The modified maize cultivars produced 5.5 bushels per acre higher than the high-amylopectin variants that were bred traditionally. They could also be created faster, proving the viability of genome editing in specific, particular fields [19]. Additionally, rice plants' ABA response can be reduced to increase output. Rice plants that had class I PYL genes simultaneously mutated using CRISPR/Cas9 produced higher yields as compared to the control. Triple knockout of PYLs 1, 4, and 6 increased yield by 30% in well-watered conditions [20]. It is exciting to observe how the yield is impacted by these PYL genes that encode ABA in less ideal circumstances. According to a recent study, wheat PYL1-1B (TaPYL1-1B), which showed better ABA sensitivity, photosynthetic capacity, and WUE under drought circumstances, is responsible for enhanced production and drought resistance. The flowering repressor SELF-PRUNING 5G (SP5G) gene was knocked out to develop tomato plants with quick flowering, resulting in an earlier yield with determined growth [21]. This method also produced bigger yields of tomatoes. The morphology of the plant was altered to a bushier form with more branches as a result of mutations within the SELF-PRUNING (SP) gene, in

contrast. The resulting mutants with two changes had an earlier fruit ripening and flowering time than the control lines. Another study found that increasing tomato yield beneath heat stress required CRISPR-based SlAGL6 deletion. The tomato agl6 mutants produced fruit without seeds that were the same weight and shape as the WT and demonstrated facultative parthenocarpy lacking any pleiotropic effects.

2. **Tolerance to Biotic and Abiotic Stresses:** By focusing on the features mostly regulated by negatively regulatory genes, genome editing technologies have demonstrated considerable promise in improving crop resistance to a variety of biotic and abiotic challenges. For instance, the CRISPR-Cas9 technique has been effectively used by DuPont scientists to produce novel ARGOS8 variations, a negative regulator of responses to ethylene in maize crops. The 5′-UTR regions of the native ARGOS8 gene have been attempted to be replaced by the native maize GOS2 promoter or by homology-directed DNA repair. The research has aided in the development of novel ARGOS8 variations for the breeding of drought-tolerant maize crops. Similar to this, the targeted mutation of the rice gene OsERF922 that encodes for the ERF transcription factor increased resistance to the rice blast fungus disease [22]. Citrus cultivars' CsLOB1 canker susceptibility gene was altered in a recent study using CRISPR/Cas9 technology [23]. Three homeoalleles (TaMLO-A, TaMLO-B, and TaMLO-D) of the MLO gene, which provides resistance to the growth of powdery mildew in bread wheat, were effectively edited using the CRISPR/Cas system. The CRISPR/Cas9 technique has been used to increase crop survival under challenging environmental conditions. The CRISPR/Cas9 technique was used to create rice that is resistant to salinity. The researchers discovered that rice with the OsRR22 gene knocked out grew more vigorously than wild-type rice under salt conditions. The CRISPR/Cas9 technique was used to create a targeted osnac041 mutant that had a greater plant height than the wild type [24]. Other research has shown that the RAV (related to ABI3/VP1) transcription factor family members are involved in the adaptation to salinity stress. For instance, the OsRAV2 gene was activated when the rice was subjected to salt stress. sgRNA that targets the GT-1 region of the promoter in order to ascertain the function of the GT-1 element in the OsRAV2 gene. They discovered that the OsRAV2 gene was essential for responding to salt stress since the mutant lines were unable to express it.Salt-hypersensitive phenotypes were seen in the CRISPR/Cas9-mediated OsGT-2 knockdown lines. Other crops than rice have also benefited from CRISPR/Cas9 genome editing, including soybean, wheat, tomato,and maize.

Plant physio-biochemical functions are disturbed by drought stress, which limits plant development and yield. It has been demonstrated that a number of phytohormone signaling pathways and genes are essential for drought stress responses. One of these, abscisic acid (ABA), controls how much water is used and how the plant reacts to drought stress. Therefore, by focusing on the genes implicated in ABA signaling, various studies have been carried out to enhance drought tolerance in crops. For instance,OsABA8ox2, which encodes ABA 80 -hydroxylase, is a factor in rice's ability to withstand drought. The OsABA8ox2 deletion lines produced by CRISPR/Cas9 showed higher drought-induced ABA in roots and triggered root development that was helpful for drought tolerance, according to the authors. In contrast, rice overexpressing OsABA8ox2 demonstrated hypersensitivity to drought stress and reduced root elongation. The CRISPR/Cas9 technology was used to introduce a mutation in the protein Improved response to ABA1 (ERA1), which codes for the -subunit of the farnesyltransferase protein, in the *Japonica rice* cv. Nipponbare [25].

