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**CHAPTER-1**

# Genetic and Genomic Innovations in Plant Sciences

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## Summary

Current advances in the biotechnology of plants are revolutionizing agriculture through making exact genetic modifications that cannot be accomplished by conventional breeding. Genome editing instruments, which include ZFNs, TALENs and the CRISPR-Cas system, enable the targeted editing of DNA to enhance yield, nutritional quality and resistance to stress. Through transformation methods such as Agrobacterium-mediated delivery, biolistic delivery, PEG-mediated delivery and nanotechnology required genes are transferred in many crops. Moreover, genomic selection has become an important breeding technique which speeds up breeding process. Nevertheless, despite the obstacles of off-target edits, regulatory obstacles and public distrust, the newest development like base editing, prime editing, RNA interference and nanoparticle-based transgene delivery is more accurate and promising to be transgene free in crop enhancement. The future is focused on developing a combination of leading-edge molecular technologies and environmentally friendly agriculture to produce climate-resistant, high yield and nutritionally enhanced crops. These genetic and genomic breakthroughs will be the main solution in ensuring world food distribution amidst the changing environmental conditions.

**Keywords: CRISPR-Cas System, Genomic Selection, Transgenic Plants / GMOs**

## Introduction

Gene editing in plants uses special techniques to make precise changes in the plant's genes. Unlike traditional breeding approaches, which takes many years and many plant generations to achieve desired traits. These methods modify genetic sequences to enhance, important plant features like disease resistance, yield, nutritional content, and environmental adaptability (Bortesi & Fischer, 2015; Chen, Wang, Zhang, Zhang, & Gao, 2019).

One of the main technologies used for gene editing is called the CRISPR-Cas system. It is popular because it is easy to use, works well, and is very different and efficient. This system uses a guide RNA to find the exact location in the plant's DNA. Then, the Cas enzyme cuts the DNA at that

spot. After this, the plant's natural repair system fixes the cut, which can add, remove, or change tiny parts of the DNA (Jinek et al., 2012). This helps make better plants that can resist pests, climate change, increased productivity, and improved nutritional profiles (Miller et al., 2011).

Introduction of transgenic technology enabled the insertion of foreign DNA into plant genomes, creating genetically modified organisms (GMOs) with enhanced traits such as herbicide tolerance and pest resistance. Even though GMOs work well, they face rules and worries from people about safety for health and the environment.

**Significance of Gene Editing in Plants**

Modern gene editing is very accurate and changes specific DNA parts and edited crops that resist diseases, climate change, and grow more food. Plants edited without adding foreign DNA face fewer rules than regular GMOs, so they are accepted and used more widely. New tools like base editors and prime editors allow even more precise and varied changes to plant genes for better crops (Anzalone et al., 2019; Waltz, 2016).

Here are some genomes editing tools that are widely used:

**Zinc Finger Nucleases (ZFNs):**

Zinc Finger Nucleases (ZFNs) are specially made proteins that combine two parts: a zinc finger domain that binds to DNA, and a DNA-cutting part called FokI endonuclease. Each zinc finger can recognize a short DNA sequence of three base pairs. When several zinc fingers are connected, they can identify a longer and very specific DNA sequence. Two ZFNs bind to opposite strands of DNA at nearby sequences. This brings the FokI enzymes close together, allowing them to cut both strands of the DNA at that exact location, creating a double-strand break (DSB). Because the zinc finger parts can be customized, ZFNs have high precision in targeting specific DNA sites. These proteins are designed to create precise breaks in the DNA, which can then be used for accurate gene editing. However, the process of designing and building these proteins is complex, which has made it difficult for ZFNs to be used widely in research and applications (Kim, Lee, Kim, Cho, & Kim, 2009).

**Transcription Activator-Like Effector Nucleases (TALENs):**

TALENs are made using DNA-binding parts taken from proteins called transcription activator-like effectors (TALEs), which come from bacteria that cause disease in plants. Each TALE unit binds to one DNA base, making TALENs easier to design and customize compared to Zinc Finger Nucleases (ZFNs). Like ZFNs, TALENs have a FokI enzyme that cuts the DNA when two TALENs join together on opposite DNA strands. Their design is simpler and more modular, allowing TALENs to target more DNA sequences with high accuracy. Because they are easier to create than ZFNs, TALENs have been used for precise gene editing in many kinds of plants. However, they still face challenges related to their cost and complexity (Cermak et al., 2011).

**CRISPR-Cas System:**

The CRISPR-Cas system comes from bacteria, where it works as an immune defense to protect against viruses. Scientists changed it to use for gene editing. This system has two main parts: a guide RNA (gRNA) and a Cas protein (usually Cas9). Together, they form a complex that can find and cut DNA at specific places by making double-strand breaks. CRISPR-Cas is easier, faster, and cheaper to design and use compared to older methods. It can also edit many genes at the same time (called multiplexing). This system is very efficient and can make changes without adding extra foreign DNA, which is called transgene-free editing. Since it was adapted for gene editing in 2013, CRISPR-Cas has greatly improved the way scientists edit genomes by using customizable guide RNAs, allowing precise and multiple gene changes more easily (Jinek et al., 2012; Shan et al., 2013).

**The CRISPR-Cas system changes plant DNA using a clear and strong step-by-step process:**

A guide RNA that matches a specific 20-letter sequence in the plant's DNA. This DNA spot must be next to a short pattern called the protospacer adjacent motif (PAM), usually the letters 'NGG' for the common Cas9 enzyme. The Cas9 protein joins with the guide RNA to form a complex. This complex searches the plant's genome to find DNA sequences that match the guide RNA (Fig 1). When the complex finds a perfect or close match with the PAM nearby, Cas9 cuts both strands of the DNA three letters before the PAM sequence, causing a double-strand break. The plant cell tries to fix this break using natural repair methods: Non-Homologous End Joining (NHEJ): This is a quick but error-prone repair that often adds or removes small DNA parts, which can disrupt a gene. Homology-Directed Repair (HDR): This method uses a similar DNA template to make precise changes like swapping letters or adding new genes. The edited plants can have specific changes like turning off unwanted genes or adding good ones to improve traits.

