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19. Genetic Engineering for Enhancing Crop Traits

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Abstract:

Feeding the ever-growing population is a major challenge, especially in light of rapidly changing climate conditions. Genetic engineering has revolutionized agriculture by employing advanced techniques such as recombinant DNA technology, gene cloning, and genome editing tools, this genetic engineering techniques allows for the direct manipulation of DNA, which includes inserting new genes, deleting or silencing existing genes and editing genes to alter their function to achieve desired traits. Genetic engineering for crop improvement is a transformative approach that enhances agricultural productivity and sustainability as it enhances desirable traits such as increased yield, pest resistance, and tolerance to environmental stresses like drought and salinity and optimize nutritional content, thereby contributing to global food security. This technology allows for precise and targeted alterations, bypassing the limitations of traditional breeding methods. Genetic engineering has also enabled the development of crops with enhanced nutritional content, such as vitamin-enriched rice. However, the application of genetic engineering in agriculture raises ethical, ecological, and socio-economic concerns, particularly related to the potential impacts on biodiversity, food security, and the environment. Despite these challenges, genetic engineering holds significant promise for addressing global food demands and mitigating the effects of climate change, making it a crucial tool for the future of agriculture.

Keywords:

Genetic engineering, recombinant DNA technology, genome editing, gene cloning.

19.1 Introduction:

The practice of agriculture has been fundamental to human civilization, providing the food and resources necessary for societal development. As the rapidly increasing global population and a many competitive dairy products and meat are driving the agricultural

production and expanding the demand for feed, food, biofuels, and livestock (Ray *et al*. 2013). The global population is projected to reach over 9 billion (cucina *et al*. 2021) by 2050, leading to a potential 100–110% increase in demand for crop production. This will result in a 38–67% rise in the effective production of staple crops like rice (*Oryza sativa*), wheat (*Triticum aestivum*), maize (*Zea mays*), and soybean (*Glycine max*). (Ray *et al*. 2013; Röös *et al*. 2017). Furthermore, the ongoing increase in population has a specific influence on the climate, the environment, and the amount of arable land. These factors will ultimately impact agricultural yield (Tian *et al*. 2021). In certain regions, the fundamental issue of sustenance is no longer a cause for worry, but hidden hunger (insufficient intake of essential nutrients and trace elements in the human body) remains a secondary problem (Zhu *et al*. 2017). Enhancing crops in terms of production, nutritional value, resilience to biotic and abiotic stress, and environmental adaptation can help address these global issues.

Crop improvement encompasses a variety of methods, both traditional and modern, aimed at enhancing the characteristics of plants, which are benefitable for human needs. The primary methods include traditional breeding, mutagenesis, polyploidy induction, tissue culture techniques, and genetic engineering. Traditional breeding methods, while effective, have limitations in terms of time, as the process is time-consuming, often taking many years to develop a new variety and also, it relies on the natural genetic variation within a species, which may not always encompass the traits needed to address specific challenges, such as resistance to new pests or diseases. These challenges necessitate innovative solutions to enhance crop yields, nutritional value, and resilience to environmental stresses. One such solution is genetic engineering, a powerful tool that has revolutionized the field of crop improvement.

The genetic engineering technique allows for the direct manipulation of DNA. This includes inserting new genes, deleting or silencing existing genes and editing genes to alter their function to achieve desired traits, commonly referred to as genetic alteration. In 1983 the first genetically modified and herbicide-resistant tobacco was developed (Herrera-Estrella *et al*. 1983), marking the beginning of era of plant genetic engineering. The first batch of transgenic crops was commercially cultivated in 1996 (Mackelprang *et al*. 2021), and genetically engineered crops started being introduced to the market. Since then, rapidly developing genetic engineering technologies have played an irreplaceable role in improving crop yields and quality.

Genetic engineering for insertion of novel genes for an organism from related or unrelated organisms relies on several techniques like *Agrobacterim* and Virus mediated gene transfer, gene gun method, electroporation, microinjection, PEG method and many more whereas, to delete or silence the existing genes and editing genes of an organism relies on gene editing tools like zinc-finger nucleases (ZFNs), transcription activator-like effector nucleases (TALENs) and clustered regularly interspaced short palindromic repeats (CRISPR) techniques (Gaj *et al*. 2013).

Genetic engineering is a transformative approach to crop improvement and can be complement to traditional methods as it offering solutions to many of the challenges faced by traditional breeding methods in terms of speed, precision and the ability to create genetic variation. Genetic modifications in crops have enhanced yield and productivity, pest and disease resistance, herbicide tolerance, improved nutritional content, enhanced tolerance to

abiotic stresses like drought and salinity, post-harvest improvements, production of pharmaceuticals and industrial products, phytoremediation *etc*.

Genetic engineers have pioneered techniques in genetic recombination to modify gene sequences in a variety of organisms, including plants and animals, enabling the expression of specific characteristics. The field of genetic engineering is expanding as interdisciplinary teams of engineers and scientists collaborate to pinpoint the positions and functions of distinct genes within the DNA of various organisms. After the classification of each gene, engineers devise methods to modify them, resulting in the creation of organisms that offer advantages, such as cattle that yield larger quantities of meat, bacteria capable of producing fuels and plastics, and crops resistant to pests.

While genetic engineering offers significant benefits, it also presents challenges and considerations like biosafety and regulatory issues, ethical and socioeconomic concerns, gene flow and resistance development among the crops.

In general, genetic engineering represents a transformative approach to crop improvement, offering unprecedented opportunities to enhance agricultural productivity, sustainability, and resilience. As we navigate the complexities and controversies associated with this technology, it is essential to weigh the potential benefits against the risks and ensure that its development and deployment are guided by sound science and ethical considerations.

Table 19.1: History of Plant Genetic Engineering in Crop Improvement

1953	:	Watson and Crick unveiled the DNA double helix structure, which provided the molecular basis for inheritance of genetic information.
1973	:	Stanley Cohen and Herbert Boyer develop the technique of recombinant DNA, allowing DNA from different organisms to be spliced together. This marks the beginning of genetic engineering.
1977	:	Plant transformation techniques for inserting foreign DNA into plant cells are developed, primarily using *Agrobacterium tumefaciens.*
1982	:	First genetically modified (GM) plant created by inserting an antibiotic resistance gene from bacteria into a tobacco plant using *Agrobacterium tumefaciens*.
1983	:	The U.S. Supreme Court allows the patenting of genetically modified organisms, leading to the first patents for genetically modified plants.
1986	:	The first field trials of genetically modified plants began to test herbicide-resistant tobacco plants.
1994	:	The Flavr Savr tomato becomes the first genetically engineered food crop approved for commercial production and sale in the U.S., designed to have a longer shelf life.
1995	:	The U.S. approves the commercial cultivation of Bt corn, which is engineered to express a bacterial toxin that protects the plant from insect pests.