Due to the improved ABA sensitivity and drought tolerance of the rice osera1 mutant lines, ERA1 may be a suitable gene for improving drought tolerance in crops. Another work by [26] revealed that the stomatal density (SD) of the CRISPR/Cas9-edited rice plants was more than eight times lower in the OsEPFL9 (Epidermal Patterning Factor like-9) mutants. The modified rice lines may withstand drought stress thanks to the decreased SD. When SD was reduced by 50% in barley and wheat, it significantly reduced carbon assimilation and conductance and improved water use efficiency (WUE) under ideal conditions[27]. Similar to this, in well-watered conditions, a CRISPR-based grapevine VvEPFL9-1 deletion lowered SD by 60% and resulted in reduced carbon absorption compared to WT. In tomatoes, slmapk3 mutants created using CRISPR/Cas9 demonstrated that SlMAPK3 is implicated in drought response; under drought stress, the slmapk3 mutants displayed more pronounced wilting signs and experienced cell membrane damage. CRISPR/Cas9 technology was employed in several research to lessen mineral toxicity. For instance, [28] created low cesium-containing rice seedlings by employing the CRISPR/Cas9 system to inactivate the K+ transporter OsHAK1. In rice, OsARM1 and OsNramp5 knockouts demonstrated enhanced arsenic tolerance and decreased cadmium accumulation, respectively.

3. **Improving Resistance to Plant Pathogens:** Utilizing CRISPR/Cas9, genome editing in rice has produced impressive results in the fight against illness. The SWEET gene family in many plants encodes sucrose transporters, which are used by the majority of pathogens. To build resistance to bacterial leaf blight, CRISPR/Cas9 was used in two trials to target the promoter coding area of a few OsSWEET genes. By utilizing CRISPR/Cas9 to disable the OsERF922 gene, which the plant uses to express the ethylene response, the disease's effects on the plant were lessened, increasing its resistance to it [22]. Additionally, plants resistant to the rice tungro virus were produced by CRISPR/Cas9 editing of the eIF4G eukaryotic elongation factor in rice [29]. This approach is demonstrated by the editing of the rice gene bsr-k1, which binds to and promotes the amplification of defense-related genes. Edited rice plants were resistant to both leaf blast and bacterial leaf blight by "turning off" these essential defense genes. The transgenic lines exhibit a better yield of 50% when exposed to rice leaf blast in the field without influencing other agronomic characteristics [30]. A similar approach has also been used on other crops to increase their resistance to disease. For instance, in tomatoes, a single locus was changed to produce broad-spectrum resistance.

The edited lines of the SlDMR6-1 mutations by CRISPR/Cas9 develop resistance to *Phytophthora capsici*, *Pseudomonas syringae*, and *Xanthomonas* spp. while maintaining a higher salicylic acid level in the plant and significantly reducing disease symptoms and pathogen abundance [31]. By altering the defense-related gene MORC1 in barley, researchers were able to boost the plant's resistance to *Fusarium graminearum* and barley powdery mildew [32]. The authors also demonstrated that the altered barley plants contained less fungal DNA and had fewer blemishes. Targeting the Mildew-Resistance Locus (MLO) and other loci's homologs improved the resistance to various fungi infections in several species. Wheat can become more resistant to powdery mildew by having CRISPR/Cas9 simultaneously target the three MLO homologs, TaMLO-A, TaMLO-B, and TaMLO-D. Another illustration is the Tomelo transgene-free tomato, which was created by employing CRISPR/Cas9 to target the SlMlo1 gene and is resistant to powdery mildew disease [33]. To increase resistance to the powdery mildew disease, simultaneously modified the three homologs of the wheat TaEDR1 gene.