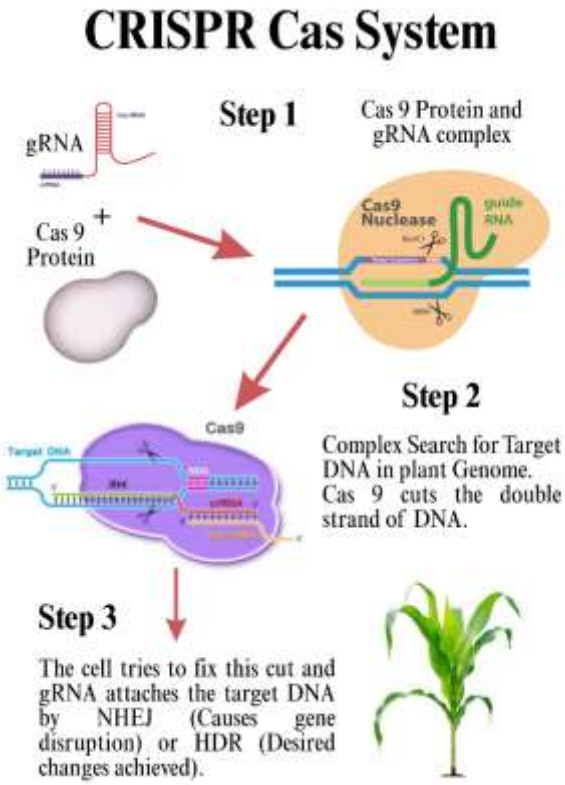


**Fig 1.** Elaborates the CRISPR Cas System in Plnats

## Transformation Methods in Plants

### Agrobacterium-Mediated Transformation

Agrobacterium tumefaciens is still one of the most effective and versatile vectors for stable genetic transformation of plants. It allows T-DNA delivery into plant genomes of host plants through a natural infection process, but more specifically in dicots, although technological breakthroughs have now made it accessible to monocots as well (Azizi-Dargahlou & Pouresmaeil, 2024). This process allows for precise low-copy integration with minimal disruption to the genome and thus is highly suitable for crop improvement and functional genomics applications (Hwang, Yu, & Lai, 2017).

Both transient and stable expression systems are possible through this method. Transient expression methods, including agroinfiltration, are widely applied in the rapid analysis of gene function, protein expression, and virus-induced gene silencing (VIGS) in organisms like Nicotiana benthamiana and Sorghum bicolor (Li et al., 2024).

### Biolistic (Gene Gun) Transformation

Biolistic transformation, also known as particle bombardment, entails bombarding DNA-coated gold or tungsten particles into plant tissue with the aid of high-speed delivery systems. The technique circumvents species-specific barriers and has been of importance in transforming chloroplast genomes and recalcitrant crops such as maize and rice (Hwang et al., 2017). Notwithstanding its high degree of versatility, the method tends to result in multiple insertions and possible genome rearrangements, which tend to complicate downstream expression and stability (Demirer et al., 2021). Recent advances in nano-biolistic utilizing atomized particles are designed to minimize cellular injury and enhance transformation efficiency (Feng et al., 2013).

**PEG-Mediated Transformation**

Polyethylene glycol (PEG)-mediated transformation is a chemical method that is mostly applied to protoplasts, plant cells lacking cell walls. Through this process, DNA or RNA is absorbed directly into protoplasts in a PEG and calcium ion-enriched medium. It is extensively applied in transient expression studies and has emerged as a useful tool for the delivery of CRISPR/Cas systems (Feng et al., 2013).

**Protoplast-Based Systems**

Protoplasts provide an amenable platform for measuring gene function, promoter expression, and genome editing systems, particularly CRISPR-Cas9. Without a cell wall, nucleic acids or ribonucleoprotein complexes (RNPs) can be readily taken up. A major limitation still is the low efficiency of plant regeneration of protoplasts in most species (Cunningham, Demirer, Goh, Zhang, & Landry, 2020). Nevertheless, protoplast systems are still indispensable for high-throughput screening of sgRNA efficiency, subcellular localization, and protein interaction studies.

**Nanotechnology in Gene Editing**

Nanotechnology has provided novel devices for non-integrative biomolecule delivery, such as CRISPR elements. Nanoparticles like carbon nanotubes, mesoporous silica, and liposomes enable the delivery of DNA, RNA, and protein complexes through plant cell walls without transgenic integration (Cunningham et al., 2020; Demirer et al., 2021). Magnetic nanoparticles, or magnetofection systems, have been found to facilitate CRISPR RNP delivery through pollen grains, paving the way for targeted DNA-free gene editing with transgene footprint avoidance (Demirer et al., 2021). Though obstacles such as cytotoxicity and species-specific uptake hurdles exist, nanoparticle-mediated delivery is promising for targeted and environmentally friendly genome editing.

**Applications of CRISPR in Crop Improvement & Functional Genomics**

CRISPR-Cas systems revolutionized plant genetic modification because of their simplicity, programmability, and high specificity. CRISPR has also been effectively utilized in crop improvement to improve drought tolerance, disease resistance, grain quality, and yield traits in different crops (Ahmar et al., 2021). Multiplexing strategies are now capable of simultaneous targeting of various genes, enabling faster polygenic trait development. In functional genomics, CRISPR offers a versatile technology to generate loss-of-function mutants, regulatory element modifications, and base editing, disclosing gene functions and pathways with unprecedented accuracy (Ahmar et al., 2021).

