Plant Tissue Culture

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***Abstract*— Plant tissue culture is a modern biotechnological technique that involves the in vitro cultivation of plant cells, tissues, or organs under controlled aseptic and nutrient-rich conditions. It exploits the principle of totipotency, enabling the regeneration of whole plants from a small explant. This method has wide applications, including micro propagation of disease-free and genetically uniform plants, production of secondary metabolites, conservation of endangered species, germplasm storage, and crop improvement through genetic engineering. Tissue culture has revolutionized agriculture, horticulture, forestry, and pharmaceutical industries by providing rapid, large-scale, and sustainable propagation methods, making it a vital tool in plant science and biotechnology.**

I. INTRODUCTION

Plant tissue culture is a branch of plant biotechnology that involves growing plant cells, tissues, or organs in a sterile, controlled environment on a nutrient medium. The technique is based on the principle of totipotency, which is the ability of a single plant cell to regenerate into a complete plant. By providing the right balance of nutrients, plant growth regulators, and aseptic conditions, plant tissue culture enables the mass production of genetically uniform and disease-free plants within a short time. This method plays a significant role in agriculture, horticulture, forestry, and pharmaceuticals, as it supports crop improvement, conservation of rare and endangered species, and large-scale production of valuable secondary metabolites. Tissue culture has thus become an indispensable tool in both research and commercial applications.

II. HISTORY OF PLANT TISSUE CULTURE

The origin of plant tissue culture dates back to the early 20th century, when German botanist Gottlieb Haberlandt (1902) first proposed the concept of totipotency, suggesting that a single plant cell has the potential to regenerate into a whole plant. Although his initial attempts to culture isolated cells were unsuccessful, his idea laid the foundation for future research. In the 1930s, Philip R. White and Nobécourt developed techniques to culture isolated plant tissues on nutrient media. A major breakthrough came in 1939 when Gautheret and Nobécourt successfully During the 1950s and 1960s, Skoog and Miller discovered the role of plant hormones, especially auxins and cytokinins, in regulating cell division and differentiation, which greatly advanced the field. Later, Murashige and Skoog (1962) developed the widely used MS medium, which provided a standardized nutrient environment for in vitro culture. Since then, plant tissue culture has evolved into an essential tool for micropropagation, genetic engineering, germplasm conservation, and production of bioactive compounds, revolutionizing both plant science research and commercial agriculture.

Early Concepts (1830s – 1900s)

- 1838–1839 → Schleiden and Schwann proposed the cell theory every plant cell is capable of independent existence and growth.
- 1885 → Gottlieb Haberlandt (known as the "Father of Plant Tissue Culture") first suggested the concept of totipotency. He attempted to culture isolated single cells of plants but failed due to lack of proper nutrient media.

Development Phase (1900–1940)

- 1902 → Haberlandt attempted to culture isolated leaf cells of Coffea arabica and Tradescantia.
- 1922 → Kotte *and* Laibach successfully cultured plant embryos.
- 1925 → Hanning grew isolated embryos on a simple nutrient medium.
- 1934 → Gautheret in France and *White* in the USA successfully cultured plant tissues (callus culture).

- 1939 → Van Overbeek showed coconut milk (endosperm extract) promotes embryo development

III. ESTABLISHMENT PHASE (1940–1960)

- 1941 → White standardized nutrient medium for root culture.
- 1948 → Skoog and Tsui demonstrated the role of auxins in tissue culture.
- 1954 → Miller et al. discovered cytokinins (kinetin), revolutionizing organogenesis.
- 1957 → Skoog and Miller proposed the hormonal balance concept:
  - High auxin: root formation
  - High cytokinin: shoot formation

Expansion Phase (1960–1980)

1962 → Murashige and Skoog developed the cultured plant callus tissue, demonstrating sustained growth.

- 1960s–70s → Development of micropropagation techniques for rapid clonal propagation.
- Somatic embryogenesis and haploid culture methods were established.

