Chapter-1

Scope of Plant Biotechnology: Plant Tissue Culture, Organ Culture & Embryo Culture.

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ABSTRACT

Plant biotechnology refers to a scientific discipline that utilizes technological and biological methods to alter plants for particular aims. It integrates knowledge from plant biology, genetics, molecular biology, and biochemistry to improve crops, create new plant varieties, and enhance agricultural sustainability. Crops that are genetically modified organisms (GMO crops) are agricultural plants whose DNA has been altered through genetic engineering methods. It is aimed to add new traits that the species does not naturally possess. Genetically modified plants known as transgenic plants contain a gene or genes that have been artificially introduced from another organism (often from a different species) to provide the plant with new characteristics not naturally present in that species.

KEYWORDS: Biotechnology, Plant Biotechnology, Scope, Plant Tissue Culture (PTC), Organ culture, Embryo culture.

INTRODUCTION

While multiple definitions of plant biotechnology exist, it is most often viewed as the genetic engineering of plants through recombinant DNA. A variety of scientific methods and techniques for screening and genetically manipulating plants to create beneficial or useful plant/plant products are included in Plant Biotechnology. Plant biotechnology involves using genetic engineering and tissue culture techniques to develop genetically modified plants that possess new or improved desirable characteristics. Among the desirable traits are improved yields and quality, as well as enhanced resistance to negative influences like diseases, pests, and harsh environmental conditions such as freezing temperatures, droughts, and salinity. With plant biotechnology, it is possible to produce in plants useful proteins that are encoded by genes from animals, humans, or microbes. It has been demonstrated by plant biotechnology that all of these objectives can be achieved, at least for the types of plants on which attempts have been made. Scientific techniques that can be used to create cellular- and molecular-based technologies aimed at enhancing plant productivity by improving the quality of plant products and minimizing environmental constraints on plant productivity are encompassed by plant biotechnology. With the help of plant biotechnology, breeders can make precise genetic alterations to introduce valuable traits into plants, exceeding all prior expectations.

PLANT BIOTECHNOLOGY

A variety of scientific methods and techniques for screening and genetically manipulating plants to create beneficial or useful plant/plant products are included in Plant Biotechnology. The

effectiveness of these tools and techniques could be enhanced by nanotechnology. In the near future, it is expected that plant biotechnology will play a crucial role in the increasing crop production. Mineral assimilation enhancement must address the challenges of reducing fertilizer use in developed countries, environmental conservation, sustainable agricultural practices, and the creation of low-input, high-performance crops in regions where soil infertility hinders productivity.

MAJOR MILESTONES

Plant biotechnology is fundamentally based on the concepts of cellular totipotency and genetic transformation. These concepts originate from the Cell Theory proposed by Matthias Jakob Schleiden and Theodor Schwann, and from Frederick Griffith's discovery of genetic transformation in bacteria, respectively.

- In the 1830s and 1840s, Schleiden and Schwann proposed the Cell Theory, which established the cell as the basic unit of life.
- This concept, along with Haberlandt's demonstration of plant cell totipotency in 1902, laid the groundwork for plant tissue culture and later genetic engineering.
- During 1960s, after decades of work, Norman Borlaug creates dwarf wheat that increases yields by 70 percent, launching the Green Revolution that helps save millions of lives.
- In the 1970s, recombinant DNA (rDNA) technologies were developed (Stanford University and the University of California) which opened up new possibilities for genetic engineering in plants.
- In 1980s, the first transgenic plants were developed by inserting bacterial genes into tobacco.
- Golden Rice, a genetically engineered crop designed to generate beta-carotene and combat vitamin A deficiency, was created in 1999.