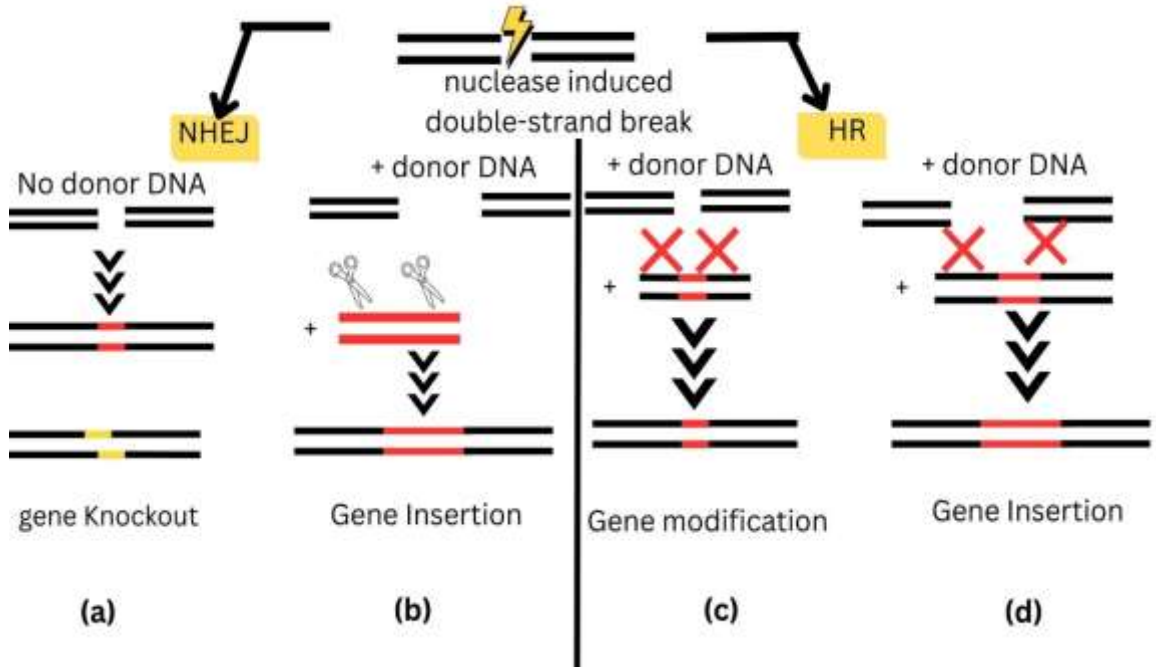


**Fig 2.** Plant Genome Editing

**Challenges and Limitations of Gene Editing in Plants**

Even though tools like CRISPR-Cas and other gene editors are designed to be very precise, sometimes they cut DNA at places that are similar but not the intended targets. This can cause unexpected changes, which might harm the plant or cause problems with regulations. Designing and testing new ZFNs is difficult, takes a long time, and costs a lot. Also, they might accidentally target wrong DNA sites if the zinc fingers do not bind perfectly. **TALENs** are easier to design than ZFNs but still require much work and time. Their large size can make delivering them into some plants harder. **CRISPR-Cas System** is simpler and faster but can still have off-target effects if guide RNAs are not carefully designed. There are also concerns about rules and safety depending on the country. Different countries have different rules about gene-edited plants. Some treat plants without foreign DNA as non-GMOs, but others have strict controls. Public opinions, ownership of technology, and social or economic effects also make it more complicated. Many crops have

complicated genetics, like having multiple copies of genes or traits controlled by many genes which makes gene editing harder.

**Table 1**: Genome Editing Techniques and their mechanism

| Technology/Method | Mechanism | Advantages | Limitations |
|---|---|---|---|
| **Zinc Finger Nucleases (ZFNs)** | DNA-binding zinc fingers fused to FokI enzyme create DSBs | High specificity with custom DNA recognition | Complex design; expensive; off-target effects possible |
| **TALENs** | TALE repeats target DNA bases; FokI enzyme creates DSBs | Easier to design than ZFNs; customizable and precise | Still labor-intensive; delivery can be challenging due to size |
| **CRISPR-Cas9** | gRNA guides Cas9 to target DNA; induces DSBs | Simple, fast, cheap; multiplexing possible; transgene-free edits | Off-target cuts if gRNA not optimized; regulatory concerns |
| **Agrobacterium-mediated Transformation** | T-DNA delivered through natural infection process | Low copy integration; high efficiency; suitable for stable transformation | Limited to some species (mainly dicots); size of DNA inserts |
| **Biolistic (Gene Gun)** | DNA-coated particles shot into plant cells | Applicable to monocots; chloroplast transformation possible | Multiple insertions; genomic rearrangements; tissue damage |
| **PEG-Mediated (Protoplast)** | DNA uptake by protoplasts in presence of PEG and $Ca^{2+}$ | Ideal for transient expression; efficient delivery of CRISPR components | Regeneration of whole plants from protoplasts is difficult |
| **Nanoparticle-based Delivery** | CRISPR components loaded | | |

**Genomic Selection:**

A method of breeding uses DNA information to know or predict an individual's breeding value for a particular trait (Cooper et al., 2014). Genomic selection works because DNA markers spread across whole genome and can capture all possible genetic variation and allowing us to predict the breeding value without knowing the genes that are exactly responsible for trait (Kumar et al., 2024).

**Methods of Genomic Selection:**

A powerful tool that uses molecular data of markers to predict the breeding value of individuals in breeding programs. Process relies on both trait and genetic information models, then these are used to predict values of those traits who have not been measured. The method of genomic selection is statistical, learning, building and testing models.