1996	:	Monsanto introduces Roundup Ready soybeans, which are engineered to be resistant to the herbicide glyphosate, allowing farmers to control weeds without harming the crop.
2000	:	Golden Rice is engineered to produce beta-carotene, a precursor to vitamin A, aimed at reducing vitamin A deficiency in developing countries.
2002	:	GM cotton was first approved in India.
2003	:	Global adoption of GM crops increases rapidly, with countries like the U.S., Brazil, and Argentina leading in the cultivation of GM soybeans, corn, and cotton.
2004	:	Europe establishes strict regulations on the cultivation and import of GM crops, reflecting public concerns about the safety and environmental impact of genetic engineering.
2013	:	The CRISPR-Cas9 technology emerges as a revolutionary tool for genetic engineering, enabling precise and efficient modification of plant genomes.
2016	:	The Arctic Apple, a non-browning apple genetically modified to resist browning after being cut, is approved for sale in the U.S.
2018	:	The U.S. Department of Agriculture (USDA) announces that it will not regulate plants that could have been developed through traditional breeding methods but were created using gene editing, paving the way for faster commercialization of gene-edited crops.
2020	:	Gene editing, using CRISPR and other techniques, begins to be used for crop improvement, with several gene-edited crops, like soybeans with healthier oil profiles, entering the market.
2021	:	The first CRISPR-edited crop, a high-fiber wheat variety developed by the company Calyxt, is commercialized in the U.S.
2021	:	Researchers increasingly focus on using gene editing to develop crops that are resilient to climate change, including drought-resistant maize and heat-tolerant wheat.
2022 and beyond	:	Advances in synthetic biology enable the creation of novel plant traits, such as enhanced photosynthesis and biofortified crops, pushing the boundaries of what is possible in plant genetic engineering.

19.2 Genetic Engineering:

Genetic engineering, also known as genetic modification or genetic manipulation, is a set of techniques and technologies that allow scientists to directly manipulate an organism's DNA (deoxyribonucleic acid) to modify its genetic makeup (Lodish *et al.* 2000).

DNA is the molecule that contains the genetic information of living organisms and is responsible for their traits and characteristics. Genetic engineering enables scientists to introduce, delete, or modify specific genes within an organism's DNA.

19.2.1 Techniques in Genetic Engineering:

A. Recombinant DNA and Gene Cloning Technology: This involves cutting and splicing DNA from one organism and inserting it into the DNA of another organism. This can be done to introduce desired traits or characteristics into the recipient organism

Recombinant DNA technology: Recombinant DNA technology is a powerful and fundamental technique in molecular biology that allows scientists to manipulate DNA molecules, specifically combining DNA from different sources to create novel genetic combinations. It involves cutting and splicing DNA fragments from one organism and inserting them into the DNA of another, resulting in recombinant DNA molecules. This technology has had a profound impact on various fields,

The Key Steps in Recombinant DNA Technology are as Follows:

1. Isolation of DNA: DNA is extracted from both the source organism containing the gene of interest and the recipient organism or vector.
2. Cutting DNA: DNA molecules are cleaved at specific sites using restriction enzymes, which act like molecular scissors. This creates fragments with "sticky ends" that can base-pair with complementary ends of other DNA fragments.
3. Insertion of DNA: The gene of interest or a DNA fragment from the source organism is inserted into the DNA of the vector. This can be done by matching the sticky ends and using DNA ligase to seal the junction, forming a recombinant DNA molecule (Venter *et al*. 2007).
4. Selection of host cells transformed by recombinant DNA.
5. Identification of clone having the gene of interest and isolation of gene.
6. Replication and cloning: The recombinant DNA is introduced into a host organism, often a bacterium (such as *Escherichia coli*), which can replicate the recombinant DNA along with its own. This allows for the production of multiple copies of the inserted DNA
7. Expression of genes: Depending on the application, the cloned DNA can be expressed, leading to the production of specific proteins. This is particularly important in biotechnology for producing pharmaceuticals, enzymes, and other valuable products (Perlak *et al*. 1990). Recombinant DNA technology has had significant applications in agriculture, leading to the development of genetically modified (GM) crops with various traits and characteristics. These genetically engineered crops aim to address agricultural challenges, improve crop productivity, and reduce the need for chemical pesticides.

Plant genetic engineering involves transfer of specifically constructed gene assemblies into plants through various transformation techniques. There are two main methods of plant transformation *viz*; direct and indirect DNA transfer methods. Direct DNA transfer methods include Particle Bombardment or Biolistic Method, Electroporation, Liposomes, Chemical mediated DNA transfer, Microinjection, Sonication and Silicon carbide whiskers, while, indirect DNA transfer include *Agrobacterium tumefaciens* mediated transformation and virus mediated transformation. These techniques will be discussed in detail in subsequent sections.

Direct Gene Transformation Methods:

The term direct gene transfer refers to the introduction of naked foreign DNA directly into the plant genome. It can be done either in protoplast or plant tissue.

I. DNA Transfer in Plant Tissue:

a. Particle Bombardment (Biolistic):

In biolistics technology, DNA is coated onto gold or tungsten micro-particles and bombarded at high velocity in a stream of helium into intact cells or tissues (Sanford *et al.* 1987; Sanford 1990; Fu *et al* 2000; Wu *et al.* 2016). Biolistic process is subdivided into two stages: (i) coating metal particles (microprojectiles) with nucleic acid, and (ii) accelerating the coated microprojectiles to velocities appropriate for penetration of target cells or tissues without excessive disruption of biological integrity (Sanford *et al.* 1990).

b. Silicon Carbide Whiskers:

Silicon carbide readily gives sharp cutting edges due to its great intrinsic hardness and fractures (Greenwood *et al*. 1984). The study using scanning electron microscopy on whisker-treated BMS ceils described by (Kaeppler *et al.* 1990) indicated that a SiC whisker might have penetrated the wall of a maize cell. The surface of SiC whiskers is negatively charged, unlike asbestos fibers (Appel *et al*. 1988). Presence of negative surface charge probably results in low affinity between DNA molecules (which are also negatively charged) and whiskers in neutral pH medium (Wang *et al*. 1995).

