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BIOTECHNOLOGY AND GENOMICS IN AGRICULTURE

Ranjana Chauhan[1], Rashami Manaware[2] and Pratibha Yadav[3]

[1]School of Biotechnology, DAVV, Indore, Madhya Pradesh, India
[2, 3]Mata Jijabai Govt. PG Girls College, Indore, Madhya Pradesh, India
Corresponding Email: ranjana.indore83@gmail.com

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Abstract

The world's population is expected to reach over 9 billion people by 2050, which will make it extremely difficult for agricultural experts to meet the rising demand for food. To achieve this need, agricultural production must rise by 60% over 2007 levels. As a result of this change, investments in conventional inputs like pesticides and fertilizers are being replaced by technology-driven solutions, especially genetic changes, which can increase yields while using fewer resources. As governments prepare for a changing climate and expanding populations, food and nutrition security are now important issues in global debates. Meeting the food needs of present generations without sacrificing the potential of future generations to do the same is a major concern in emerging nations. It is crucial to guarantee sufficient crop output in the present and the future. In order to ensure future food supply, creative ways must be pursued, as current agricultural technologies will not be sufficient to meet future output needs. Breeders have worked for years, but traditional methods still have many problems. In this regard, biotechnology is essential to enhancing food, feed, and fuel to sustain the world's growing population. Current limits could be overcome and plant breeding could be much improved in previously unthinkable ways with the help of modern biotechnology and genome editing technologies. Crop genomes can be accurately altered by combining cutting-edge genome editing tools with high-throughput omics technologies (backed by next-generation sequencing). This makes it possible to develop crop types with particular characteristics and increased climate adaptation.

Key words: Biotechnology, genome editing technologies, next-generation sequencing, plant breeding.

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Introduction

Agriculture has undergone a radical change thanks to groundbreaking scientific discoveries like biotechnology and genomics. While genomics focuses on comprehending and modifying an organism's genetic composition, biotechnology exploits biological systems and creatures to create agricultural breakthroughs. These disciplines work together to address urgent global issues like sustainability, climate

change, and food security.

Fundamental Concepts

Manipulating living organisms to increase crop yields, strengthen resistance to pests and diseases, and lessen environmental effects is the core of biotechnology in agriculture. Important instruments include CRISPR-Cas9, a precise genome-editing technique that has transformed genetic research, and genetic engineering, which introduces or modifies particular genes to give desired features. The study of an organism's entire genetic makeup, or genomics, has allowed scientists to pinpoint the genes causing particular characteristics, such resilience to illness or drought. The quick identification of these genes and their roles is made possible by cutting-edge genomic technologies like bioinformatics and high-throughput sequencing.

In order to create better crop types, boost their nutritional content, and shield them from biotic and abiotic challenges, genomics is essential. By comprehending the underlying biological mechanisms, crop cultivars can be improved to display desired features through the use of genomics. Application of genomics in crop breeding is vital for improving existing varieties and addressing the increasing demands of a growing world population (Sinha et al., 2023).

The goal of translational genomics is to convert genetic ideas into useful instruments. Translational genomics seeks to improve plant breeding operations with effectiveness, efficiency, and precision by using the vast quantity of genetic information that is already available. Phenotypic selection is the mainstay of traditional breeding techniques, and it requires a lot of time and resources (Choi, 2019). Using omics data and cutting-edge bioinformatics approaches, translational genomics provides an alternate strategy to overcome the shortcomings of traditional breeding programs. The analysis of genetic data has been completely transformed by next-generation sequencing (NGS) technology, which makes it possible to gather extensive omics data and enhances the precision of translational genomics techniques. Single nucleotide polymorphisms (SNPs), conserved orthologous set (COS) markers, cleaved amplified polymorphic sequences (CAPS), insertions/deletions (indels), simple sequence repeats (SSRs), and sequence-characterized amplified regions (SCARs) are among the functional molecular markers that have been made easier to study the genome sequencing (Satam et al., 2023).