**Statistical Approaches:**

Using statistical approaches to predict the genomic value key method includes are G-BLUP a method estimates genetic marker impact and predict the breeding value by using genomic relationship. Assume equal marker effects. Single-Step GBLUP method merges all possible available genotypic and phenotypic data from both genotyped and ungenotyped into single analysis. Bayesian method assigns prior possibilities to marker effects and good for more complex genetic pattern capturing (Crossa et al., 2017); (Nakaya & Isobe, 2012).

**Machine learning Methods:**

Machine learning approaches to predict the genomic value are deep learning a method in which neural network used in model to know the complex relationships between traits and genetic markers. Second method is machine learning algorithms, a machine learning techniques like random forests and support vector machine can also be applied (Alemu et al., 2024).

**Training and Validation:**

Training and validation also very important in genomic selection, Training population involve in prediction model which is created from group of individuals by using both genotypic and phenotypic data. While validation population involves only genotypic data of an individuals whose predicted values used to check model's accuracy. In this training data is split into two subsets, some for validation and other for training, to know the model's predictive ability called cross validation (Heslot, Yang, Sorrells, & Jannink, 2012).

**Important Consideration for Accuracy:**

Marker data with high density, often from whole genome sequencing or from SNP chips, is important for precise predictions. Training population size is directly proportional to the accurate prediction; larger training size led to more accurate predictions. Genetic relationships within or between populations is crucial for effective model training understanding (Crossa et al., 2017).

**Useful Aspects and drawback of genomic selection:**

Genomic selection is a powerful tool that reduces cycle times and significantly accelerates genetic gain, but implementation of it comes thorough different sets of challenges including significant negative impact on unselected characters or traits.

**Advantages of genomic selection:**

With genomic selection breeder gets genetic improvement at much quicker pace than traditional breeding especially for those traits having low heritability. With the help of genomic selection breeder can get genetic improvement in early development of individuals and reduce the time duration between the generation. At early stages genotyping can be investment, but it can reduce the cost that comes on phenotyping, leads to overall beneficial. Genomic selection is more accurate for selecting individuals in breeding program especially for those traits that are challenging to measure traditionally. Individuals selected on the base of genomic selection are better suited to wide range of environment conditions. Genomic selection can effectively tackle with the trait that is influenced by multiple genes, that are normally difficult during traditional methods (Jannink, Lorenz, & Iwata, 2010).

**Limitations of genomic selection:**

Selection for some specific traits can adversely affect the other traits especially if they are negatively correlated. Genomic selection can induce rapid changes in gene frequencies; it may impact long-term accuracy of prediction. Under genomic selection, influence of epistatic interaction becomes more prominent, it may affect prediction reliability. Genotyping cost is still a significant challenge for implementation. Precise genomic prediction depends upon well-characterized and large training population. Genomic selection demands robust infrastructure for data analysis, genotyping and phenotyping successful implementation. Genomic selection necessitates careful precautions of various factors such as breeding population, specific trait and genotyping strategies (Nakaya & Isobe, 2012).

**Table 2:** Difference in Traditional and Genomic Breeding

| Feature | Traditional breeding | Genomic breeding |
|---|---|---|
| **Speed** | Slow | Fast |
| **Accuracy** | Lower | Higher |
| **Trait selection** | Limited traits can be naturally select | Wide range, also those with low heritability |
| **Gene pool** | Leads towards narrow gene pool | Gene pool can be broadened |
| **Cost** | Initially less expensive | More expensive |

| | | |
|---|---|---|
| **Technology** | Relies on natural selection/process | Uses advance technologies |

**Challenges of genomic selection:**

Genomic selection for plant breeding facing various challenges for its widespread adaptation such as Inherit difficulty in precise predicting breeding values, complex relationship between genotype and environment, demanding nature of high output phenotyping and need of robust training population (Crossa et al., 2017).

**Genotypic obstacles:**

Many important traits in plants are controlled by numerous genes with minute effects, make genomic data challenging but make very precise prediction. Plant's performance can be varied from region to region due to environmental effects, genomic selection model account accurately these interactions for reliable prediction. Traits having low heritability are very harder to predict through genomic selection because contribution of environment is more as compared to genetics. The degree of linkage disequilibrium between QTLs and markers, directly impact prediction accuracy (Nakaya & Isobe, 2012).

**Phenotypic obstacles:**

To obtain precise and consistent phenotypic data for big population remains a major problem for genomic selection despite advancements. Traits demanding specialized equipment's and high expertise, they required large amount of money. Efficient storage, processing, and analytical solution, they are very complex and very difficult to manage (Fasoula, Ioannides, & Omirou, 2020).

**Training population limitation**

It includes many factors such as size and density of reference population. It is also affected by genomic relationship between training population and target breeding population. The quality of data is also a major challenge when use genomic methods for selection (Alemu et al., 2024).

**Transgenic plants**

Transgenic plants are those whose DNA has been altered by using the methods of genetic engineering. Transgenic plants are genetically engineered plants that have one or more genes from unrelated species through biotechnological approaches. These genes can be originated from bacteria, virus, animals and even from other plant species, these genes are often referred to as Transgenes. The goal of creating the transgenic plants is to incorporating the desired characteristics and make it useful and productive that are impossible to achieve through traditional breeding practices. Common features include drought tolerance, herbicide tolerance, improved nutritional profile, increase yield, longer shelf life and resilience to biotic and abiotic challenges. Additionally, these plants can be created to express foreign proteins that possess the commercial and medicinal values. Through the direct transformation of plant genomes, this technology enabled the

researchers to overcome the limits of conventional breeding. This advancement has transformed the ways of farming and has the profound implications for industrial uses, food security and environmental sustainability. In 1983 the first transgenic plant was documented. Since then, several genetically engineered proteins have been expressed in a number of significant agronomic plant species, such as canola, corn, tobacco, tomato, banana, alfalfa and potato. For human immunization tobacco plants were traditionally utilized but banana and potatoes are also considered (Rani & Usha, 2013).