II. DNA Transfer in Protoplasts:

a. Electroporation:

Electroporation involves the treatment of plant cells with short high voltage electric pulses, which causes brief permeability of the plasmalemma for high molecular particles, such as DNA (Bates *et al.* 1989). The DNA movement is via pores formed after electric pulses in the cytoplasmic membrane (Sowers *et al.* 1992). The pores are of temporal character and they are related to the increased dipole moment of hydrophilic heads building cell membrane lipids. The dipole heads of phospholipids dislocate in the direction of the electric field, which causes breaks in the continuity of the cell membrane (Kinosita *et al.* 1977; Neumann *et al.* 1982; Neumann *et al* 1996, Wojcik *et al*. 2015).

b. Liposome-Mediated Transformation:

Liposome-mediated transformation delivers the functional DNA into the cell by the more natural processes of endocytosis and lipid-plasmalemma fusions. DNA fragments on treatment with liposomes gets encapsulated inside liposomes. These liposomes have the ability to attach to cell membranes and merge with them for the purpose of transferring DNA fragments. Thus, the DNA enters the cell and then to the nucleus. It is relatively non-toxic simple to perform with readily available chemical reagents.

c. Chemical Mediated DNA Transfer:

Calcium phosphate mediated gene transfer, DEAE-Dextran mediated gene transfer and polyethylene glycol mediated gene transfer are common chemical methods used in transformation experiments.

d. Microinjection:

The microinjection needle is made by drawing out a heated glass capillary to a fine point. Using a micromanipulator (a mechanical device for fine control of the capillary) the needle has been inserted into the nucleus of the host cell which is held on a glass capillary by gentle suction

e. **Sonication:**

Sonication (ultrasound) can alter the transient permeability of plasma membrane to facilitate uptake (Tachibana *et al*. 1999). Compared to other direct DNA delivery methods, such as particle gun bombardment, electroporation and microinjection, the ultrasound treatment may be simpler to carry out. Sonication, however, could cause cell damage or ever rupture (Liu *et al*. 2006). Ultrasound has been reported to mediate gene uptake in plant protoplast, suspension cells and intact pieces of tissues.

Indirect Gene Transformation Methods:

***Agrobacterium Tumefaciens* Mediated Transformation:**

Agrobacterium sp are gram negative plant pathogenic soil bacteria which, naturally infect dicotyledonous plants. These are examples of natural plant transformation where in the bacterial genes are successfully incorporated into the genome of higher plants. These genes are for production of phytohormones resulting in rampart proliferation of cells and for synthesis of a special class of compounds called opines which are used as food by the bacteria. Agrobacterium tumefaciens causes crown gall disease and Agrobacterium rhizogenes causes Hairy roots disease. These diseases are caused by the action of a plasmid in which the genes for pathogenicity and opine metabolism are present. In A. tumefaciens it is Ti-plasmid and in A. rhizogenes it is Ri-plasmid (Wang et al. 1995; Otten et al. 2008; Kuzmanović et al. 2015).

Ti plasmid: (Ti = Tumor Inducing):

It is a large circular plasmid (~200 kb in size) containing several regions of importance:

- **Transfer or T-DNA**: Is a region of the plasmid that is transferred from the bacteria to the host plant cell during the infection process and stably integrated into one of the host's chromosomes. T-DNA is ~25-kbp long and is bordered on both sides by two 25-bp direct repeats called left and right borders. Between the borders are several genes: Genes for synthesis of plant growth hormones cytokinin and auxin. Massive production of these hormones at the site of infection causes the surrounding plant cells to divide

and create the tumour gall. Genes for synthesis of a specific type of opine, either the octopine (In octopine Ti plasmid) or nopaline (In nopaline Ti plasmid) type (Gelvin *et al*. 2003).

- **Virulence or Vir Region**: Region that contains many genes required for the infection process that is transfer of T-DNA into the plants. There are six operons/distinct loci in nopaline Ti plasmid (vir A, B, C, D, E and G) and eight operons in octopine Ti plasmid (vir A, B, C, D, E, G, F and H). They are required for the transfer and integration of the T-DNA into host. vir region does not have to be physically connected to the T-DNA to work; region can work in Cis (on a same plasmid) or Trans (on a separate plasmid) which forms basis for the construction of binary Ti plasmids (Gelvin *et al*. 2003).

Course of Events During Agrobacterium Infection: Agrobacterium present in the soil detects dicot plants susceptible to infection by the secretion of polyphenols such as acetosyringone or hydroxyl acetosyringone from the roots or from wound sites. Bacteria move up chemical gradient of polyphenols to find the plant. Polyphenols binds to a receptor encoded by vir A gene. Binding activates vir A which then activates the vir G protein by phosphorylation. Both vir A and G are constitutively expressed. Vir G protein is a transcription factor which then initiates transcription of the rest of vir genes on Ti Plasmid as well as vir genes on the Agrobacterium chromosome (*ChvA*, *chvB* and *pscA*).

Specific vir gene products then cut T DNA at left and right borders (Vir D1, D2, C). Single stranded copies of the T DNA region are synthesized, creating the T-strand. T-strand is coated with single stranded DNA binding proteins (Vir E) and the ss DNA/Vir E complex is shuttled out of the bacterium and transferred to plant cell where it is integrated in the host chromosome. Process similar to bacterial conjugation. Once integrated in the plant chromosome, T-DNA genes become active, producing the oncogenic proteins for the synthesis of auxins and cytokinins, thus forcing the cells to proliferate. The opine synthesis enzyme is also produced and the manufactured opines are used as food for bacteria.

Steps in Agrobacterium Mediated Transformation:

1. Preparation of Agrobacterium containing Ti plasmid and gene to be transferred with marker genes and unique restriction site and preparation of explants. Our gene of interest is inserted into Ti plasmid by disarming it (removal of oncogenes).
2. Co-cultivation: Incubate Agrobacteria with explants (plant tissue) wounded in some way to facilitate entry of bacterium into the plant. Acetosyringone is added.
3. Plating explants on media containing antibiotic to kill remaining Agrobacterium. (Carbencillin, Cefotaxime etc is used).
4. Plating explants on media containing a balance of plant hormones to allow explants to divide and form callus tissue.
5. Selection: Plating callus on media containing suitable toxin (*e.g.,* kanamycin, phosphinothricin) to kill all non-transformed cells (do not contain the NPT II gene present in T-DNA region)
6. Transferring individual callus onto appropriate media with right hormone balance to allow regeneration of callus cells into intact plants.
7. Transformed plants will be hemizygous for inserted gene. Self-pollination with convert some progeny into homozygous transformed lines.

Virus Mediated Gene Transformation:

Plant viruses like Caulimo virus and Gemini virus can be used for plant transformation. In this technique, recombinant DNA is packed into head of the virus and allowed to infect the plants through which foreign DNA is transferred into plants.