Using CRISPR/Cas9 technology, [34] altered the rice cv. "Zhonghua 11" genome to prevent the sugar transporter OsSWEET14 from carrying out its normal functions. This gene has been identified as a key susceptibility gene for the bacterial blight (*Xanthomonas oryzae*pv. oryzae, or Xoo) disease. Edited plants demonstrated a high level of resistance to the Asian Xoo strain PXO86 and the African Xoo strain AXO1947. OsSWEET14 function was disrupted in the field, increasing plant height without affecting yield. In other research, the impact of GE-driven mutations on the target attribute was directly assessed by field trials. By focusing on three recognized broad-spectrum blast-resistant genes in rice, Bsr-d1, Pi21, and ethylene-responsive factor 22 (ERF922), [35] created single and triple mutants.

The best blast resistance was shown by the erf922 mutants, which were similar to triple mutants. This was likely caused by the overexpression of genes linked to the SA- and JA-pathways, but all single and triple mutants showed greater resistance to rice blast compared with wild-type plants. GE has already targeted the OsERF922 gene to increase resistance to rice blast [22]. Both controlled and field environments were used in both investigations to examine mutant plants for disease response. Additionally, the field tests made clear that there were no compromises made between resistances and essential agricultural features. These findings are quite intriguing and pave the way for a more extensive and long-lasting analysis of these mutant materials to determine the effects of these mutations on disease resistance over a wide range of regions and years. In fact, a long-lasting broad-spectrum resistance is anticipated for genes like OsERF922 that do not code for tight pathogen-receptor-specific nucleotide-binding domain leucine-rich repeat (NLR) receptors.

4. **Improvement of Quality-Related Traits:** The development of high amylose-containing rice plants by targeting starch-associated genes (SBEII b and SBEI) by CRISPR/Cas9-mediated genome editing has made it feasible to improve the nutritional qualities of starch in rice grains. Myo-inositol 1, 2, 3, 4, 5, and 6-hexakisphosphate, an anti-nutritional molecule called Phytic acid (PA), was discussed in relation to maize. The PA content of maize seeds was decreased by developing two gRNAs that target the ZmIPK (inositol phosphate kinase) gene, which catalyzes a crucial step in the PA biosynthesis pathway. PA is poorly digested in humans and poses harm to the environment. One of the most crucial quantitative characteristics in the production of rice is grain weight. Rice production can be enhanced by increasing the grain weight. Targeting three important genes (GW2, GW5, and TGW6) that adversely affect rice grain weight, CRISPR/Cas9-mediated multiplex editing of genes was applied for quick pyramiding to increase grain weight in LH422 [36].DET1 gRNA1 has the highest genome editing efficiency. To investigate the effects of mutations on tocopherol accumulation, *N. benthamiana* leaves were infiltrated with GmDET1-GFP OE and GmDET1 gRNA1 constructs. Results showed a significant increase in tocopherol content (51.74 ppm) compared to the control (45.6 ppm). also found a similar trend in tocopherol accumulation in transient *N. benthamiana*leaves infiltrated with GmDET1-GFP OE and GmDET1 gRNA1 constructs. Silencing the DET1 gene through the RNAi approach increased the expression of photo-regulated genes involved in the antioxidant compound synthesis, leading to significant increases in tocopherol, isoflavones, carotenoid, saponins, and anthocyanins[37].

VIII. IMPROVEMENT OF AGRONOMIC TRAITS THROUGH INTRAGENESIS /CISGENESIS

GMOs (genetically modified organisms) may provide a solution to a variety of pertinent issues impacting agriculture. However, because there is foreign DNA present, agricultural improvement by GMOs is frequently linked to safety worries, environmental dangers, and health issues. Alternative technologies have emerged as a result of these restrictions. *Cisgenesis* and *intragenesis* are two new techniques that have just been created with the intention of modifying crops. *Intragenesis* is the transfer of novel gene and regulatory sequence combinations specific to that species, as opposed to *cisgenesis*, which entails genetic modification via a whole copy of natural genes along with the regulatory elements that exclusively belong to sexually compatible plants. Because we don't yet fully understand the necessary regulatory sequences, the use of *cisgenesis* and *intragenesis* as substitutes for traditional transgenesis is now restricted to a small number of species. The grape is among the most economically important crop and one of the most widely farmed crops in the world. Its genomic sequencing has been finished, opening up new sources of knowledge for genetically modifying grape qualities to improve their characteristics. Several features have been cis- or intragenetically integrated into pertinent crops. Several features have been cis-or intragenetically integrated into pertinent crops. These species consist of potato, strawberry, apple, alfalfa, barley, and durum wheat.