Quantitative trait loci (QTLs) governing a range of agronomic variables and stress tolerances have been identified as a result of the use of genetic markers, including as SNPs and ESTs, to create physical and genetic maps. To fulfill the increasing need for food from a fast growing human population, it is essential to incorporate these QTLs into crop development projects employing transgenic technologies or marker-assisted selection/breeding (MAS or MAB). Recent developments in translational genomics and other omics methods used to improve the quantity and quality of crops and plants are the main topic of this chapter. The current methods of gene editing are also covered in this chapter. It examines the use of translational genomics in crop breeding and talks about the methods available for producing huge omics data (Ahmad, 2022).

Integration of genomics with other omics disciplines, such as proteomics (study of proteins), transcriptomics (study of RNA), and metabolomics (study of metabolites), provides a comprehensive understanding of biological systems. This holistic approach enables targeted interventions to improve agricultural productivity and sustainability.

Applications in Crop Improvement

I. Genetically Modified Organisms (GMOs) and Gene-Splicing

Genetically Modified Organisms (GMOs) represent a landmark achievement in biotechnology. By introducing foreign genes into crops, researchers have been able to develop varieties with enhanced resistance to pests, diseases, and environmental stresses. As shown in fig 1.

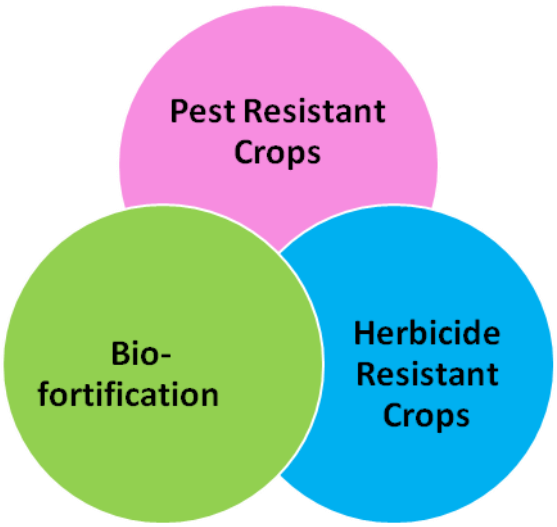

Fig: 1 Applications of GMO's in agriculture

Pest-Resistant-Crops

One of the most successful GMOs is Bt cotton, which incorporates a gene from the bacterium Bacillus thuringiensis (Bt). This gene produces a protein toxic to specific insect pests, such as bollworms, effectively reducing the need for chemical pesticides. Studies show that Bt cotton adoption has led to higher yields, reduced pesticide use, and increased profits for farmers.

Biofortification: Golden Rice

Golden Rice was developed to address vitamin A deficiency, a major health concern in many developing countries. It contains genes responsible for beta-carotene synthesis, enabling the human body to produce vitamin A**.** This innovation has the potential to reduce childhood blindness and mortality rates in vitamin A-deficient populations.

Herbicide-Resistant Crops

GM crops such a**s** glyphosate-resistant soybeans and maize have been engineered to tolerate broad-spectrum herbicides**.** This allows farmers to control weeds effectively without harming the crop, leading to simplified weed management, reduced labor costs, and increased farm productivity.

However, GMOs face criticism related to ecological risks, such as the potential for gene flow to wild relatives, the development of pest resistance, and non-target effects. Public skepticism and stringent regulatory requirements continue to challenge their widespread

adoption (Raman, 2017) .Following are the simple steps to make a GMO plant as shown in fig 2.