Some Key Applications of Recombinant DNA Technology in Agriculture Include:

1.Pest Resistance: Genetically modified (GM) crops are designed to produce proteins that are harmful to specific pests, making them resistant to insect damage. For example, Bt (*Bacillus thuringiensis*) genes have been inserted into crops like cotton and corn to produce a toxin that targets certain insects, reducing the need for chemical insecticides. The initial release of the first-generation Bt cotton (Bollgard I – BG I) expressing *Cry1Ac* occurred in India in 2002. Subsequently, the second-generation pyramided trait (Bollgard II), incorporating both *Cry1Ac* and *Cry2Ab2* (MON15985 event), gained approval in 2006 and currently dominates 95% of the cotton cultivation area in India. Research (Koch *et al*. 2015) indicates that the pyramided trait, expressing both *Cry1Ac* and *Cry2Ab* toxins, exhibits a superior phenotype compared to cotton expressing *Cry1Ac* alone. Bt cotton was successful in controlling major bollworms, including *Helicoverpa armigera*, *Earias vittella,* and *Pectinophora gossypiella,* leading to higher crop productivity and greater earnings for farmers. In the USA, Dow AgroSciences introduced wide strike cotton, containing *Cry1Ac*/*Cry1F*, in 2004. Both BG II and wide strike cotton, with multiple toxin expressions, demonstrate higher efficacy in controlling a broad range of caterpillar insects compared to BG I. Beyond cotton, Bangladesh approved and commercialized four insect-resistant Bt brinjal varieties in 2014 (Singh *et al*. 2016). Additionally, Latin America witnessed the approval of transgenic soybeans expressing *Cry1Ac/Cry1Ab* for commercial use. Notably, transgenic cotton that contain *cry1Ac* gene under the regulation of a strong constitutive Figwort Mosaic Virus (FMV) promoter exhibited elevated expression of *Cry1Ac* toxin, which is fatal to lepidopteran insects, especially *H. armigera* (Fitch *et al*. 1992).

Recognizing the importance of addressing resistance concerns, (Cheng *et al*. 1996) reported cross-resistance development between *Cry1Ac* and *Cry2Ab* in cotton-growing regions in China. Therefore, the incorporation of transgenic plants expressing insecticidal proteins (ICPs) with varied combinations becomes imperative to effectively combat a broad spectrum of harmful insect pest populations globally. Achieving this through a combination of constitutive and tissue-specific promoters would provide a significant advantage, allowing continuous gene expression and targeted organ-specific functionality.

2. Herbicide Tolerance: Some GM crops are modified to be tolerant to specific herbicides. This allows farmers to use broad-spectrum herbicides to control weeds while sparing the crop plants. For instance, glyphosate-resistant soybeans and maize have been developed, which can withstand glyphosate-based herbicides. The pivotal breakthrough unfolded during the 1990s when glyphosate-resistant (GR) crops were commercially introduced. These crops revolutionized agriculture by enabling the application of glyphosate throughout the growing season without jeopardizing crop health. Glyphosate, previously employed indiscriminately for weed control in various settings such as vineyards, orchards, rights-of-

way, industrial areas, and railroads, earned recognition as a "once-in-a-century herbicide" (Chen *et al*. 2001).

Its broad weed spectrum, reasonable cost, favourable environmental characteristics, and association with widely embraced GR crops contributed to this distinction. In plants susceptible to glyphosate, the herbicide acts by inhibiting 5-enolpyruvylshikimate-3-phosphate synthase (EPSPS), a crucial enzyme in the shikimate pathway responsible for the synthesis of aromatic amino acids and several secondary metabolites in the phenylpropanoid pathway (Lines *et al*. 2002; Tennant *et al*. 2002). The introduction of GR maize in 1998 marked a significant milestone. Maize plants were transformed with CP4 (from an *Agrobacterium species* strain) EPSPS and e35S promoter, resulting in plants exhibiting vegetative resistance to glyphosate but with reduced male fertility. The inaugural generation of GR maize, known as Roundup Ready® (RR) trait or GA21, utilized the rice actin 1 promoter to drive the gene for a glyphosate-resistant form of maize EPSPS (TIPS-EPSPS) and (ZM-EPSPS). To enhance maize tolerance to glyphosate at both vegetative and reproductive stages, a new event, NK603, was developed in 2001. This event featured two copies of a slightly modified EPSPS CP4 gene and was commercially released as part of a breeding stack with glufosinate and four insect resistance traits.

3. Disease Resistance: Genetic engineering can confer resistance to plant diseases caused by viruses, fungi, or bacteria. For instance, papaya resistant to the papaya ringspot virus and potatoes resistant to late blight disease have been developed using recombinant DNA technology. The creation of transgenic papaya to counteract PRSV infection followed the successful development of transgenic tobacco, which expressed the CP gene of the tobacco mosaic virus, demonstrating disease resistance. Transgenic papaya with PRSV-resistant CP genes was developed using a gene transfer system involving immature zygotic embryos and a plasmid construction containing the neomycin phosphotransferase II (nptII) gene. This ground breaking study was the first to showcase the effectiveness of CP-mediated resistance in controlling PRSV (Azad *et al*. 2013). Subsequently, utilized the CP gene from the Taiwanese strain of PRSV, constructed with a Ti binary vector pBGCP through Agrobacterium-mediated transformation, to develop PRSV-resistant transgenic papaya (Davis *et al*. 1999). The pursuit of PRSV-resistant transgenic papaya has expanded, with various researchers employing different explants and plasmids containing the neomycin phosphotransferase II (nptII) gene (Fitch *et al*. 2005; Papolu *et al*. 2016; Beyer *et al*. 2002). The global adoption of CP-mediated protection against PRSV is evident, and CP genes have become the preferred agents for developing PRSV-resistant papaya (Zhang *et al*. 2011). Resistance against root-knot nematode (*Meloidogyne incognita*) using Modified rice cystatin gene (OC-IΔD86); *Agrobacterium tumefaciens*-mediated gene transfer in brinjal (Chen *et al*. 2016), Resistance against *Radopholus similis* using Cystatin gene (OC-IΔD86); *Agrobacterium tumefaciens*-mediated gene transfer in banana (Park *et al*. 2005).

4. Improved Nutritional Content: Genetic modification has been used to enhance the nutritional content of crops. Golden rice, for example, has been engineered to produce beta-carotene, a precursor to vitamin A, which can help combat vitamin A deficiency in developing countries. In the process of Agrobacterium-mediated transformation, engineered bacteria facilitated the integration of their DNA into targeted rice plant embryos. This introduced DNA encompassed three essential genes: phytoene synthase (psy, derived from daffodil), phytoene desaturase (crtI from bacteria), and lycopene beta-cyclase (lcy, sourced

from daffodil). To ensure the proper functioning of these genes within the cell, scientists also incorporated additional DNA fragments, along with marker genes that aided in monitoring the presence of the inserted DNA. Subsequently, the embryos underwent growth, selection, and testing to assess their beta-carotene production. Upon reaching maturity, the genetically modified rice plants demonstrated the capability to produce and store beta-carotene within their starch (Kaur *et al*. 2010; Robson *et al*. 1996; Beyer *et al.* 2002).