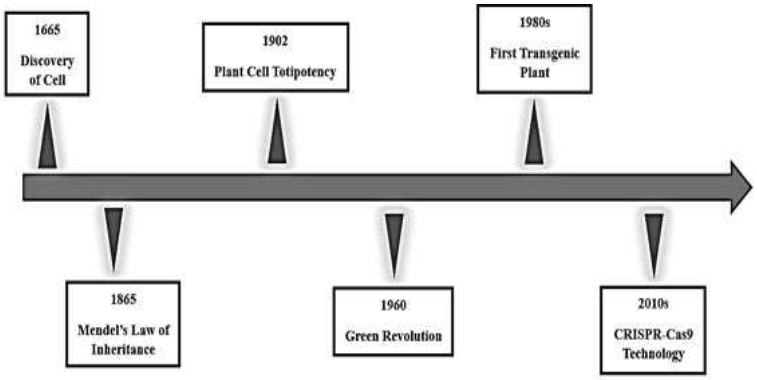

Figure 1. The flow chart represents the important discoveries and inventions which has greatly contributed toward the development of plant biotechnology.

SCOPE OF PLANT BIOTECHNOLOGY

The scope of plant biotechnology is broad and rapidly expanding, with applications in agriculture, industry, environmental management, and health. Here's an overview of its main areas:

1. Agricultural Improvements

- **Crop yield and quality enhancement:** Genetically modified (GM) crops with higher productivity, better nutritional content, and improved shelf life.
- **Pest and disease resistance:** Development of crops resistant to insects (e.g., Bt cotton), fungi, viruses, and bacteria.
- **Stress tolerance:** Engineering plants to withstand abiotic stresses like drought, salinity, and extreme temperatures.

2. Genetic Engineering

- **Transgenic plants:** Introducing specific genes to confer desired traits (e.g., herbicide resistance).
- **Gene editing tools:** Technologies like CRISPR/Cas9

for precise genetic modifications without introducing foreign DNA.

3. Plant Tissue Culture

- ***In Vitro* propagation**: The rapid clonal multiplication of uniform, disease-free plants.
- **Somaclonal variation:** Generating genetic diversity for breeding.
- **Synthetic seeds**: Somatic embryos are encapsulated for convenient storage and planting.

4. Molecular Breeding

- **Marker-assisted selection (MAS):** Using molecular markers to select desirable traits more efficiently than conventional breeding.
- **Genomic selection**: Forecasting plant performance with genome-wide markers.

5. Biopharmaceuticals and Nutraceuticals

- **Edible vaccines:** Plants engineered to produce vaccine components.
- **Phytochemicals:** Production of medicinal compounds like alkaloids, flavonoids, and essential oils.

6. Biofuels and Industrial Products

- **Bioenergy crops:** Engineering plants for higher biomass and oil production for biofuels.
- **Bioplastics and fibres:** Production of biodegradable plastics and sustainable materials.

7. Environmental Applications

- **Phytoremediation:** Using plants to clean up soil, water, and air contaminants.
- **Carbon sequestration:** Enhancing plants' ability to capture atmospheric CO, .

8. Conservation and Biodiversity

- **Cryopreservation:** Long-term storage of plant

germplasm.

- **In vitro conservation:** Maintaining rare and endangered plant species in controlled conditions.

PLANT TISSUE CULTURE

New varieties were traditionally developed using seed propagation. But at present, plantlets generated via micropropagation provide a viable option for numerous plant species. Even though micropropagation demands considerable effort, it reduces the time needed to commercialize new varieties and enables the production of disease-free plants. The contemporary field of plant biotechnology has emerged in a new era of science and technology, where the generation of secondary metabolites, significant enhancements to plant genetics, conservation of germplasm, and development of numerous disease-free and novel varieties are prioritized. The current research in plant tissue culture science includes the production of artificial seeds, biopharmaceuticals, recombinant or other therapeutic proteins, transgenic plants, and plant-made vaccines or antibodies (plantibodies).

ORGAN CULTURE

Organ culture refers to the cultivation of organs or plant parts in artificial media or a culture derived from isolated medium. Explants in organ culture can be any part of the plant, such as the shoot (for shoot tip culture), root (for root tip culture), leaf (for leaf culture), and flower (anther, ovary, ovule cultures). Studies examining dependence on growth regulators and other growth factors have demonstrated that organ culture is highly dependable. It also contributes to expanding the scope of advancements in agriculture and horticulture. The main forms of organ culture used for in vitro plant propagation include meristematic culture, shoot tip culture, nodal culture of separate lateral buds, isolated root culture, and embryo culture.