To produce a high amylopectin content, the first intragenic potato was created. The granule-bound starch synthase gene (GBSS), which is in charge of producing amylose in potatoes, served as the foundation for this strategy. The starch composition of potatoes is a crucial characteristic; however, it is currently challenging to grow tetraploid potatoes having the required levels of amylose and amylopectin. In order to generate tetraploid cultivars with all the necessary features present in the original cultivar, techniques to mute either amylopectin or amylose or artificial genes must be employed. A company called AVEBE released this potato into the field in the European Union in 2007 (B/NL/07/04), and it has T-DNA boundaries, a potato GBSS terminator or an *Agrobacterium tumefaciens* nopaline synthase gene termination for controlling gene expression. For instance, the polyphenol oxidase gene (PPO), that catalyzes the oxidative degradation of cytoplasmatic polyphenols and results in a precipitation of melanin and compromises tuber quality during storage, was silenced to reduce enzymatic browning. By introducing multiple copies of the native phytase gene, cisgenic barley was created. Phosphorus is released from phytic acid by phytase, making it available for animal absorption. In order to avoid adding phytase from microbial sources to feed and to lessen the current phosphate-related environmental pollution, this approach has been found to be effective in boosting phosphate bioavailability. The late blight disease, which affects potatoes all around the world, is brought on by the oomycete (fungus-like microorganism) *Phytophthora infestans*. R-genes, which encode proteins that give tolerance to late blight through hypersensitivity reactions, are found in many wild potato species. Through conventional breeding, R-genes from wild species have been included in potato cultivars. The initial crosses between wild species and cultivated potatoes are challenging because wild species have different ploidy levels from the grown tetraploid potato, which is why this method takes a very long time (up to 50 years).

High-Throughput Approaches for Modifying Crops through GE: The use of gene editing techniques is becoming a potent tool for altering specific genes in organisms. Although numerous techniques have been created to identify creatures that have had their genes altered,

these methods require a lot of time and effort. There haven't been many studies that look into high-throughput identification and screening methods for plants that have undergone gene editing. In order to detect both a wild-type and a gene-edited mutant, the qPCR-based approach makes use of two separately labeled probes that are inserted into a single product at the gene-editing target site. In numerous different plant species, including *Oryza sativa*, *Arabidopsis thaliana*, *Sorghum bicolor,* and *Zea mays*, we demonstrated that the qPCR-based technique can accurately differentiate CRISPR/Cas9-induced mutants from the wild-type. By directly sequencing the qPCR products of mutations caused by gene editing, the approach can also later identify the mutation type. In T0 transgenic plants, the qPCR-based technique is also sensitive enough to differentiate between heterozygous and homozygous mutations. In order to reduce public fear and settle legal disputes, there is a need for cost-effective GMO testing that may make it simpler to evaluate hazards, manage them, and monitor them after their release. This is due to the ongoing growth in GMO production as well as the diversification of traits across the globe. Today, a variety of molecular methods are available to assess the presence or absence of GMOs in samples, as well as to identify and quantify them.

However, recent highly quick and convenient technologies that are now certified globally for GM detection have replaced the time-consuming traditional PCR and ELISA-based procedures. It is envisaged that these more current methods, which are capable of absolute quantification and can produce a significant amount of data in a single experiment, will soon occupy their rightful place in the field of GMO detection and quantification. The findings made here highlight the importance of field testing of genetically engineered plants, particularly in trials conducted in a variety of settings, in order to understand how these mutations affect crops' agronomic performance traits and identify the most promising breeding approaches. A highly intriguing advance in the near future would be to evaluate, in extensive field trials, a wide range of various GE-modified lines, with various mutations in a wide range of different genes, in order to pinpoint the most productive plants. A high-throughput rice CRISPR/Cas9 mutant library that was helpful for discovering gene functions with significant potential for genetic improvement. Nearly 13,000 genes that have high levels of expression in rice shoots were chosen for targeting out of an initial list of over 50 thousand rice genes. A random sample of 200 T_0 lines out of 14,000 was phenotypically assessed in the field, and fascinating traits that might be connected to altered genes were found. The mutant library is a highly promising tool for genomics research and breeding that will soon be applied to other crops as well. The data was of excellent quality, had good coverage, and was distributed uniformly.