5. Drought and Stress Tolerance: Researchers are working on creating crops that can withstand drought, salinity, or other environmental stresses by introducing genes that help the plants cope with adverse conditions. These drought-tolerant crops can potentially increase yields in regions with water shortages. Drought tolerance in Tomato (*Solanum lycopersicum* cv. Aika Craig) using Sly-miR169c, an miR169 family member; *Agrobacterium tumefaciens*-mediated gene transfer (Zhang *et al*. 2011), Freezing and drought tolerance in China rose (*Rosa chinensis*) using RcXET and MtDREBIC genes*; Agrobacterium tumefaciens*-mediated gene transfer (Chen *et al.* 2016).

6. Extended Shelf Life: Some GM crops, such as tomatoes and potatoes, have been engineered to have an extended shelf life by slowing down the ripening process, reducing post-harvest losses. Prolonged shelf-life in tomato using *Arabidopsis thaliana* H+/cation exchanger (CAX) gene; *Agrobacterium tumefaciens*-mediated gene transfer (Park *et al.* 2005), Enhanced fruit softening in tomato using LeEXP1 gene; *Agrobacterium tumefaciens*-mediated gene transfer (Kaur *et al*. 2010), Reduction in plant height in potato using PHYA gene; *Agrobacterium tumefaciens*-mediated gene transfer (Robson *et al*. 1996).

7. Increased Crop Yields: Genetic modification can be used to improve crop yields by optimizing various traits, such as plant architecture, nitrogen utilization, and overall growth. While these applications offer potential benefits to agriculture, the adoption of GM crops is a subject of debate and regulation. Concerns related to environmental impacts, biodiversity, and human health have led to the implementation of strict regulatory frameworks in many countries to ensure the safe deployment of genetically modified organisms. The balance between the potential advantages of GM crops and their potential risks remains a critical issue in the field of agriculture and food production.

B. Modern Genetic Engineering Techniques:

Over the past 15 years, several new techniques have been developed and are being implemented to facilitate breeding of improved crop varieties. Compared with traditional breeding, these techniques increase the precision of making changes in the genomes and thereby reduce the time and effort that is needed to produce varieties that meet new requirements. A common denominator of these techniques is that they make use of a GM step, but result in products in which no foreign genes (i.e., genes other than from the species itself or from cross-compatible species) are present. GM is normally defined as an alteration of the genotype by the insertion or alteration of a specific DNA sequence using ‘recombinant DNA technologies’ involving artificial delivery systems. Early GM technology focused on the insertion of DNA from a foreign species, but there has been a

trend away from transgenics (foreign DNA insertion) to cisgenics (same species DNA insertion) and most recently to targeted mutagenesis (genome editing) of a favoured genotype.

C. Genome Editing:

Genome editing tools are advanced molecular techniques that enable precise modification of an organism's DNA, allowing for the addition, deletion, or alteration of specific genetic sequences.

These tools have revolutionized genetic research, biotechnology, and medicine. Several key genome editing technologies are notable (Karadagi *et al*. 2023).

Many technologies have been identified for broadening the genetic base through modern biotechnological method among them is genome editing technology which include site specific, precise modification of DNA sequence gene editing technology includes a set of tools such as MNs (meganucleases), ZFNs (zinc finger nucleases), TALENs (transcription activator-like effector nucleases), CRISPRs/Cas9 (clustered regularly interspaced short palindromic repeats which are associated with protein 9) (Gaj *et al.* 2013).

Table 19.2 History of Genome Editing

1985:	Discovery of Zinc finger proteins in frog oocytes.
1985:	Discovery of meganucleases in yeast.
1987:	CRISPRS described.
1994:	Meganuclease-induced DSBs enhance HDR discovered in mammalian cells.
1996:	Creation of ZFNs.
2001:	ZFN-induced DSBs enhance HDR in vivo in Xenopus sps. oocytes.
2007:	Discovery of CRISPR is bacterial immune system.
2009:	TALENs proteins described in plant pathogenic bacteria of genus *Xanthomonas* sps.
2010:	Creation of TALENS.
2012:	Discovery of CRISPR/Cas9 as programmable nuclease.
2013:	CRISPR used in vivo.
2015:	Cas9 variant with altered PAM was used.
2016:	A high-fidelity Cas9 variant was identified.
2016:	Used for Base editing.

2019:	Used for Prime editing.
2020:	Nobel Prize in Chemistry for CRISPR genome editing was given to jennifer doudna and Emmanuelle

Mega Nucleases (MegNs):

Meganucleases are endogenous sequence specific nucleases with a large recognition site 14-40 bp to cleave dsDNA to form DSBs promoting site specific gene recognition and provide a scope to engineer this type of enzyme remodelling of crop. Meganucleases are known as homing endonucleases (HEs) causing homologous recombination pathway.

HEs are small proteins (250-300 amino acids) found in prokaryotes and in unicellular eukaryotes. HEs families have been grouped into five families based on their sequence and structural motifs: LADGLIDADG, GIY-YIG, HNH, His-Cys box and PD (D/E) XK (Belfort *et al.* 2014).

The high specificity of MegNs in targeting dsDNA is a result of their exceptionally lengthy recognition sequences., ease in delivery, and production of more recombinogenic potential cytotoxicity (Gaj *et al.* 2016; Djukanovic *et al.* 2013). The main limitations of engineering MegNs is of introducing known cleavage site, separation DNA cleavage and DNA binding domains. The production customized MegNs is highly difficult and time consuming which limits its usage in genome editing. Commonly used MegNs is 1-Cre1 with a 22 bp recognition site.

Table 19.3: Genome Editing Using MegNs in Crop Improvement

Crop	MegNs	Number of bp	Target gene	Mutation	Trait	Reference
Maize	1-Cre1	22	*MS26*	Knock out	Male sterility	Nizolli *et al.* 2021
Cotton	COT-5/6	22	*EPSPS*	Knock out	Herbicide resistance	Miller *et al.* 1985

Zinc Finger Nucleases (ZFNs):

Zinc fingers are small proteins with characterized structural motifs in association of one or more zinc ions for stability, zinc finger motifs were first discovered in transcription factors of Xenopus laevis (Kim *et al.* 1996). Using an array of zinc fingers along with Fok1 cleavage domain hybrid nucleases were used for gene editing (Yin *et al.* 2017). Each zinc motif recognizes a 3-nucleotide sequence making it an efficient and site-specific genome editing tool compared to MgeNs.