1. Identifying gene of interest:
2. Cloning the gene of interest (plant transformation and insertion of gene into a transfer vector):
3. Selection of modified plant cells and their regeneration into full plants:
4. Detection of the transformation and identification of the inserted DNA fragment:
5. Performance testing of a plant:
6. Conduct a risk assessment:

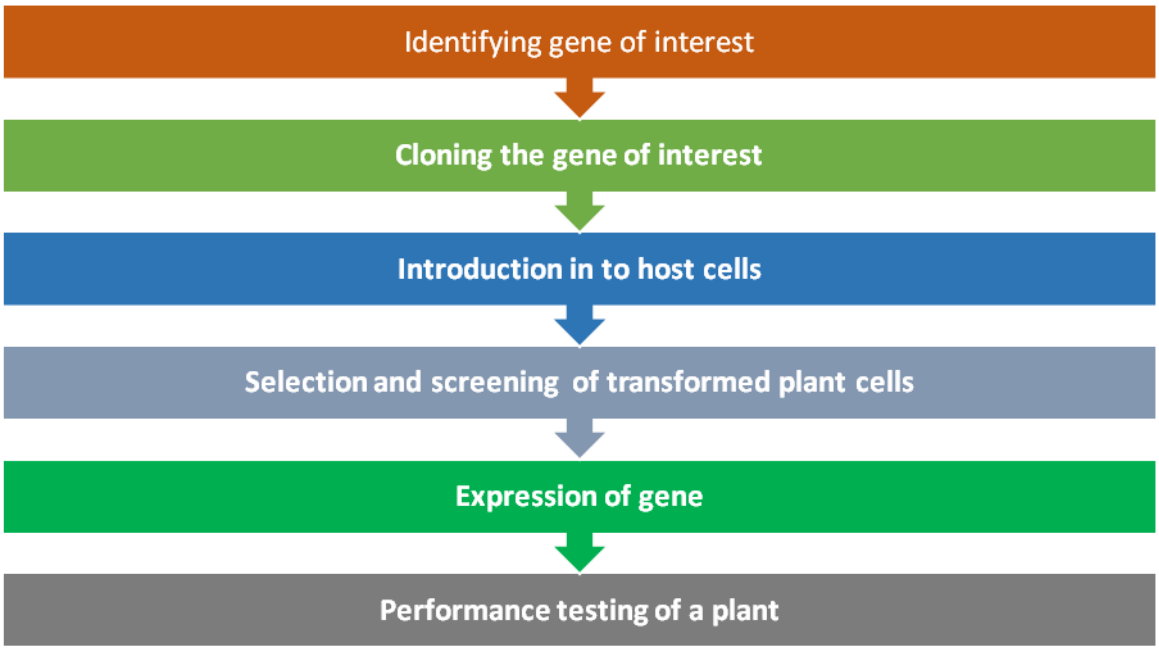

Fig: 2 Steps of GMO plant

A health and environmental risk assessment, as well as a test of the plant's overall performance, are all carried out.

Gene-Splicing and Genetic Modification

Finding, separating, and introducing a gene into a different host organism in order to exhibit a desired characteristic is known as gene-splicing. Three essential areas make up each gene: the coding sequence, which includes the instructions for producing proteins; the terminator, which indicates that transcription has ended; and the promoter, which starts transcription (Rodríguez-Molina., 2023). In order to extract a desired gene and transfer it into the DNA of another creature, genetic engineers employ restriction enzymes to cut particular DNA sequences. The discovery of these enzymes in the 1970s paved the way for the advancement of genetic engineering through the creation of recombinant DNA technology (Loenen., 2014).

The gene needs to be cloned and inserted into a host cell after it has been isolated. Plasmids, which are tiny circular DNA molecules present in bacteria, are commonly used for this. The plasmid is broken up by a restriction enzyme, producing sticky ends that make it easier for the foreign gene to implant. The plasmid is subsequently sealed by another enzyme,

ligase, creating recombinant DNA. Plasmids are perfect vectors for transferring and reproducing foreign genes because they naturally move across bacterial cells. The plasmid is replicated by the altered bacterial cells, which also copy their own DNA and the foreign gene. Gene expression, or the effective conversion of DNA into RNA and translation into a useful protein, is the ultimate aim of gene-splicing. By altering their regulatory signals to resemble bacterial genes, higher organism genes—like those encoding insulin or human growth hormone—can be produced in bacteria (Jia.,2024). Similar steps are taken in plant genetic engineering, except a whole plant must be grown from genetically altered cells. The ideal method is protoplast culture, which promotes gene transfer by removing the cell wall before adding foreign DNA. After being altered, the protoplast grows back into a complete plant that may be planted in soil to continue growing (Reed., 2021).