**Genetically Modified Organisms**

As we look to the future, one of the most pressing issues of 21$^{st}$ century is the significant rise in global food demand. United Nation study predicts that there will be 9.8 billion people on the earth by 2050. This increase poses the serious issues for food production since agricultural growth rates are not sufficient to sustain the world's population. Traditional breeding is significantly limited in its ability to exploit genes from tertiary gene pools due to the obstacles caused by reproductive isolation. Here GM technology strengthened the attainment of sustainable global food security by collaborating with agricultural improvement aided by markers and genomics

Any living thing whose genetic makeup has been artificially changed to produce desirable biological products by using the genetic engineering methods is referred to as genetically modified organism (GMO). Various transformation techniques have been used to genetically modify plants, animals and microbes for a number of uses, including medical, industrial, agricultural and environmental. Mostly the genetically modified organisms (GMOs) have been released into the environment for the production of food and feed (Gupta & Singh, 2016).

**Table 3:** How Transgenic Plants different from GMOs

| **Features** | **Transgenic plants** | **GMOs** |
|---|---|---|
| **Definition** | Plants that have had their genetic material modified by introducing the genes from unrelated species. | Organisms such as plants, animals and micro-organisms with altered genetic material. |
| **Type of organism** | Just plant | Plant, animal and microbes |
| **Source of gene** | Non- native or Foreign DNA from other species | Include native gene modification or foreign DNA |
| **Example** | BT cotton and Golden rice | Insulin producing bacteria |
| **Technique used** | Agrobacterium–mediated transformation and gene gun | Gene Editing, CRISPR and RNAi |
| **Scope** | Limited to plants | Plants, animal, fungi and bacteria |

| | | |
|---|---|---|
| **Regulatory focus** | Environmental concern, food labeling and biosafety of crop. | Biodiversity, safety of pharmaceuticals and human health. |
| **Level of controversy** | High because foreign DNA is involved from unrelated species | It may vary because gene editing is seen as less controversial than transgenics. |
| **Field of application** | agriculture | Agriculture, research, industry and medicine. |

**Advance technologies to develop Transgenic plant and GMOs**

There are the different methods that are used to develop transgenic plants and genetically modified organisms. Here are some of the methods that are widely used:

**Agrobacterium – mediated transformation**

The most common method that is used for plant transformation is the agrobacterium-mediated transformation. Agrobacteria are native to the ecology of soil. Hairy root and crown gall disease are caused by these harmful Gram-negative bacteria. In the genome of these bacteria, the genetic information of tumor growth is encoded on either a tumor inducing plasmid (Ti plasmid) or a hairy root-inducing (Ri plasmid). There are mainly two species of Agrobacterium are used one is Agrobacterium rhizogenic and Agrobacterium tumefaciens. When the harmful genes in Agrobacteria are eliminated, the discovery of these two species offer the effective vector system for the development of transgenic plants. A wide range of plants. Such as maize, tobacco, rice and barley has been successfully transformed by using this technique (Low et al., 2018).

**Gene Editing:** This technique uses the tools like CRISPR-Cas9 to modify the DNA of an organism, for example by introducing a gene that confers resistance to disease. The genome can be altered more broadly with recombinant technology, whereas gene editing is more accurate technique (Naveen & Sontakke, 2024).

**RNA interference**

This method uses small RNA molecules to suppress the expression of specific genes in an organism. It is feasible to develop crops resistant to specific viruses by doing this (Naveen & Sontakke, 2024).