Generally, a pair of ZFNs are used which bind to upstream and downstream regions of the locus depending upon the objective of gene editing program. Engineering of ZFNs involves screening of large number of ZF motifs with synergistic cross talk between them which is

in most cases absent limiting the use this tool. ZFNs are known to cause off-target gene editing due to antagonism between adjacent motifs which is dangerous for survival of individuals and is also known as the toxicity of gene editing tools. ZFNs are very small and can be inserted into vectors such as viruses for in vivo editing (Curtin).

Table 19.4: Genome Editing Using ZFNs in Crop Improvement

Crop	Target gene	Trait	Reference
Soyabean	*DCL*	Herbicide transmission	Schornak *et al.* 2006
Maize	*PAT*	Herbicide resistance	Wright *et al*. 2005
Tobacco	*GUS:PPT II*	Chromosomal breaks	Cantos *et al.* 2014
Rice	*OsQQR*	Detection of safe harbour loci Herbicide	Troder *et al*. 2022

Transcription Activator-Like Effector Nucleases (TALENs):

TALE proteins were discovered in plant pathogenic bacteria of the genus *Xanthomonas*. The bacteria utilize the sequence specific binding capacity of TALES to regulate the gene expression of the infected plant cells (Romer *et al.* 2007). TALEs unlike ZFNs contain a variable DNA binding domains and DNA cleavage which are amalgamated, DNA binding domain with multiple amino acid repeats (33-35 amino acids) almost identical to each other except for two adjacent amino acids in positions 12-13 known as repeat variable di residues (RVDs). TALENs recognize a single nucleotide corresponding to each protein module making it more specific (Cermak *et al.* 2011). The diversity of TALENs depends only on the RVDs region, as only two amino acids should be engineered it is easier and more, flexible than ZFNs (Khan *et al.* 2019). TALENs show reduced toxicity because of off-target breaks that lead to toxicity in the genome. TALENs offer another benefit, which is a higher rate of success and specificity in genome editing (Shan *et al*. 2015).

Table 19.5: Genome Editing Using TALENs in Crop Improvement

Crop	Gene	Trait	References
Rice	*OsBADH2*	Aroma in rice	Wang *et al*. 2014
Wheat	*TaMLO-A1, TaMLO-B1, TaMLO-D1*	Powdery mildew resistance	Demorest *et al.* 2016
Soyabean	*FAD2-1A, FAD2-1B*	Low polyunsaturated fat	Jung *et al*. 2016
Sugarcane	*COMT*	Production of bioethanol	Mojica *et al*. 1995
Tomato	*ANR1*	Anthocyanin accumulation	Mojica *et al*. 2000

Clustered Regularly Inter-Spaced Short Palindromic Repeats (CRISPR):

CRISPR/Cas is known as third generation gene editing tool due to its precise, efficient mode of site-specific gene remodelling. The system was developed in 2013 by jennifer doudna and emmanuelle but has its history before 20 years as follow:

1993:	The first observation of CRISPR repetitive DNA structures in *Halofera mediterra*.
1995:	short regulatory spaces motifs
2000:	It was known that these repeats were found in many microbes.
2005:	Immunity related role of CRISPR (Bolotin *et al*. 2005).
2007:	Experimental proof for immunity revealed the role of CRISPR (Barrangou *et al*. 2007).

CRISPR/Cas is based on DNA-RNA interactions and was, initially identified as defence system in bacteria that provides acquired immunity against parasites such as bacteriophages and plasmids. The CRISPR/Cas9 system consists of Cas9 nuclease and single-guide RNA (sgRNA). The engineered single RNA molecule known as sgRNA comprises CRISPR RNA and tracr RNA components.

The sgRNA identifies the target sequence through Watson-Crick base pairing and is followed by a DNA motif called a protospacer adjacent motif (PAM) to carry out its function.

The commonly used protein is Cas (SpCas9) found in Streptococcus pyogenes with specific PAM -NGG--stream of the target sequence in the genomic DNA, on the non-target strand. SpCas9 variants may increase the specificity in genome modifications. DNA cleavage is performed by Cas9 nuclease. The CRISPR system has been classified into two classes namely:

Table 19.6 Difference Between Class 1 and Class 2

Class 1 System	Class 2 System
Consists of three sub-groups namely I, III and IV	Consists of three sub-groups namely II, IV and VI
The RNA-guided target cleavage needs several effector proteins	The RNA-guided target cleavage needs several effector proteins

Table 19.7: Classification of CRISPR

		Class 1		**Class 2**		
	Type I	**Type III**	**Type IV**	**Type II**	**Type V**	**Type VI**
Protein	Cas3	Cas10	Csf1	Cas9	Cas12	Cas13
Target	DNA	RNA/DNA	-	DNA	DNA	RNA
Reference	Hillary *et al*. 2023					

Because of its high efficiency and low off target effects. CRISPR is extensively used in crop improvement to improve yield as well as quality traits

Table 19.8: Genome Editing Using CRISPR in Crop Improvement

Crop	Gene	Trait	Reference
A) Quality			
Soyabean	*FAD2-1B, FAD2-1A*	Higher oleic acid levels	Kim *et al*. 2017
Rice	*Gn1a, GS3 and DEP1*	Grain number, grain size and panicle size	Li *et al*. 2016
Wheat	*TaGASR7*	Grain length and weight	Wang *et al*. 2018
Flax	*FAD2*	Higher oleic acid levels	Jiang *et al*. 2017
Cotton	*ALARP*	Cotton fiber development	Sander *et al.* 2014
Maize	*PPR, RPL*	Reduced zein protein	Qi *et al*. 2016
Sorghum	*Whole1C*	Increase grain protein and lysine content	Li *et al*. 2018
B) Abiotic stress			
Maize	*ARGOS8*	Drought tolerance	Kaur *et al*. 2018
Rice	*OsNAC041*	Salinity tolerance	Bo *et al*. 2019
Soyabean	*Drb2a and Drb2b*	Salt and drought tolerance	Curtin *et al.* 2018
Maize	*ZmHKT1*	Salinity tolerance	Zhang *et al.* 2018
C) Biotic stress			
Wheat	*MLO-A1, TaMLO-B1 and TaMLO-D1*	Resistance to powdery mildew	Wang *et al*. 2014
Rapeseed	*WRKY70, WRKY11*	Induced resistance	Sun *et al.* 2018
Rice	*Pi-ta*	Resistance to blast	Xu *et al*. 2020
Wheat	*EDR1*	Resistance to powdery mildew	Zhang *et al.* 2017
Cotton	*Gh14-3-3*	Resistance to cotton verticillium wilt	Zhang *et al.* 2018