Controlling gene expression, or making sure that genes are only activated in particular tissues or developmental stages, is a major difficulty in genetic engineering. In order to emulate natural processes like photosynthesis activation in leaves but not in roots, scientists are trying to understand gene regulatory elements that allow targeted gene expression. Furthermore, inserting foreign genes could inadvertently alter how existing genes are expressed, resulting in trade-offs including lower crop yields or poorer storage quality. Medicine and agriculture have been transformed by genetic manipulation. The Flavr Savr tomato, the first genetically modified plant, was designed to postpone ripening. Since then, GM crops have helped to improve food security and agricultural production. While GMOs hold promise for disease resistance, enhanced nutrition, and economic benefits, careful evaluation of risks and ethical considerations remains essential for their responsible use in modern agriculture (Raman., 2017).

Genome Editing Tools and Genetic Modification

Genome editing in agriculture involves modifying the DNA of plants and animals to enhance desirable traits such as disease resistance, higher yield, improved nutritional value, and environmental adaptability. Advanced genome-editing tools like CRISPR-Cas9, TALENs, and ZFNs allow precise genetic modifications without introducing foreign DNA, making them more acceptable than traditional GMOs.

CRISPR-Cas9

- Most widely used and efficient genome-editing tool**.**
- Uses guide RNA (g RNA) to direct the Cas9 enzyme to a specific DNA sequence, where it makes a precise cut. Allows for gene knockout, insertion, or correction with high specificity.
- Used in medicine (gene therapy), agriculture (GM crops), and biotechnology.

Genome editing techniques, especially CRISPR-Cas9, allow for precise gene alterations, they have completely changed crop improvement. In contrast to conventional genetic engineering, which frequently entails adding foreign DNA, genome editing can subtly alter a plant's current DNA to resemble natural mutations.

The most effective and extensively used genome editing technology for plants is the clustered regularly interspaced palindromic repeat (CRISPR)/CRISPR-associated (Cas) protein (CRISPR/Cas). A synthetic single-guide RNA (sgRNA) and the Cas endonuclease work together to guide the Cas protein to a specific genomic DNA (gDNA) sequence, which CRISPR/Cas9 subsequently recognizes and cleaves (Karlson et al., 2021; Hamdan et al., 2022). Cas9, Cas12a, Cas12b, Cas12j (Cas), and Cas12f (CasMINI) are among the several

Cas endonucleases (Alok et al., 2021). DNA cleavage frequently results in insertions or deletions (InDels), which can cause gene knockouts and frameshift mutations. Numerous plant species, including model plants, cereal crops, oil crops, fruits, vegetables, and horticulture plants, have had their genomes edited using CRISPR/Cas9 (Okamoto et al., 2022; Yao et al., 2022). However, there is little genome editing available for vegetables, and radish genome editing has not yet been studied. In this special issue, Muto and Matsumoto modified the GLABRA1 (GL1) orthologs, RsGL1a and RsGL1b, which are known to be involved in the development of leaf trichomes in radish, using a CRISPR/Cas9 system with a sgRNA.

The majority of the mutant alleles were stably inherited in the T1 generation, and the authors discovered that T0 plants had an editing effectiveness of at least 62%. This study demonstrated the possibility of obtaining genome-edited radish that lacks T-DNA (null-segregant) as unique breeding material. This might offer a more successful and economical method of creating new radish varieties with enhanced qualities, which could have a big impact on agriculture and crop improvement. Additionally, it is anticipated that the developed technology will be applicable to other vegetables and traits, such as enhanced nutritional value, resistance to pests and diseases, and tolerance to environmental stresses—all of which are critical in addressing food security and sustainable agriculture in the face of climate change.