The first organs to be cultured were excised roots. Root tips about 10 mm in length are extracted from young seedlings grown under axenic conditions and placed in an aqueous culture medium, where they generate laterals. The main explant is now divided into sectors, each containing a portion of the main root along with some lateral roots. Each sector is periodically transferred to fresh medium to maintain a continuous supply of roots. Essential substances alone fulfil the medium requirements, yet some cultures show a positive response to the addition of auxin and other growth regulators. Although basic anatomy and metabolism remain intact, the ability to form secondary vascular tissue is gradually lost in cultured roots.

Shoot tip cultures can be classified into two categories: shoot apex culture, where a segment of the shoot tip that includes an apical meristem, a few leaf primordia, and some stem tissue is cultured. Bud can also serve as an explant. For their growth, they require exogenous gibberellin and cytokinin. To foster stem elongation, a medium devoid of exogenous growth regulators is required. Ultimately, culture requires a third medium with auxin for root initiation. The explant may consist solely of an excised and isolated apical meristematic dome, lacking any leaf primordia or stem tissue. Then, they can be cultured either on agar media or on filter paper that is soaked in a liquid medium. The cultures are referred to as apical meristem culture. These cultures necessitate the presence of exogenous hormones in their medium.

Haploid plants are produced from anthers that have been cultured on basal nutrient medium. Anthers are excised from buds chosen at the uninucleate microspore stage, when microspore mother cells are undergoing early meiosis. After sterilization, they are placed on an appropriate culture medium. Haploid plants may be formed directly from pollens in anthers, or a callus may first be created from which plants develop.

ORGAN CULTURE FOR CROP IMPROVEMENT

Organ culture, which is a type of plant tissue culture, entails the in vitro growth of particular plant organs like roots, shoots, or embryos in a sterile and controlled environment. This technique is important for crop improvement as it allows for the propagation of disease-free plants, rapid multiplication of desirable genotypes, and conservation of rare or endangered plant species. Whole plants can be regenerated from explants in organ culture, and these explants can be genetically altered to exhibit traits like pest resistance, drought tolerance, or improved nutritional value. It also facilitates research into plant developmental biology and hybrid production by addressing challenges such as embryo abortion. In summary, organ culture serves as a significant resource in contemporary plant biotechnology, aiding sustainable agriculture and food security.

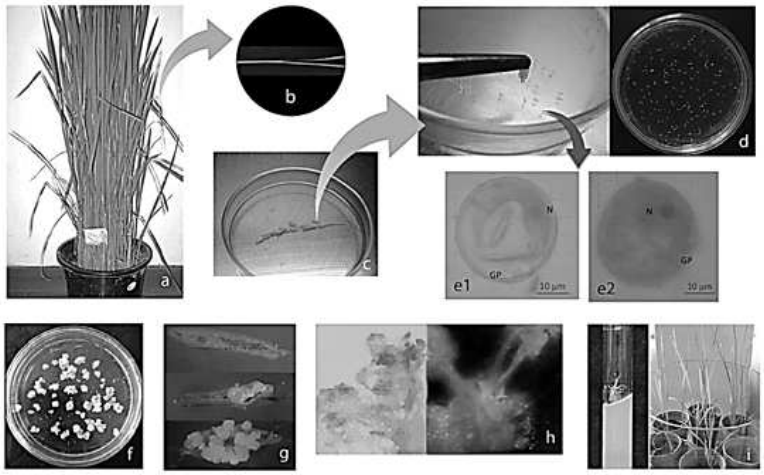

Figure 2. Stages of *in vitro* anther culture for creating doubled haploids in ***indica* rice**. **(a)** Disinfected Mother rice plants **(b)** A panicle **(c)** Surface sterilization **(d)** Anther cultured for callus formation **(e1, e2)** Microspores at uni-nucleate stage; N = nucleus; GP = germ pore; *scale bar = 10 µm* **(f, g)** Calluses development from haploid anthers **(h)** Shoot induction in

anther-derived calluses **(i)** Fully developed haploid plantlets **(Mayakaduwa, & Silva, 2023)**.