IX. CONTRIBUTING TO A SCIENCE-BASED REGULATORY FRAMEWORK FOR GENOME-EDITED CROPS

The development of novel breeding technologies and the crops and products that result from them depends on the regulatory and popular acceptance of genome-edited plants, but these processes are still challenging. Genome-edited crops are currently regulated using process- or product-based regulatory techniques. While Canada, the United States, and Argentina support the product-based approach, the European Union employs process-based regulations, and the majority of other nations have not yet developed their regulatory structures. Using the product-based strategy, numerous modified plants have already received complete approval. The majority of the genome-edited plants in the regulatory pipeline awaiting approval were submitted by public research organizations and small to medium-

sized businesses. However, if a rigid regulatory approach is used and modified plants are treated as GMOs, it would result in enormous cost constraints that would only be acceptable to major multinational corporations. For transgene-free modified products, policies should be reasonable and simple to understand. A comprehensive, universal regulatory strategy might not be appropriate because genome editing is a molecular toolbox rather than a single technology. To accommodate both current and emerging technologies, a tiered regulatory structure should be adopted. To maintain open communication and regulatory openness, further work is required. Fact- and science-based public communication is preferred [38]. The demand for expanded food production is greatest in developing and impoverished nations, thus we should open the discussion and engage with all viewpoints. However, it will undoubtedly be difficult to resolve the competing interests of various parties.

Under the "Manufacture, Import,Export, Use, and Storage of that are harmful Microorganisms/ Genetically Engineered Organisms or Cells, Rules, 1989" published by the Ministry of Environment, Forest and Climate Change (MoEF&CC), Government of India, all activities involving genetically engineered organisms or cells and potentially hazardous microorganisms and products thereof are subject to regulation in India.The Ministry of Environment, Forests, and Climate Change issued Office Memorandum F. No. C - 12013/3/2020-CS-III dated 30.03.2022, which limits the regulation of the act of genome editing of plants being carried out under containment, until free from exogenous implemented DNA, to be applied to all rules under Rules, 1989 with the exception of rules 7 – 11 (both inclusive), on which exemption from regulation has been granted under Rule 203.These regulations shall be effective for all currently underway, duly approved plant genome editing activities conducted in containment settings under the direction of the appropriate IBSCs, as well as for those that will be conducted moving forward in India. The RCGM may create standard operating procedures (SOPs) and criteria that must be followed for IBSCs to be able to regulate biosafety when necessary.

X. CONCLUSIONS

The use of plant biotechnology could help with a number of issues facing agriculture and society. Utilizing excellent zinc, iron, proteins, anthocyanins, vitamins, carotenoids, and other nutrients to enrich food crops, GM methods are utilized to reduce production losses caused by a variety of challenges. GM crops can help commercial agriculture, overcome a number of present problems. They are expected to be among the most creative and rapidly expanding global industries, with advantages for growers, consumers, and major national economies. New GM crops with stacking HR characteristics and GM cultivars with improved glyphosate resistance are being developed commercially. This strategy won't, however, result in a decrease in the overall amounts of herbicides used in agriculture. A wide range of prospects for plant breeding is made possible by the advancement of genome editing technology in plants. Genome editing's effective, focused, and targeted mutagenesis has created the groundwork for a number of next-generation breeding techniques that will transform agriculture in the future. All methods must be investigated in order to utilize plant genome editing to its fullest potential. Crops can be intelligently developed with a variety of genetic features thanks to genome editing. When employed for quick plant breeding, these accurate and effective approaches produce results that are comparable to those of traditional breeding. Therefore, it could be argued that achieving food security for the present and future generations can be accomplished through the sustainable integration of traditional agricultural practices with contemporary biotechnology. However, it is crucial that the growth and

development of a GM crop undergoextensive biosafety assessments on individual cases and are closely monitored for multiple generations underneath field conditions before being approved for commercial cultivation. In order to maximize the potential of biotechnology for the benefit of humanity, GM crops must become an integral part of our daily lives.

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