In a different work, Cao et al. targeted the RS2 and RS3 genes involved in raffinose synthesis in soybeans using a multiplex CRISPR/Cas9 technique. They demonstrated the superior editing effectiveness of the single transcriptional unit (STU) and two-component transcriptional unit (TCTU) methods with tRNA as the cleavage point. Additionally, Cao et al. successfully induce mutations at RS2 and RS3 using the TCTU-tRNA method, which causes a considerable decrease in raffinose family oligosaccharides and a high level of sucrose in soybeans. According to their research, the multiplex CRISPR/Cas9 method may be a viable means of enhancing the quality of soybeans for monogastric animal and human consumption. The use of CRISPR/Cas technology in maize crops has demonstrated encouraging results. With an emphasis on gene function and creating new germplasm for enhanced yield, specialized corns, plant architecture, stress response, and haploid induction, Wang et al. provide an overview of the present uses and prospects of CRISPR/Cas technology in maize.

TALENs (Transcription Activator-Like Effector Nucleases)

TALENs are genome-editing tools that use engineered proteins to cut DNA at specific locations, allowing for targeted genetic modifications. They consist of two main parts:

- TALEs (Transcription Activator-Like Effectors): These are proteins that recognize specific DNA sequences.
- FokI Nuclease: An enzyme that cuts the DNA at the targeted site once the TALE proteins have bound to it.
- Uses engineered proteins (TALEs) fused with a nuclease (FokI**)** to target specific DNA sequences.
- More precise than CRISPR but requires complex protein design.
- Used in gene therapy and plant biotechnology.

ZFNs (Zinc Finger Nucleases)

- Uses zinc-finger proteins fused with FokI nuclease to recognize and cut specific DNA sequences.

- One of the first genome-editing tools, but less used now due to complexity and off-target effects.
- Applied in gene therapy (e.g., HIV resistance).

Mega nucleases (Homing Endonucleases)

- Naturally occurring enzymes that recognize long DNA sequences (12-40 base pairs).
- High specificity but limited flexibility compared to CRISPR and TALENs.

Prime Editing

- A more precise version of CRISPR that edits DNA without making double-strand breaks.
- Uses a modified Cas9 enzyme fused to a reverse transcriptase, allowing direct base Changes.

Prime Editor Complex

- A Cas9 nickase (a modified Cas9 enzyme that cuts only one DNA strand).
- A Reverse Transcriptase (RT) enzyme, which copies the desired genetic change into the DNA.
- A Prime Editing Guide RNA (pegRNA) that directs the editor to the target sequence and carries the desired edit.

Editing Process

- The pegRNA guides the Cas9 nickase to the target DNA site.
- Instead of cutting both strands (as in CRISPR-Cas9), it makes a single-strand break (a "nick").
- The Reverse Transcriptase copies the corrected DNA sequence into the cell.
- The cell naturally repairs the DNA, incorporating the precise genetic change. Promising for correcting genetic mutations without introducing random errors.

Base Editing

Different types of genomes editing tool as shown in fig 3

- A refined CRISPR technique that directly converts one DNA base to another (e.g., C to T or A to G) without cutting the DNA.
- Reduces unintended mutations and improves accuracy.
- Useful for treating genetic diseases like sickle cell anemia.

Each tool has its strengths and limitations, with CRISPR-Cas9 being the most commonly used due to its ease, efficiency, and cost-effectiveness.

Applications of Genome Editing In Crops Include

- **Disease Resistance**: Researchers have developed disease-resistant varieties by editing genes associated with susceptibility. For instance, CRISPR-edited tomatoes resistant to powdery mildew demonstrate the potential of this technology.
- **Abiotic Stress Tolerance**: Crops edited for enhanced tolerance to drought, salinity, and extreme temperatures are critical for agriculture in the face of climate change. Drought-tolerant rice and heat-resistant wheat are notable examples.

- **Nutritional Enhancement**: Genome editing has been used to increase the nutritional value of crops. For example, biofortified rice varieties with higher iron and zinc levels address micronutrient deficiencies in vulnerable populations.