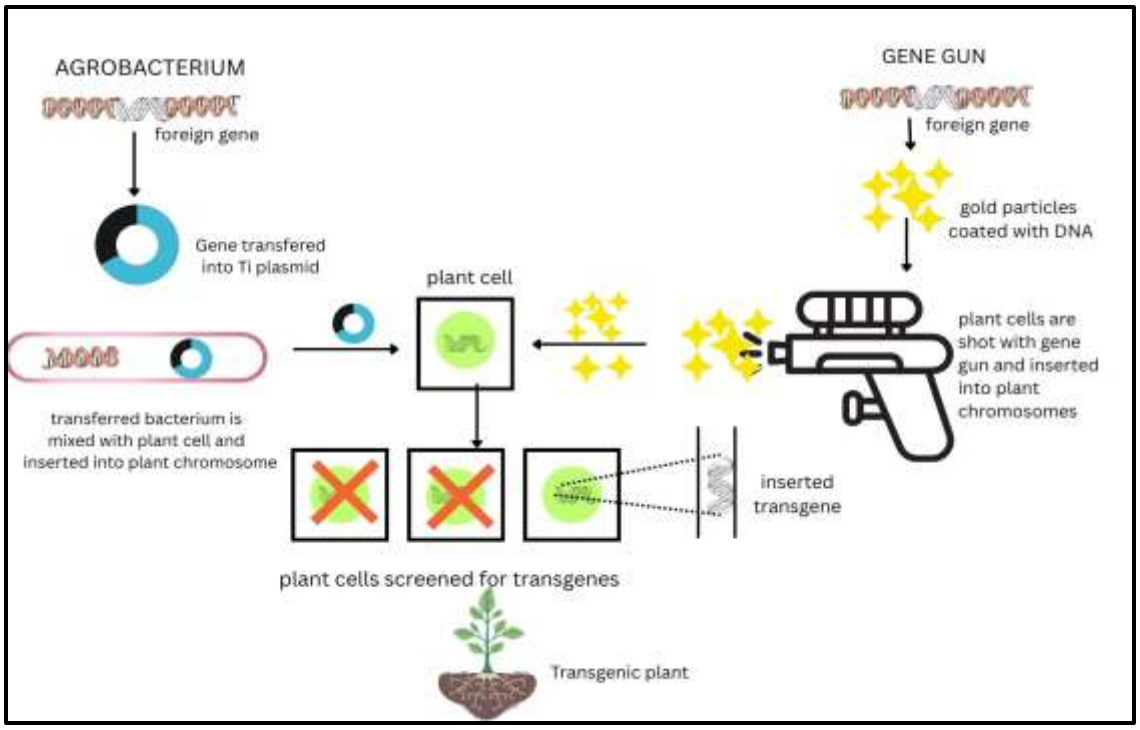


**Fig 3.** Advance technologies to develop Transgenic plant and GMOs

**Controversies related to Genetically Modified Crops**

Genetically modified plants are the biotechnological crops developed through using advance techniques are preferred to cultivate in recent era (Isaaa, 2019). In general terms these plants are high yielding, increased resistance to disease and pests and are resistant to climatic changes. The excessive use and cultivation of GM crops has increased conflict regarding to their biosafety (Snow & Palma, 1997). These conflicts include the impacts on soil, long term effects on human health and ethical concerns. The impact of genetically modified crops on soil includes the degradation of soil fertility, decrease the activity of soil micro-organisms, transfer of genes in other species and development of resistant pests (Sisterson, Carrière, Dennehy, & Tabashnik, 2005).

Micro-organisms are very essential component of rhizosphere the underground place where soil and plant roots integrate with each other (Luo et al., 2017). Plants release a fix amount of carbon which is about 20% through their roots which is utilized by micro-organisms for their activity (Haichar et al., 2008). Plants also release essential metabolites along with carbon that provide nutrients to the micro-organisms. Therefore any change in plant at genome or gene level may affect the release of carbon and nutrients which is alternatively effecting the microbial life in soil (Guyonnet, Cantarel, Simon, & Haichar, 2018). For example, Bt cotton carries crystalline protein which is released when Cry gene is expressed in plants. This protein is not toxic when freely available but as it enters the gut of susceptible insects it causes the death of insect. Although Bt has advantages such as less use of pesticides but it carries many side effects for soil microbial life

(Fig 40). Research reveals that Bt decreases soil bacteria by 14% (Tarafdar, Rathore, & Shiva, 2012). As soil microbial life is changed by the use of GM crops it effect the soil fertility and develop resistant in pests.

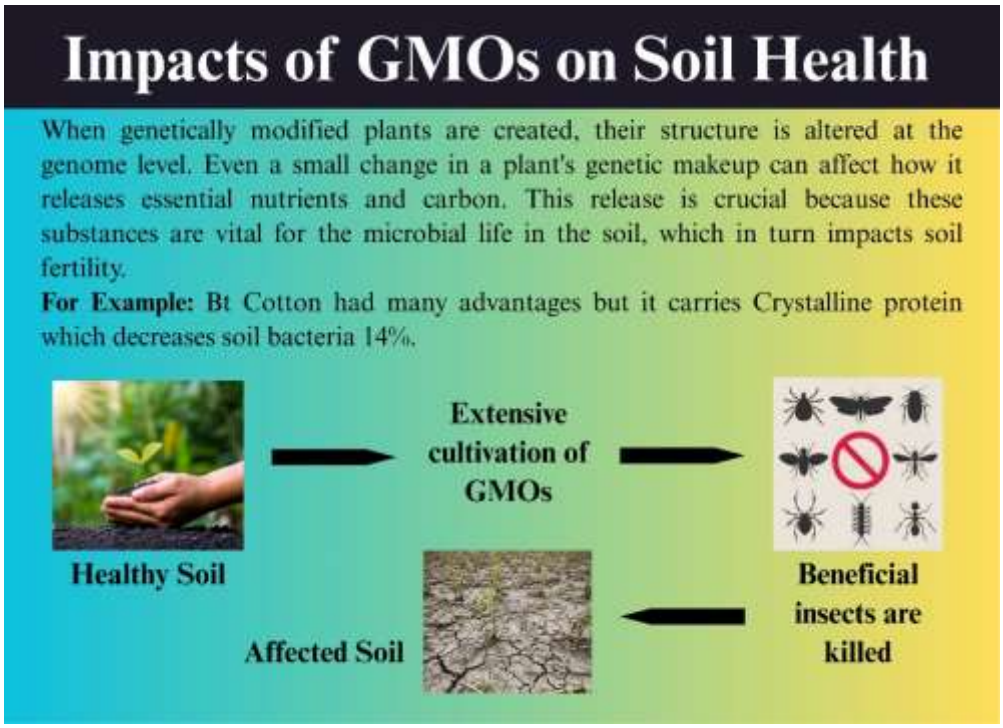


**Fig 4:** Impacts of GMOs on Soil Health

Genetically modified products have potential benefits but it may cause health risks not short term but may be long term affects. As transgenic products are developed by making changes at gene level might a product contain a gene that is not its part naturally. The foreign inserted genes are beneficial but may cause some side effects in some edible products (Uzogara, 2000). World Health Organization has shown the results that there is no any history which reveals the safe use genetically modified products. It also shows that the changes made at gene level cause physiological effects on human health (Cellini et al., 2004). When genetically modified products are produced antibiotic resistant genes are used as markers to identify the gene transfer which is associated to specific traits. If such genes are transmitted to disease causing pathogen it may develop resistance against antibiotics. In future such genes may be transmitted to humans through bacteria which cause severe health issues. For example, mad-cow a disease of cattle is transmitted to humans who consumed the meat of that cow and get severe infections and eventually death with in a period of two months (Vasil, 1998). Sometimes when researchers are working to develop GMOs there is the occurrence of allergic reactions when a foreign gene is inserted in to a new body and as a resultant this allergy is transmitted to humans. For example, StarLink is a genetically modified corn and it contains Cry9C protein which is an allergenic (Batista et al., 2005).