IV. PRINCIPLE OF PLANT TISSUE CULTURE

The fundamental principle of plant tissue culture is cellular totipotency, which is the inherent capacity of a single plant cell to regenerate into a complete plant under appropriate conditions. In practice, a small piece of plant tissue, known as an explant, is placed on a sterile, nutrient-rich medium containing essential minerals, vitamins, carbohydrates, and plant growth regulators. By manipulating the ratio of hormones such as auxins and cytokinins, scientists can induce the explant to undergo cell division, callus formation, organogenesis (formation of shoots and roots), or somatic embryogenesis. Controlled aseptic conditions are essential to prevent microbial contamination, while regulated light, temperature, and humidity provide an environment suitable for growth. Thus, plant tissue culture relies on the combination of totipotency, nutrient supply, hormonal balance, and aseptic technique to regenerate plants and produce useful compounds in vitro.

- Totipotency All plant cells contain the full set of genetic material, and under proper conditions (nutrients + hormones), they can regenerate into a whole plant.
- Dedifferentiation: Mature cells can revert back to an undifferentiated state (callus formation).
- Redifferentiation: Undifferentiated cells later specialize into organs like roots, shoots, or embryos.

V. PLANT TISSUE CULTURE TECHNIQUES

Plant tissue culture involves a wide range of techniques depending on the type of plant material used and the purpose of the culture. One of the most common methods is callus culture, where small pieces of plant tissue (explants) are placed on a nutrient medium supplemented with plant growth regulators, leading to the formation of an unorganized mass of cells called callus. This is often the starting point for regeneration and genetic studies. From callus, cell suspension culture can be established by transferring friable callus into a liquid medium under agitation, producing dispersed cells that are especially useful for large-scale production of valuable secondary metabolites.

Another important technique is embryo culture, where isolated embryos from seeds are grown in vitro to

- MS medium, the most widely used medium in plant tissue culture.

not survive naturally. Similarly, anther and pollen culture allow the development of haploid plants directly from male gametophytic tissue, which are then doubled to produce homozygous lines beneficial in plant breeding. Protoplast culture and fusion involve the enzymatic removal of cell walls, allowing fusion between different species or varieties to create novel hybrids through somatic hybridization.

In addition, organ culture focuses on the growth of specific plant organs such as shoots, roots, or ovules in controlled conditions to study their development. A related technique, somatic embryogenesis, involves the formation of embryo-like structures from somatic cells that can regenerate into full plants, providing a powerful tool for clonal propagation and synthetic seed production. Finally, meristem and shoot tip culture, commonly used in micropropagation, exploits the virus-free meristematic tissue of plants to produce large numbers of genetically identical and disease-free plants within a short period.

Together, these techniques have transformed agriculture, horticulture, forestry, and biotechnology by enabling large-scale propagation, genetic improvement, conservation of endangered species, and production of bioactive compounds.

VI. BASIC REQUIREMENTS FOR PLANT TISSUE CULTURE

Plant tissue culture requires a set of essential components and conditions to ensure the successful growth of plant cells, tissues, or organs in vitro. The first requirement is a suitable explant, which is the piece of plant material (such as leaf, stem, root, or meristem) used to initiate the culture. A sterile environment is crucial, as contamination by bacteria or fungi can easily destroy the culture; therefore, aseptic conditions must be maintained using laminar airflow chambers, sterilized instruments, and surface-sterilized explants. The explant is placed on a nutrient medium, such as Murashige and Skoog (MS) medium, which supplies essential mineral salts, vitamins, carbohydrates (usually sucrose), and growth regulators. The balance of plant hormones, particularly auxins and cytokinins, is critical for directing callus formation, root initiation, or shoot regeneration.

In addition to chemical requirements, physical conditions also play an important role. Cultures must be maintained at an optimal temperature (generally 24–28 °C), light intensity and photoperiod (commonly 16 hours light and 8 hours dark), and relative humidity suitable for growth. Proper equipment and facilities such as autoclaves, culture vessels, incubators, and microscopes are also necessary for successful operation. overcome seed dormancy or rescue hybrids that may avoid contamination (using laminar airflow autoclaving)

- Nutrient Medium: Provides minerals, vitamins, sugars, amino acids, and plant growth regulators.
  - MS medium (Murashige & Skoog, 1962) is most widely used.
- Growth Regulators (Hormones):
  - Auxins → promote root formation & callus induction
  - Cytokinins → promote shoot formation
  - Gibberellins → elongation and embryo development
  - ABA (Abscisic Acid) → promotes dormancy and embryo maturation
- Physical Factors: Controlled light, temperature (24–28°C), humidity, and pH (~5.6).