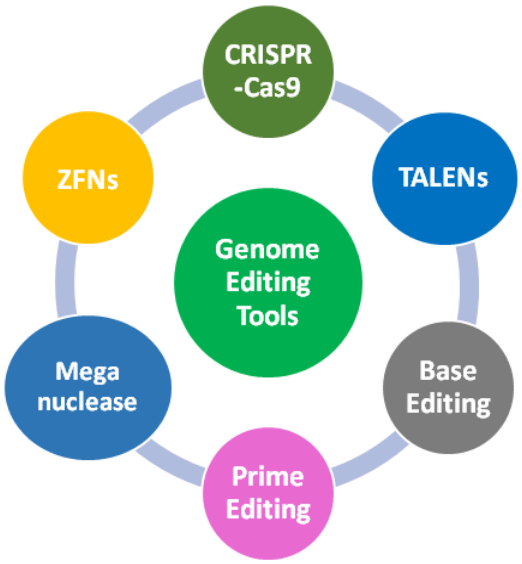

Fig: 3 Different types of Genomes editing tools

- **Yield Improvement**: By modifying genes involved in plant growth and development, researchers have achieved significant yield increases. Genome-edited maize and rice varieties with optimized plant architecture exemplify this approach.

CRISPR-Cas9's precision, efficiency, and cost-effectiveness make it a game-changer in agriculture. Its applications are expanding rapidly, offering solutions to challenges that were previously insurmountable.

Marker-Assisted Selection (MAS)

Marker-Assisted Selection (MAS) combines traditional breeding methods with molecular biology techniques to accelerate the development of improved crop varieties. Breeders can choose plants that possess desired qualities by detecting genetic markers linked to those traits, eliminating the need for lengthy field testing. Through marker-assisted selection (MAS), DNA markers have the potential to significantly improve the accuracy and efficiency of conventional plant breeding. Numerous mapping investigations of quantitative trait loci (QTLs) in different crop species have produced a multitude of relationships between DNA markers and traits. There are five main elements to consider when utilizing DNA markers in MAS: dependability, the amount and quality of DNA needed, the technical technique for marker testing, the level of polymorphism, and cost (Mackill & Ni 2000).Simple sequence repeats (SSRs), sometimes referred to as microsatellites, are the most widely used markers in main cereals (Gupta & Varshney 2000). These markers usually show a high degree of polymorphism, are co-dominant in inheritance, are reasonably simple and affordable to utilize, and are highly dependable (reproducible). Sequence-tagged sites (STS), sequence-characterized amplified regions (SCAR), and single nucleotide polymorphism (SNP) markers are extremely useful for marker-assisted selection (MAS) because they are

derived from particular DNA sequences of markers like restriction fragment length polymorphisms (RFLPs) connected to a gene or quantitative trait locus (QTL) (Shan et al., 1999; Sanchez et al., 2000; Sharp et al., 2001).

Key applications of MAS include

- **Disease Resistance**: MAS has been instrumental in developing rice varieties resistant to bacterial blight, a major disease affecting rice production in Asia (Fiyaz et al., 2022).
- **Abiotic Stress Tolerance**: Breeding programs have utilized MAS to develop crops tolerant to drought, salinity, and submergence. For instance, Sub1 rice varieties can survive prolonged flooding, ensuring stable yields in flood-prone regions (Jiang et al., 2020).
- **Quality Improvement**: MAS has enabled the development of crops with improved grain quality, such as aromatic rice and high-oil maize.
- **Yield Enhancement**: By selecting for traits associated with high yields, breeders have achieved significant productivity gains in staple crops like wheat and maize.

MAS is particularly valuable in crops with long breeding cycles, such as fruit trees, where traditional methods are time-consuming and labor-intensive. By reducing the time required to develop new varieties, MAS contributes to the rapid adoption of improved crops.

Micropropagation

Plant tissue culture is a biotechnological technique involving in vitro methods to enhance crop improvement, increase genetic variability, and improve plant health. It plays a crucial role in modern agriculture by facilitating genetic enhancement, pathogen elimination, and large-scale plant production. Tissue culture techniques are available for most crops, though optimization is still needed for cereals and woody plants. When combined with molecular techniques, tissue culture enables gene transfer for incorporating desirable traits.