Genome editing has a wide range of applications in agriculture, revolutionizing the way crops and livestock are bred and managed. It allows for the precise modification of an

organism's DNA, enabling the development of crops and animals with improved traits, increased resistance to pests and diseases, and enhanced nutritional content. Here are some key applications of genome editing in agriculture:

1. Crop Improvement:

a. **Pest Resistance**: Genome editing is used to introduce genes that confer resistance to pests and diseases. For example, crops can be engineered to produce insecticidal proteins to combat specific pests, reducing the need for chemical pesticides.
b. **Herbicide Tolerance**: Crops can be modified to withstand herbicides, making it easier for farmers to control weeds without harming the crop plants.
c. **Disease Resistance**: Plants can be engineered to resist various diseases caused by viruses, fungi, or bacteria, reducing yield losses and the need for chemical treatments.
d. **Drought and Stress Tolerance**: Genome editing can help develop crops that are more resilient to environmental stressors like drought, salinity, and extreme temperatures.
e. **Improved Nutritional Content**: Researchers use genome editing to enhance the nutritional content of crops, as seen in the development of Golden Rice with increased levels of provitamin A (beta-carotene).

The use of genome editing in agriculture has the potential to increase food production, reduce the environmental impact of farming (such as by decreasing the need for pesticides), and enhance the nutritional value of crops. However, regulatory, ethical, and safety considerations remain important in its application to ensure responsible use and minimize unintended consequences. As a result, various countries have implemented regulations and guidelines to govern the use of genome-edited organisms in agriculture.

D. Transplastomic Approaches:

Beyond traditional transgenic approaches through nuclear transfer of foreign genes, plant biologists have found plastids to be suitable organelle to carry foreign genes. Plastids are specific to plant kingdom with exceptions of some photosynthetic bacteria. The genome of plastid offers several advantages over nuclear genome. As plastids behave like prokaryotic endosymbiotic bacteria, it has retained many prokaryotic features. Rather than non-homologous end joining, as in the nucleus, homologous recombination is used to integrate any foreign genetic material into the plastid genome. This ensures increased specificity of transgene insertion, reduced position effect and reduced overlapping. Again, in plastid transcription occurs in a stretch of operons as in prokaryotes, which makes is possible to insert multiple genes at once by constructing a suitable operon and engineer complete metabolic pathways. In this context plastid (chloroplast) genome of higher plants is an appealing target for metabolic engineering via genetic transformation.

Pioneering transformation of plastid was implemented in the unicellular green alga *Chlamydomonas reinhardtii* (Boynton *et al.* 1988), followed by plastid transformation in the flowering plant tobacco (Svab and Maliga 1993). The major problems to be addressed in devising a suitable method for plastid transformation are the efficiency of tissue culture, selection (along with selectable marker genes) and regeneration protocols.

There are numerous methods for plastid transformation designed by Bock 2015, Ruf *et al.* 2019, Kwak *et al*. 2019, Thagun *et al.* 2019, LaManna *et al.* 2022 etc. To reduce the metabolic burden, the foreign gene can be also put under an inducible system. This has been achieved in several instances, *viz*., Muhlbauer and Koop, 2005 by using lac repressor and operator, Lossl *et al.*, 2005 by using ethanol-inducible T7 RNA polymerase, Emadpour *et al.*, 2015 by using a theophylline-inducible riboswitch etc. Besides being the main hub for photosynthesis, the plastid is the place for several other metabolic pathways, viz., de novo synthesis of fatty acid, shikimate and 2-C-methyl-D-erythritol-4-phosphate (MEP) etc.

These pathways give opportunity to manipulate the production of many metabolites like plastoquinones, tocopherols, chlorophylls, as well as plant hormones (e.g., abscisic acid, strigolactones and gibberellins) (Yang *et al.*, 2022).

Applications of Transplastomics:

1. **Crop Improvement:**

a) Pest-Resistant Crops- In 1999, Kota *et al.* produced tobacco transplastomic lines showing overexpression of the Cry2Aa2 protoxin which conferred high toxicity against many insect pests and was successful to impart resistance in tobacco. Similarly in 2006, Chakrabarti *et al.* reported control of potato tuber moth by transferring cry9Aa2 gene into the plastid genome of tobacco. Plastid engineering was also shown successful in controlling *Helicoverpa armigera* by using l*epidopteran chitin synthase (Chi), cytochrome P450 monooxygenase (P450)* and *V-ATPase* dsRNAs (Jin *et al.*, 2015). Again Zhang *et al*. (2015) showed full crop protection in field condition against Colorado potato beetle by expression of long ds-RNAs in plastid of potato.

b) Herbicide-Resistant Crops- In 2001, Lutz *et al.* produced transplastomic tobacco lines expressing a bacterial *bar* gene (*b-bar1*) which conferred field-level tolerance to herbicide Liberty. In 2007, Dufourmantel *et al.* introduced the *hppd* gene from *Pseudomonas fluorescens* tobacco and soybean plastids which provided them with strong tolerance to isoxaflutole. In 2019, Stavridou *et al*. developed transplatomic tobacco lines overexpressing a theta class GST from *Arabidopsis thaliana AtGSTT1* which conferred tolerance towards herbicide Diquat.

2. **Nutritional Enhancement**- Wurbs *et al.* (2007) presented plastid expression of a bacterial lycopene *β-cyclase* in tomato-mediated conversion of lycopene to β-carotene and resulted in fourfold enhanced provitamin- A content in fruits. Apel and Bock (2009) produced transplastomic tomato by overexpressing β-cyclase that resulted in an increase of up to 50% in provitamin A. Further in an instance, expression of *γ-tocopherol methyltransferase* (γ-TMT) and *tocopherol cyclase* (TC) in tobacco and lettuce plastids resulted in improved α-tocopherol levels (Yabuta *et al.*, 2013).

3. **Pharmaceutical Production**- Del *et al.* (2012) expressed *Toxoplasma gondii* GRA4 antigen in tobacco chloroplast, and immunization elicited mucosal immune response resulting in production of specific IgA, interferon (IFN-γ), and interleukin (IL-4 and IL-10). Tobacco expressing human interleukin-2 (targeted to plastids) induced in vitro proliferation of IL-2-dependent murine T lymphocytes (Zhang *et al.* 2014). Transplastomic plants have been produced to express antigenic vaccines such as multi-epitope DPT (also known as

DTwP), a class of combination vaccines effective against three infectious diseases in humans: diphtheria, pertussis (whooping cough) and tetanus (Soria-Guerra *et al.,* 2009).