VII. PROCESS OF PLANT TISSUE CULTURE

The process of plant tissue culture involves several systematic steps carried out under sterile and controlled conditions. It begins with the selection of explant, a small piece of plant tissue such as a leaf, stem, root, or meristem. The explant is then surface sterilized using disinfectants like ethanol or sodium hypochlorite to eliminate microbial contamination. After sterilization, the explant is placed onto a sterile nutrient medium (such as Murashige and Skoog medium) that provides essential minerals, vitamins, sugars, and growth regulators like auxins and cytokinins.

Once placed on the medium, the explant undergoes cell division and forms an unorganized mass of cells called callus. By adjusting the concentration of hormones in the medium, the callus can be induced to undergo organogenesis (formation of shoots and roots) or somatic embryogenesis (development of embryo-like structures). The regenerated shoots and roots are then transferred to a fresh medium for further growth. Finally, the complete plantlets are moved to acclimatization or hardening stage, where they are gradually adapted to external environmental conditions in soil, greenhouse, or field.

Thus, the process of plant tissue culture follows a sequence of explant selection → sterilization → inoculation on medium → callus induction → regeneration (shoot/root formation) → acclimatization, leading to the successful development of whole plants from small tissue pieces.

Callus Culture

A callus is an unorganized mass of cells that develops when cells are wounded. When the explant is cultivated on media that promote the development of

- Explant: The plant part used to start culture (leaf, stem, root, bud, anther, embryo, etc.).
- 2Aseptic Conditions: Sterile environment to cytokinins. Using plant growth hormones, callus can multiply continuously or be directed to develop organs or somatic embryos

Cell Suspension Culture

Small fragments of loose friable callus can be cultured as cell suspension cultures in a liquid medium. Cell suspensions can be maintained as batch cultures grown in flasks for long periods. A portion of callus tissue can be transferred into a liquid medium, and when subjected to continuous shaking, single-cell cultures and suspension cultures can be cultivated from callus cultures. The growth rate of the suspension-cultured cells is generally higher than that of the solid culture.

Anther/Microspore Culture

The culture of anthers or isolated microspores to produce haploid plants is known as anther or microspore culture. Embryos can be produced via a callus phase or be a direct recapitulation of the developmental stages characteristic of zygotic embryos. Compared to traditional breeding methods, microspore culture enables the creation of homozygous plants in a very short time. These homozygous plants are useful tools in plant breeding and genetic studies.

Protoplast Culture

Protoplasts contain all the components of a plant cell except for the cell wall. Protoplasts can be used to create somatic hybrids and regenerate whole plants from a single cell. Cell walls of explant can be removed either mechanically or enzymatically. Protoplasts can be cultured either in liquid or solid medium. Protoplasts embedded in an alginate matrix and then cultured on a solid medium have better success rates of regeneration. Although protoplasts appear to be a very appealing method for regenerating plants and transferring genes, they are extremely delicate.

Embryo Culture

It is a technique in which isolated embryos from immature ovules or seeds are cultured in vitro. For species whose seeds are dormant, resistant, or prematurely sterile, embryo culture has been used as a helpful tool for direct regeneration. In plant breeding programs, embryo culture goes hand in hand with in vitro control of pollination and fertilization to ensure hybrid production. In addition, direct somatic embryos and embryogenic calluses can be produced from immature embryos.