Sometimes GMOs are carcinogenic contain such genes which are toxic to human health and may cause diseases like cancer.

The consumer behavior towards the use of GM products depends on the risk assessment of such products. There are some ethical concerns related to the use of GM products. Studies revealed that some peoples prefer the use of GM products just based on their life style but some don't prefer to use such products instead of knowing their potential benefits (Ozkok, 2015). A study was carried which reveals that 40% of people do not prefer the use of GMOs besides knowing their benefits. The results also shows that US shows positive behavior towards the use of transgenic plants as compared to other countries (Christoph, Bruhn, & Roosen, 2008). Besides the benefits of genetically modified products there are some health concerns and ethical issues associated with the use of GMOs.

## Future directions

The future of plant transgenic and gene editing technologies is to incorporate next-generation molecular tools with environment friendly agricultural practices in order to meet global food security needs. The emerging technologies, including base editing, prime editing and RNA-guided genome editing, will provide efficient, reversible and transgene-free editing, minimizing the regulatory barriers. The blending of nanotechnology-based delivery systems and the advances in CRISPR can improve efficiency, particularly in recalcitrant crops, as well as reduce off-target activities. In addition, integrating genomic selection with machine learning and big data analytics will speed up breeding schemes by anticipating complex traits across diverse environments. Strategies to enhance regeneration systems, provide biosafety and advance socially acceptable innovation through open policies and public participation should also be the focus of future research. This integrated strategy will ensure climate-resilient, high-yielding and nutritionally enhanced crops.



## REFERENCES

Ahmar, S., Mahmood, T., Fiaz, S., Mora-Poblete, F., Shafique, M. S., Chattha, M. S., & Jung, K.-H. (2021). Advantage of nanotechnology-based genome editing system and its application in crop improvement. *Frontiers in Plant Science, 12*, 663849.

Alemu, A., Åstrand, J., Montesinos-Lopez, O. A., y Sanchez, J. I., Fernandez-Gonzalez, J., Tadesse, W., . . . Crossa, J. (2024). Genomic selection in plant breeding: Key factors shaping two decades of progress. *Molecular Plant, 17*(4), 552-578.

Anzalone, A. V., Randolph, P. B., Davis, J. R., Sousa, A. A., Koblan, L. W., Levy, J. M., . . . Raguram, A. (2019). Search-and-replace genome editing without double-strand breaks or donor DNA. *Nature, 576*(7785), 149-157.

Azizi-Dargahlou, S., & Pouresmaeil, M. (2024). Agrobacterium tumefaciens-mediated plant transformation: a review. *Molecular Biotechnology, 66*(7), 1563-1580.

Batista, R., Nunes, B., Carmo, M., Cardoso, C., São José, H., de Almeida, A. B., . . . Oliveira, M. M. (2005). Lack of detectable allergenicity of transgenic maize and soya samples. *Journal of Allergy and Clinical Immunology, 116*(2), 403-410.

Bortesi, L., & Fischer, R. (2015). The CRISPR/Cas9 system for plant genome editing and beyond. *Biotechnology advances, 33*(1), 41-52.

Cellini, F., Chesson, A., Colquhoun, I., Constable, A., Davies, H., Engel, K. H., . . . Leguay, J.-J. (2004). Unintended effects and their detection in genetically modified crops. *Food and Chemical Toxicology, 42*(7), 1089-1125.

Cermak, T., Doyle, E. L., Christian, M., Wang, L., Zhang, Y., Schmidt, C., . . . Voytas, D. F. (2011). Efficient design and assembly of custom TALEN and other TAL effector-based constructs for DNA targeting. *Nucleic acids research, 39*(12), e82-e82.

Chen, K., Wang, Y., Zhang, R., Zhang, H., & Gao, C. (2019). CRISPR/Cas genome editing and precision plant breeding in agriculture. *Annual review of plant biology, 70*(1), 667-697.

Christoph, I. B., Bruhn, M., & Roosen, J. (2008). Knowledge, attitudes towards and acceptability of genetic modification in Germany. *Appetite, 51*(1), 58-68.

Cooper, M., Messina, C. D., Podlich, D., Totir, L. R., Baumgarten, A., Hausmann, N. J., . . . Graham, G. (2014). Predicting the future of plant breeding: complementing empirical evaluation with genetic prediction. *Crop and Pasture Science, 65*(4), 311-336.

Crossa, J., Pérez-Rodríguez, P., Cuevas, J., Montesinos-López, O., Jarquín, D., De Los Campos, G., . . . Beyene, Y. (2017). Genomic selection in plant breeding: methods, models, and perspectives. *Trends in plant science, 22*(11), 961-975.

Cunningham, F. J., Demirer, G. S., Goh, N. S., Zhang, H., & Landry, M. P. (2020). Nanobiolistics: an emerging genetic transformation approach *Biolistic DNA Delivery in Plants: Methods and Protocols* (pp. 141-159): Springer.

Demirer, G. S., Silva, T. N., Jackson, C. T., Thomas, J. B., W. Ehrhardt, D., Rhee, S. Y., . . . Landry, M. P. (2021). Nanotechnology to advance CRISPR–Cas genetic engineering of plants. *Nature Nanotechnology, 16*(3), 243-250.