Protoplast, anther, microspore, ovule, and embryo culture techniques contribute to genetic variation, including haploid production. Somaclonal and gametoclonal variations from cell cultures have significant crop improvement potential. Additionally, single-cell and meristem cultures help eradicate pathogens, enhancing the yield of established cultivars.

A major commercial application of tissue culture is micropropagation, the in vitro clonal propagation of plants from small tissue samples. It surpasses traditional asexual propagation by ensuring rapid multiplication of genetically identical plants. Micropropagation rejuvenates aging cultivars and accelerates the development of new, stress-resistant varieties. Large-scale micropropagation laboratories are essential for commercial ornamental and agricultural crop production. As plant breeding advances, tissue culture technologies are expected to have an increasing impact on global agriculture and food security.

Steps involved in Micropropagation:

Steps involve in micropropagation as shown in Fig 4

- **Explant selection:** Choosing a healthy plant part (like a shoot tip) from the desired parent plant.
- **Surface sterilization:** Disinfecting the explant to eliminate contaminants.
- **Initiation of culture:** Placing the explant on a nutrient-rich agar medium in a controlled environment.

- **Multiplication phase:** Promoting shoot proliferation by adjusting hormone levels in the medium to produce multiple shoots from the initial explant.
- **Rooting:** Transferring shoots to a different medium to induce root development.
- **Acclimatization:** Gradually adapting the plantlets to outdoor conditions before transplanting to soil.

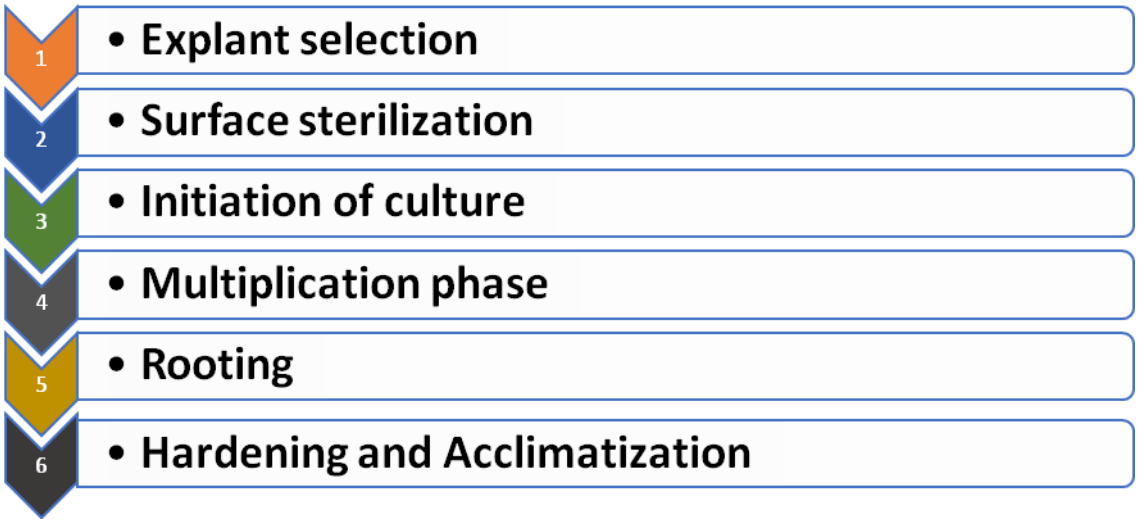

Fig: 4 Steps involved in micropropagation

Synthetic Biology

Synthetic biology applies engineering principles to biology, enabling the design and construction of novel biological systems. In agriculture, synthetic biology holds immense promise for addressing global challenges (Benner and Sismour. 2005).
Applications in crop improvement include:

- **Enhanced Photosynthesis**: Researchers are engineering photosynthetic pathways to increase the efficiency of light capture and carbon fixation. These advancements have the potential to significantly boost crop yields.
- **Nitrogen Fixation**: Synthetic biology aims to engineer crops capable of fixing atmospheric nitrogen, reducing the need for chemical fertilizers. Nitrogen-fixing maize and wheat are under development, offering sustainable solutions for smallholder farmers.
- **Biosynthesis of Secondary Metabolites**: Plants produce a wide range of secondary metabolites with applications in nutrition, medicine, and pest management. Synthetic biology enables the scalable production of these compounds, reducing reliance on chemical inputs.
- **Resistance to Emerging Threats**: Synthetic biology can rapidly respond to emerging pests and diseases by designing new resistance mechanisms. For example, engineered RNA interference (RNAi) pathways provide targeted control of specific insect pests.
- Despite its potential, synthetic biology faces challenges related to public perception, biosafety, and regulatory approval. Addressing these issues is critical for the widespread adoption of synthetic biology in agriculture.

Omics Technologies in Crop Improvement

Omics technologies, including genomics, transcriptomics, proteomics, and metabolomics, provide a systems-level understanding of plant biology. These tools enable the

identification of key genes, pathways, and networks involved in desirable traits (Roychowdhury., 2023).

Applications of omics technologies include:

- **Stress Tolerance**: Transcriptomics has revealed gene expression changes in response to abiotic stresses, guiding the development of resilient crops.
- **Nutritional Enhancement**: Metabolomics has identified pathways involved in the biosynthesis of essential nutrients, enabling the biofortification of crops like rice, maize, and cassava.
- **Disease Resistance**: Proteomics has uncovered proteins involved in plant defense mechanisms, informing the design of disease-resistant varieties.
- **Yield Improvement**: Integrating omics data has enabled the identification of yield-related genes, accelerating breeding programs for staple crops.
- Omics technologies are integral to modern crop improvement, offering insights into complex traits that were previously difficult to study (Tian et al., 2020).

Biotechnology in Sustainable Agriculture

Climate-Resilient Agriculture

Climate change poses significant challenges to agriculture. Biotechnology offers solutions through the development of crops that can withstand extreme weather conditions. For example, drought-tolerant maize varieties developed using genetic engineering ensure stable yields in water-scarce regions (Villalobos., 2022).

Biofertilizers and Biopesticides

Microbial genomics has enabled the development of eco-friendly biofertilizers and biopesticides. These products reduce chemical inputs, improve soil health, and minimize environmental pollution. For instance, nitrogen-fixing bacteria enhance soil fertility naturally, reducing dependence on synthetic fertilizers.

Carbon Sequestration and Soil Health

Biotechnological innovations contribute to carbon sequestration and improved soil health. Engineered microbial communities can capture atmospheric carbon, enhancing soil organic matter and mitigating climate change effects.

Public Perception of Biotechnology

Public acceptance of biotechnology varies widely. While GMOs and genome-edited crops have demonstrated significant benefits, skepticism persists due to concerns about safety, environmental impact, and ethical implications. Transparent communication and robust regulatory frameworks are critical to building trust (Lucht.,2015)

Economic Impacts

Biotechnology has transformed agriculture, benefiting farmers through increased yields and reduced input costs. In developing countries, biotech crops have improved livelihoods by addressing local challenges such as pests and diseases. However, issues such as corporate control over seed patents and high technology costs need to be addressed to ensure equitable benefits.

Ethical Dilemmas

Ethical considerations include the potential loss of biodiversity, unintended ecological effects, and the morality of modifying living organisms. Addressing these concerns requires collaboration among scientists, policymakers, and ethicists to develop responsible practices and regulations.

Conclusion

Biotechnology and genomics have ushered in a new era of agricultural innovation, addressing critical challenges such as food security, climate change, and sustainability. This chapter has explored their fundamental concepts, applications, ethical considerations, and future directions. The path forward requires interdisciplinary collaboration, public engagement, and sound policies to harness these technologies responsibly. By doing so, we can unlock their full potential to create a more resilient, equitable, and sustainable agricultural future.

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