Meristem Culture

Using apical meristem tips, it is possible to produce disease-free plants. This technique can be referred to as meristem culture, meristem tip culture, or shoot tip culture, depending on the actual explant used. Plant apical meristems make good explants for the cultivation of virus-free plants. Hence, this method is usually used to eliminate viruses in many species. undifferentiated cells, a callus is formed. The majority of callus cells are formed with the aid of auxins and

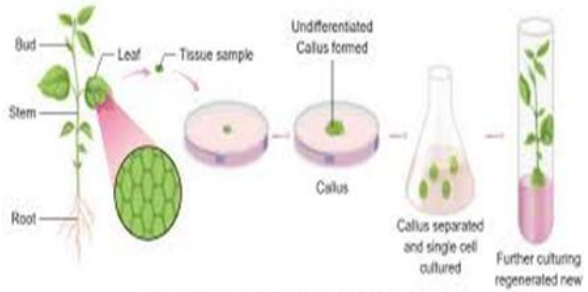


Figure 5.3: Basic steps in Plant tissue culture technology

VIII. REGENERATION METHODS OF PLANTS IN CULTURE ORGANOGENESIS

In plant tissue culture, it refers to the formation of either shoot or root. The equilibrium of auxin and cytokinin and the tissue's capacity to react to phytohormones during culture are key factors in in-vitro organogenesis. In-vitro organogenesis can be of two types

Indirect organogenesis involves the formation of organs indirectly via a callus phase. For the production of transgenic plants, induction of plants through a callus phase has been used. Either the callus is transformed, plants are regenerated, or the primary explant is transformed, and the callus is formed, and then shoots are cultivated from the explant. It is more important for transgenic plant production.

Direct organogenesis involves direct bud or shoots formation from the tissue without a callus stage. Plants are usually propagated by direct organogenesis for improved multiplication rates and production of transgenic plants but mainly for clonal propagation.

Somatic Embryogenesis

Somatic embryogenesis is a nonsexual developmental process that produces a bipolar embryo with a closed vascular system from the somatic tissues of a plant. It

has become one of the most powerful techniques in plant tissue culture for mass clonal propagation. Somatic embryogenesis may occur directly or via a callus phase. For clonal propagation, direct somatic embryogenesis is preferred since there is less chance of somaclonal

Indirect somatic embryogenesis is usually used in the selection of desired somaclonal variants and for the production of transgenic plants.

Encapsulated somatic embryos are known as synthetic seeds. Synthetic seeds have multiple advantages. They are easy to handle, they can potentially be stored for a long time, and there is potential for scaleup and low cost of production.

Rooting of shoots

The success of acclimatization of a plantlet greatly depends on root system production. Rooting of shoots can be achieved in vitro or ex-vitro.

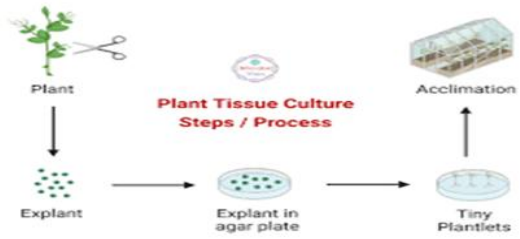
Ex vitro rooting involves pretreating the shoots with phenols or auxins and then planting them directly in soil under high humidity, which significantly lowers the cost of manufacturing. This technique also allows simultaneous acclimation of the rooted shoots.

In vitro rooting consists of rooting the plants in axenic conditions. Despite the cost factor, in vitro rooting is still a common practice in many plant species.

Several factors are known to affect rooting. The most important factor is the action of endogenous and exogenous auxins. Phenolic compounds are also known to have a stimulatory effect on rooting. Phloroglucinol, a root promoter, is reported effective in root development. Catechol, a strong reducing agent, has been reported to regulate IAA oxidation.