Fasoula, D. A., Ioannides, I. M., & Omirou, M. (2020). Phenotyping and plant breeding: Overcoming the barriers. *Frontiers in Plant Science, 10*, 1713.

Feng, Z., Zhang, B., Ding, W., Liu, X., Yang, D.-L., Wei, P., . . . Mao, Y. (2013). Efficient genome editing in plants using a CRISPR/Cas system. *Cell research, 23*(10), 1229-1232.

Gupta, R., & Singh, R. L. (2016). Genetically modified organisms (GMOs) and environment *Principles and applications of environmental biotechnology for a sustainable future* (pp. 425-465): Springer.

Guyonnet, J. P., Cantarel, A. A., Simon, L., & Haichar, F. e. Z. (2018). Root exudation rate as functional trait involved in plant nutrient-use strategy classification. *Ecology and Evolution, 8*(16), 8573-8581.

Haichar, F. e. Z., Marol, C., Berge, O., Rangel-Castro, J. I., Prosser, J. I., Balesdent, J., . . . Achouak, W. (2008). Plant host habitat and root exudates shape soil bacterial community structure. *The ISME journal, 2*(12), 1221-1230.

Heslot, N., Yang, H. P., Sorrells, M. E., & Jannink, J. L. (2012). Genomic selection in plant breeding: a comparison of models. *Crop science, 52*(1), 146-160.

Hwang, H.-H., Yu, M., & Lai, E.-M. (2017). Agrobacterium-mediated plant transformation: biology and applications. *The arabidopsis book, 15*, e0186.

Isaaa. (2019). Global status of commercialized biotech/GM crops in 2019: Biotech crops drive socio-economic development and sustainable environment in the new frontier: ISAAA Ithaca, NY.

Jannink, J.-L., Lorenz, A. J., & Iwata, H. (2010). Genomic selection in plant breeding: from theory to practice. *Briefings in functional genomics, 9*(2), 166-177.

Jinek, M., Chylinski, K., Fonfara, I., Hauer, M., Doudna, J. A., & Charpentier, E. (2012). A programmable dual-RNA–guided DNA endonuclease in adaptive bacterial immunity. *science, 337*(6096), 816-821.

Kim, H. J., Lee, H. J., Kim, H., Cho, S. W., & Kim, J.-S. (2009). Targeted genome editing in human cells with zinc finger nucleases constructed via modular assembly. *Genome research, 19*(7), 1279-1288.

Kumar, R., Das, S. P., Choudhury, B. U., Kumar, A., Prakash, N. R., Verma, R., . . . Das, R. (2024). Advances in genomic tools for plant breeding: harnessing DNA molecular markers, genomic selection, and genome editing. *Biological Research, 57*(1), 80.

Li, Q., Wang, X., Teng, C., He, X., Fu, X., Peng, W., . . . Lyu, S. (2024). An Improved and Simplified Agrobacterium-Mediated Genetic Transformation Protocol for Solanum nigrum with a Shorter Growth Time. *Plants, 13*(15), 2015.

Low, L.-Y., Yang, S.-K., Kok, D.-X. A., Ong-Abdullah, J., Tan, N.-P., & Lai, K.-S. (2018). Transgenic plants: gene constructs, vector and transformation method *New visions in plant science*: intechopen.

Luo, J.-Y., Zhang, S., Zhu, X.-z., Lu, L.-m., Wang, C.-y., Li, C.-H., . . . Zhou, Z.-g. (2017). Effects of soil salinity on rhizosphere soil microbes in transgenic Bt cotton fields. *Journal of Integrative Agriculture, 16*(7), 1624-1633.

Miller, J. C., Tan, S., Qiao, G., Barlow, K. A., Wang, J., Xia, D. F., . . . Hinkley, S. J. (2011). A TALE nuclease architecture for efficient genome editing. *Nature biotechnology, 29*(2), 143-148.

Nakaya, A., & Isobe, S. N. (2012). Will genomic selection be a practical method for plant breeding? *Annals of botany, 110*(6), 1303-1316.

Naveen, A. K., & Sontakke, M. (2024). A review on regulatory aspects, challenges and public perception in acceptance of genetically modified foods. *Food science and biotechnology, 33*(4), 791-804.

Ozkok, G. A. (2015). Genetically modified foods and the probable risks on human health. *Int J Nutr Food Sci, 4*(3), 356-363.

Rani, S. J., & Usha, R. (2013). Transgenic plants: Types, benefits, public concerns and future. *Journal of Pharmacy Research, 6*(8), 879-883.

Shan, Q., Wang, Y., Li, J., Zhang, Y., Chen, K., Liang, Z., . . . Qiu, J.-L. (2013). Targeted genome modification of crop plants using a CRISPR-Cas system. *Nature biotechnology, 31*(8), 686-688.

Sisterson, M. S., Carrière, Y., Dennehy, T. J., & Tabashnik, B. E. (2005). Evolution of resistance to transgenic crops: interactions between insect movement and field distribution. *Journal of Economic Entomology, 98*(6), 1751-1762.

Snow, A. A., & Palma, P. M. (1997). Commercialization of Transgenic Plants: Potential Ecological Risks. *BioScience, 47*(2), 86-96. doi:10.2307/1313019

Tarafdar, J. C., Rathore, I., & Shiva, V. (2012). Effect of Bt-transgenic cotton on soil biological health. *Applied Biological Research, 14*(1), 15-23.

Uzogara, S. G. (2000). The impact of genetic modification of human foods in the 21st century: A review. *Biotechnology advances, 18*(3), 179-206.

Vasil, I. K. (1998). Biotechnology and food security for the 21st century: a real-world perspective.

Waltz, E. (2016). Gene-edited CRISPR mushroom escapes US regulation. *Nature, 532*(7599), 293.