EMBRYO CULTURE

Hannig conducted the first embryo cultures in 1904. The seed is cut open to remove the embryo, which is then cultured on a specialized medium. The media requirements differ depending on the species, the stage of the excised embryo, and the experiment's objective. The richness and composition of required media vary among young heterotrophic embryos, globular embryos, and undifferentiated preglobular embryos.

Nucellar tissue removed from ovules (either pre- or post-pollination) and cultured on an appropriate medium first generates a callus, from which numerous tumor-like pseudo bulbils emerge. These bulbils develop into embryos and eventually seedlings. While embryogenesis in the Nucellar tissue of pre-fertilized ovules occurs only with malt extract and adenine, post-fertilization Nucellar tissue from ovules requires casein hydrolase supplementation for proper development.

It is relatively easy to raise mature seeds by culturing ovaries when the embryo is at the globular stage or later in its development. This requires a rather uncomplicated nutritive medium. Seeds from plants with reduced embryos, as well as seeds from parasites, have also been cultured for different purposes. It is quite common to culture ovaries in order to improve fruit quality or study fruit physiology. After pollination, ovaries are cultured. A simple medium containing mineral salts and sugar, sometimes enhanced with vitamins, glycine, and yeast extract, is sufficient. Fruits derived from ovary culture typically have a maximum size that is smaller than that of naturally occurring fruit. Embryo culture techniques are also been implemented for restoration of diversity of many rare and endangered forest plant species.

EMBRYO CULTURE FOR CROP IMPROVEMENT

Embryo culture, a method of plant tissue culture, entails the aseptic cultivation of plant embryos (extracted from seeds) on nutrient media in a controlled lab environment. This technique is particularly useful in enhancing crops because it enables the recovery of embryos from wide crosses that would otherwise not succeed due to post-fertilization obstacles, like embryo abortion. Embryo culture facilitates the transfer of advantageous traits such as disease resistance, stress tolerance, and enhanced yield from wild relatives to cultivated crops by enabling the development of hybrid plants from interspecific or intergeneric crosses. Additionally, it shortens breeding cycles by speeding up seed germination and overcoming seed dormancy. Therefore, embryo culture is essential for increasing genetic diversity and promoting the creation of improved crop varieties.

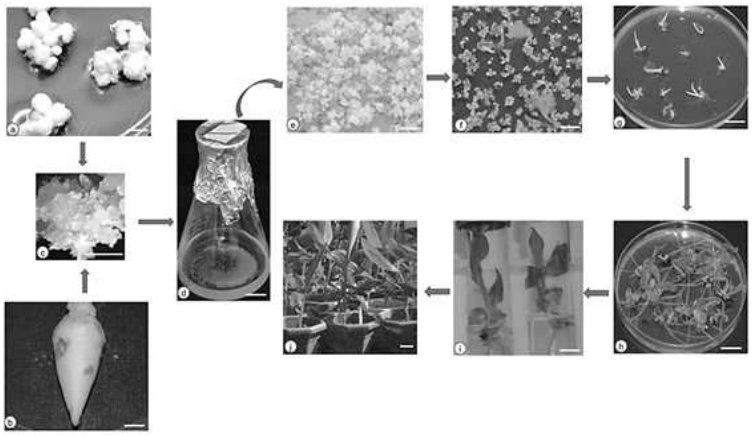

Figure 3. Different stages of somatic embryogenesis and plant regeneration in *Musa* spec. (banana). **(a)** Callus development from multiple meristems explants, **(b)** Male bud, **(c)** Friable embryogenic ideal callus, **(d)** Embryogenic cell suspension, **(e, f, g)** embryo development and maturation, **(h)**

Embryos germinating into plantlets and shoots, **(i)** Fully developed plantlets with roots, **(j)** Complete plants transferred to pots in the greenhouse **(Adero et al., 2023)**.

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