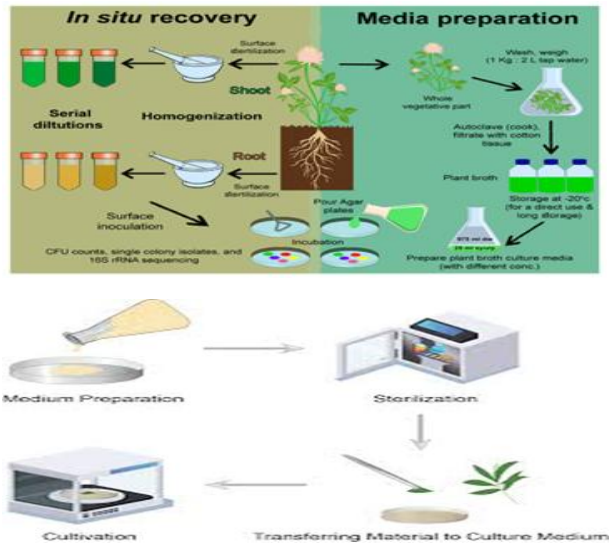
Acclimation / Acclimatization

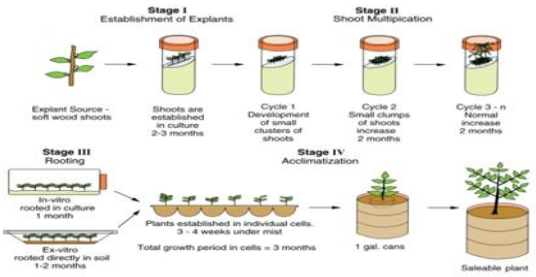
Once plants are generated by tissue culture, they have to be transferred to the greenhouse or field. This requires that the plants be hardened-off before transfer to the field. To reduce water loss during acclimatization, plants are initially transferred to a greenhouse or growth chamber. The relative humidity outside the vessels is often significantly lower than the humidity inside the vessels. Once the plants are acclimatized under greenhouse conditions, they are ready for transfer to the field.



IX. MEDIA PREPARATION IN PLANT TISSUE CULTURE

Media preparation is one of the most critical steps in plant tissue culture, as the growth and development of cultured tissues depend on the nutrient composition of the medium. A standard medium, such as Murashige and Skoog (MS) medium, contains essential mineral salts (macro- and micronutrients), vitamins, amino acids, a carbon source (usually sucrose), and plant growth regulators like auxins and cytokinins in specific concentrations. To prepare the medium, the required chemicals are accurately weighed, dissolved in distilled water, and adjusted to the desired pH (generally 5.6–5.8). A gelling agent such as agar is often added to solidify the medium, although liquid media are used for suspension cultures. The medium is then dispensed into culture vessels, such as test tubes, jars, or flasks, and sterilized in an autoclave at 121 °C under 15 psi pressure for about 15–20 minutes. Once sterilized, the medium is allowed to cool and solidify before use. Proper media preparation ensures that plant cells receive the necessary nutrients and hormones for callus induction, organogenesis, embryogenesis, and complete plant regeneration.

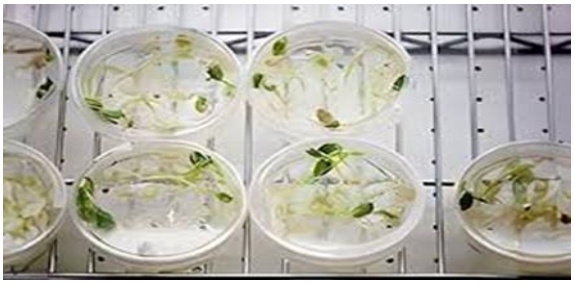




X. TYPES OF PLANT TISSUE CULTURE MEDIA

Plant tissue culture media are specially formulated nutrient solutions that provide essential minerals, vitamins, carbon sources, and growth regulators for in vitro growth. Several types of media have been developed depending on the plant species and the purpose of culture. The most widely used is Murashige and Skoog (MS) medium, which has high salt concentration and supports rapid growth and regeneration. Gamborg's B5 medium is commonly used for cell suspension cultures and protoplast cultures because of its suitable composition of vitamins and nitrogen sources. Nitsch and Nitsch (NN) medium is often used for anther and pollen cultures to produce haploid plants. White's medium, one of the earliest formulations, is useful for root culture studies. N6 medium, developed by Chu, is mainly used for cereal crops like rice in anther and cell culture studies. In addition, SH (Schenk and Hildebrandt) medium is frequently used for callus induction and somatic embryogenesis.