4. **Environmental Sustainability**: Tobacco plants were engineered to produce biodegradable plastics (PHAs) in their chloroplasts, offering a sustainable alternative to petroleum-based plastics (Somleva *et al*., 2013). Plastid engineering also aids in phytoremediation. For example, expression of bacterial genes such as *mer A* coding for mercuric ion reductase and *mer B* coding for organomercurial lyase into the chloroplast of tobacco plants resulted in plants being highly tolerant to phenylmercuric acetate (PMA) and $HgCl_2$ (Ruiz *et al.,* 2011).

E. Base Editors:

Base editors are a more recent addition to genome editing tools, capable of converting one DNA base pair into another without causing double-strand breaks. They offer precision and have the potential to correct single-point mutations associated with genetic diseases.

19.3 Comparison Between Direct Gene Transfer, Indirect Gene Transfer, and Genome Editing:

Table 19.2: Comparison Between Direct Gene Transfer, Indirect Gene Transfer, and Genome Editing:

Feature	Direct Gene Transformation Methods	Indirect Gene Transformation Methods	Genome Editing
Genetically modified organisms	Not required	Required	Not required
Plasmid	Required	Required	Required
Efficiency	Generally lower due to lack of targeted insertion	Higher due to targeted gene insertion and stable integration	High precision with variable efficiency depending on the editing tool and target site
Risk of transfer of non-desirable DNA fragment	High	Low	Almost negligible
Stability of Integration	Often less stable, may require selection markers	More stable due to integration via homologous recombination	Very stable, depending on the success of the editing and repair mechanisms
Species specificity	Non specific	specific	Non specific

Feature	Direct Gene Transformation Methods	Indirect Gene Transformation Methods	Genome Editing
Sequence information	Not required	Not required	Required
Regulatory Concerns	Considered a GMO; regulatory scrutiny varies	Considered a GMO; regulatory scrutiny varies	Considered a GMO; regulatory scrutiny varies
Cost and Complexity	Generally lower cost but higher technical skill needed for some methods	Moderate cost, well-established protocols	Can be expensive and technically challenging, especially for precise edits
Time Required	Fast, but often requires screening for successful transformants	Takes longer due to tissue culture steps	Varies, can be relatively fast but depends on the complexity of the edit
Applications	Suitable for simple gene transfers, gene expression studies	Widely used in plant transformation, transgenic crop development	Gene therapy, crop improvement, knockout or knock-in studies, functional genomics
Techniques	Particle Bombardment or Biolistic Method, Electroporation, Liposomes etc.	Agrobacterium mediated transformation	ZFN, TALENs, CRISPR/Cas

19.4 Biosafety in Genetic Engineering:

Biosafety in genetic engineering refers to the practices, regulations, and principles designed to prevent or minimize potential risks associated with genetic modification (GM) technologies.

These risks can be to human health, the environment, and biodiversity (Indira *et al.*, 2005). Biosafety measures aim to ensure that the development and use of genetically modified organisms (GMOs) are conducted safely and responsibly.

19.4.1 Key Aspects of Biosafety in Genetic Engineering:

1. **Regulatory Frameworks**: Many countries have established regulatory bodies to oversee the development, release, and commercialization of GMOs. These regulations often require risk assessments, public consultations, and compliance with safety standards.
2. **Risk Assessment**: This is a critical component of biosafety, involving the evaluation of potential risks that a GMO may pose. This includes considering the likelihood of gene

flow to wild relatives, potential impacts on non-target organisms, and any unforeseen effects on ecosystems.

3. **Containment and Monitoring**: In research and development stages, GMOs are often kept in controlled environments like laboratories or greenhouses to prevent unintended release. Continuous monitoring is conducted to ensure that any potential risks are identified and mitigated early.
4. **Ethical Considerations**: Ethical issues in genetic engineering, such as the potential for unforeseen long-term impacts on biodiversity, are integral to biosafety discussions. Public involvement in decision-making is often encouraged to address these concerns.
5. **International Guidelines**: The Cartagena Protocol on Biosafety, an international agreement under the Convention on Biological Diversity (CBD), provides a framework for the safe handling, transfer, and use of GMOs, with a particular focus on transboundary movements.
6. **Public Perception and Communication**: Public understanding and acceptance of GMOs can significantly impact biosafety practices. Transparent communication about the benefits and risks of genetic engineering is crucial for fostering trust and informed decision-making.
7. **Biocontainment Strategies**: These include physical, chemical, and biological methods to prevent the spread of GMOs outside their intended environment. Examples include gene drives, sterility genes, and other genetic safeguards.
8. **Environmental Impact**: Assessing the potential impact of GMOs on ecosystems, including soil health, water quality, and non-target species, is a crucial aspect of biosafety. Measures are taken to minimize any negative effects.

In summary, biosafety in genetic engineering ensures that the development and use of GMOs are conducted in a way that protects human health and the environment, balancing innovation with caution.

19.5 Future Prospects:

There is a need for development of bioinformatic design tools which can be used to increase efficiency and decrease off-target effects. The tools depend on the activity prediction models and off-target detection algorithms. A substantial bottleneck to the implementation of CRISPR tools in agriculture is the effective packaging and delivery of CRISPR-Cas complex to the targeted plant cells. Novel delivery methods need to be established to achieve high-efficiency genome editing in plants. Thus, the outlook for improvement in reducing the size of presented Cas proteins or the innovation of smaller Cas9 proteins is needed. Genome editing is a promising technology with the ability to contribute to food generation for the use of the rising population. However, the biosafety, social and ethical concerns remain about the usage of genome editing in plants. Therefore, intragenesis, cisgenesis (use of intraspecific genes for transformation) and clean gene technology (marker gene free technology) should be given more emphasis which makes approval process less risky. Nevertheless, there is a need to revise the regulations of genome-edited plants and to enlighten the general community about their characteristics. A sustainable future for agriculture can now be imagined along with the responsibility of continuously resolving both scientific and public concerns about its usage.

19.6 Conclusion:

Genetically modified organisms (GMOs) have the potential to greatly benefit humanity by enhancing food quality and availability, improving medical care, and promoting environmental sustainability. To fully realize these benefits, it's crucial to exercise caution and assess the risks associated with each new GMO on a case-by-case basis. Genetic engineering technologies can play a role in enhancing crop productivity, addressing issues like bacterial wilt, late blight, drought stress, lodging resistance, and nutritive quality in native crops. It's essential to establish effective biotechnology policies, biosafety measures, and regulatory frameworks for GMO introduction, research, and release. Additionally, efforts should be made to expand applications like plant tissue culture, microbial product development, vaccine production, and diagnostics. Harnessing the country's biodiversity through in vitro conservation, molecular characterization, and marker-assisted breeding is vital. Developing national expertise in recombinant DNA technology research, including containment greenhouse facilities, is necessary, along with securing sufficient financial resources from both the public and private sectors.

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