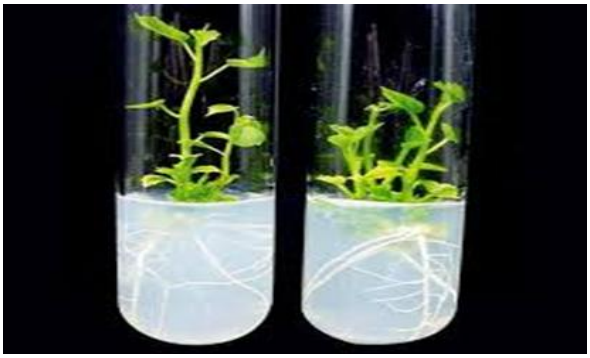
Each medium differs in its concentration of salts, vitamins, and organic additives, making them suitable for specific tissues, organs, or species. By modifying these media with the right balance of auxins, cytokinins, and other supplements, researchers can direct plant tissue development toward callus formation, organogenesis, or somatic embryogenesis, depending on experimental needs.



XI. COMPOSITION OF MEDIA FOR PLANT TISSUE CULTURE

Plant tissue culture media are artificial nutrient formulations that provide all the essential requirements for the growth and differentiation of plant cells in vitro. The composition typically includes inorganic nutrients, organic nutrients, a carbon source, growth regulators, and sometimes other additives. Inorganic nutrients supply macronutrients (such as nitrogen, phosphorus, potassium, calcium, magnesium, and sulfur) and micronutrients (such as iron, manganese, zinc, boron, copper, and molybdenum), which are vital for metabolic and structural functions. Organic nutrients include vitamins (e.g., thiamine, nicotinic acid, pyridoxine), amino acids (e.g., glycine), and organic acids that support enzymatic activities and cell growth. A carbon source, usually sucrose (2–3%), provides energy for heterotrophic growth since isolated plant tissues cannot perform full photosynthesis in vitro.

Plant growth regulators such as auxins (IAA, NAA, 2,4-D) and cytokinins (BAP, kinetin) are critical in directing morphogenesis, while gibberellins and abscisic acid are occasionally used for specific developmental responses. A gelling agent such as agar (0.7–0.8%) is added to solidify the medium, though liquid media are also used for cell suspensions. The pH of the medium is usually adjusted to 5.6–5.8 before sterilization. In some cases, additives such as activated charcoal, casein hydrolysate, or coconut water are used to improve growth and regeneration. Together, these components create an optimal environment for callus induction, organogenesis, embryogenesis, and complete plant regeneration.



XII. ADVANTAGES OF PLANT TISSUE CULTURE

Plant tissue culture offers several important advantages over conventional methods of plant propagation. One of the major benefits is the rapid clonal multiplication of plants, where a large number of genetically identical plants can be produced in a short time from a small explant. This ensures uniformity in growth and yield. Another significant advantage is the production of disease-free plants, since meristem culture eliminates systemic pathogens such as viruses. Tissue culture also plays a crucial role in the conservation of rare, endangered, and threatened plant species through in vitro germplasm storage and cryopreservation.

In agriculture and horticulture, this technique helps in the year-round production of plants, independent of seasonal or climatic constraints. It is also a valuable tool in plant breeding and crop improvement, as it enables the development of haploids, somatic hybrids, and genetically engineered plants. Furthermore, cell and organ cultures are used for the production of secondary metabolites such as alkaloids, flavonoids, and other bioactive compounds that are difficult to obtain through conventional cultivation. The method also allows for the rescue of hybrid embryos and overcoming of seed dormancy, which is useful in breeding programs.

In plant breeding programs, techniques like anther and pollen culture help in developing haploid plants that can be converted into homozygous lines, accelerating crop improvement. Embryo culture is useful for rescuing hybrid embryos that normally would not survive, thus overcoming barriers in interspecific and intergeneric hybridization. Tissue culture also enables somatic hybridization and genetic transformation, making it a key tool in developing transgenic plants with improved traits such as pest resistance, stress tolerance, and enhanced nutritional value.

On the industrial side, plant cell and organ cultures are exploited for the production of secondary metabolites like alkaloids, flavonoids, steroids, and other bioactive compounds used in pharmaceuticals, cosmetics, and food industries. Furthermore, tissue culture techniques allow for year-round production of plants, independent of seasonal limitations, and support large-scale reforestation and ornamental plant propagation.

Overall, the applications of plant tissue culture extend from basic research in plant development to commercial-scale production, conservation, crop improvement, and pharmaceutical industries, making it one of the most impactful techniques in modern plant science.

XIII. CONCLUSION

Plant tissue culture is a powerful and versatile technique that has revolutionized modern plant science. By utilizing the principle of totipotency, it enables the regeneration of entire plants from small explants under sterile and controlled conditions. This method provides numerous benefits, including rapid large-scale propagation of disease-free plants, conservation of endangered species, genetic improvement through breeding and transformation, and commercial production of valuable secondary metabolites. Although challenges such as high cost, contamination risks, and technical expertise are involved, the advantages far outweigh the limitations. Today, plant tissue culture plays a vital role in agriculture, horticulture, forestry, and pharmaceutical industries, contributing to sustainable crop production, biodiversity conservation, and global food security.

Applications of Plant tissue Culture

Plant tissue culture has become an indispensable tool in agriculture, horticulture, forestry, and biotechnology due to its wide range of applications. One of the most important uses is in micropropagation, where large numbers of genetically uniform and disease-free plants can be rapidly produced from small explants, ensuring high-quality planting material. It is also widely used in the production of pathogen-free plants, as meristem culture eliminates viruses and other systemic infections. In conservation biology, tissue culture plays a vital role in the germplasm conservation and rescue of endangered and rare plant species, as well as in the storage of genetic resources through cryopreservation.

REFERENCES

Textbooks

[1] Reinert, J. & Bajaj, Y.P.S. (1997). *Applied and Fundamental Aspects of Plant Cell, Tissue, and Organ Culture*. Springer-Verlag, Berlin.

[2] Gamborg, O.L. & Phillips, G.C. (1995). *Plant Cell, Tissue and Organ Culture: Fundamental Methods*. Springer.

[3] Narayanaswamy, S. (1994). *Plant Cell and Tissue Culture*. Tata McGraw Hill Publishing Co.

[4] Razdan, M.K. (2003). *Introduction to Plant Tissue Culture*. Oxford & IBH Publishing, New Delhi.

[5] George, E.F., Hall, M.A. & De Klerk, G.J. (2008). *Plant Propagation by Tissue Culture* (3rd ed.). Springer.

[6] Kalyan Kumar De (2017). *An Introduction to Plant Tissue Culture*. New Central Book Agency

Research Papers / Reviews

[1] Haberlandt, G. (1902). "Kulturversuche mit isolierten Pflanzenzellen" – The pioneering paper introducing the concept of totipotency. Sitzungsberichte der Akademie der Wissenschaften Wien, Mathematisch-Naturwissenschaftliche Klasse.

[2] Murashige, T. & Skoog, F. (1962). “A revised medium for rapid growth and bioassays with tobacco tissue cultures.” Physiologia Plantarum, 15: 473–497.

[3] White, P.R. (1934). “Potentially unlimited growth of excised tomato root tips in a liquid medium.” Plant Physiology, 9: 585–600.

[4] Gautheret, R.J. (1939). “Sur la possibilité de culture indéfinie de tissus de tubercule de carotte.” Comptes Rendus de l'Académie des Sciences, Paris, 208: 118–120.

[5] Thorpe, T.A. (2007). "History of plant tissue culture." Molecular Biotechnology, 37(2): 169–180.

[6] Pierik, R.L.M. (1987). In Vitro Culture of Higher Plants. Martinus Nijhoff Publishers.

Web Resources (Peer-reviewed / Educational)

[1] FAO Biotechnology in Food and Agriculture – Plant Tissue Culture section: http://www.fao.org/biotech

[2] NCBI Bookshelf – Biotechnology (Open-access chapters on tissue culture methods).

[3] SpringerLink and ScienceDirect journals for latest plant biotechnology applications.