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Implementations of Plant Tissue Culture Technique in Producing Hybrid Plants: (Article Review)

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Abstract

Plant tissue culture methods have greatly improved the process of producing hybrid plants. These techniques, which include callus culture, somatic embryogenesis, Anther and Pollen Culture to Production of Haploid Plants, somatic hybridization by protoplast fusion and Fertilization conducted in a test tube, hairy roots provide reliable means of creating a large number of consistent, superior hybrids. With the use of micropropagation, hybrid plants can be quickly multiplied from a small tissue sample, guaranteeing excellent yield and genetic consistency. The Somatic embryogenesis process aids in the formation of embryos from somatic cells, making the manufacturing of hybrid plantlets scalable and effective. Embryo rescue gets around obstacles to hybridization by promoting the development of embryos from crossings that might not otherwise mature. In order to produce hybrids with desired features, callus culture aids in the regeneration of plants from undifferentiated cell masses. When combined, these methods improve hybrid plant production's efficiency, homogeneity, and viability while removing historical barriers and raising the bar for creative crop breeding and conservation. This abstract emphasizes the main techniques and advantages of using plant tissue culture to produce hybrid plants, highlighting the significance of this practice for contemporary horticulture and agriculture.

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1. Introduction

In the development of hybrid plants, plant tissue culture techniques have become essential due to their potential to provide several advantages such as enhanced efficiency, uniformity, and the capacity to reproduce plants with certain desirable characteristics. By providing mechanisms for rapid multiplication and exact control over the propagation process, these strategies facilitate the mass creation of hybrids [1]. Micropropagation is a method employed to generate a substantial quantity of genetically indistinguishable plants from a limited tissue sample. In hybrid plant production, this is especially advantageous since it guarantees consistency and the creation of plants of superior quality. Somatic embryogenesis is the process of generating embryos from somatic cells, which are non-reproductive cells. The quick generation of embryos and subsequent plant lets allows for the bypassing of the necessity for conventional seed development, therefore conferring advantages to the production of hybrid plants. During hybridization, especially between distant relatives or species, embryos may not undergo appropriate development. Embryo rescue includes the transfer of these embryos to an appropriate culture medium in order to facilitate their development into viable plants, therefore surmounting obstacles to hybridization. Callus culture is the cultivation of undifferentiated plant cells (callus) derived from ex plants on a

nutrient-rich media. It is possible to stimulate this callus to undergo differentiation into shoots and roots, therefore promoting the regeneration of hybrid plants [1,2]. Tissue culture modalities enable the quick and effective generation of substantial quantities of hybrid plants. This is particularly advantageous in commercial agriculture where consistency and magnitude are crucial. Hybrid plants generated via tissue culture exhibit genetic uniformity, so guaranteeing a consistent level of quality and performance in the plants [3]. In order to preserve and propagate rare or endangered hybrid plants that are challenging to replicate using traditional methods, tissue culture techniques can be employed. Advancements in embryo rescue techniques can effectively address obstacles to hybridization, such as incompatibility between parent plants, therefore enabling the development of hybrids that may otherwise be unattainable [4].

2. Basic Concepts

2.1. Crop Improvement and Plant Tissue Culture

1. **Crop Improvement:** Crop improvement is the act of strengthening the genetic traits of crops in order to augment its production, quality, resistance to illnesses, and adaptation to environmental conditions [5]. This procedure may be accomplished by several techniques; including conventional Reproduction this approach entails the selection of plants possessing desirable traits and their cross-breeding to generate offspring with enhanced characteristics. Genetic Modification, including technologies like gene editing (CRISPR), enables precise alterations of crop genomes to introduce or enhance specific traits. Biotechnology, on the other hand, employs tools such as marker-assisted selection and transgenic technologies to enhance crops [6, 7].
2. **Plant Tissue Culture:** Plant tissue culture refers to a set of laboratory methods employed to cultivate plant cells, tissues, or organs in a sterile environment using a nutritional culture media. This technique is crucial for the multiplication, genetic alteration, and conservation of plants [8].Micro propagation is the culture of a substantial number of plants from tiny tissue samples. This technology is employed for large-scale cultivation of crops and the multiplication of uncommon plants. Somatic Pre implantation development Developmental embryo genesis is the process of generating embryos from somatic cells, which can subsequently mature into fully formed plants and calluses. Culture refers to the process of cultivating callus, a collection of undifferentiated cells, from plant tissues, with the intention of stimulating the development of shoots and roots [9, 10].

2.2. Production of Haploid Plants

In contrast to diploid plants, which typically have two sets of chromosomes, haploid plants possess only one set. The generation of haploid plants is a noteworthy accomplishment in the field of plant breeding and biotechnology, mostly because of its practical advantages in genetic research, crop enhancement, and the establishment of homozygous lines. Haploid plants are highly advantageous in breeding operations due to their ability to facilitate the early formation of homozygous lines by successive chromosomal doubling [11,12].

2.2.1. Techniques for Producing Haploid Plants

Anther culture is the process of cultivating anthers, which are the pollen-producing structures of the flower, in order to stimulate the development of haploid plants. It finds widespread application in crops such as rice and barley [13]. Anther culture is a method employed in plant tissue culture to stimulate the reproductive growth of haploid plants from the microspores present in anthers. The aforementioned technique is very advantageous in the field of plant breeding and genetic research due to its ability to efficiently generate homozygous lines (Figure1).

- Procedure: The anthers are removed from flowers and cultivated on an appropriate medium that facilitates the growth of micro spores into callus and ultimately into plant lets.
- Application: It is extensively employed in crop breeding for rice, wheat, and barley to generate haploid plants that can be subsequently doubled to form homozygous diploids. [14]

Advantages of anther culture include a substantial reduction in breeding program time and an improvement in genetic variety [15]. The microspore culture technique, like anther culture, entails the cultivation of isolated microspores to form haploid plants. It is especially efficient in species such as *Brassica* [16]. The process of somatic embryogenesis involves the generation of haploid plants from somatic cells, typically achieved using tissue culture techniques [17].

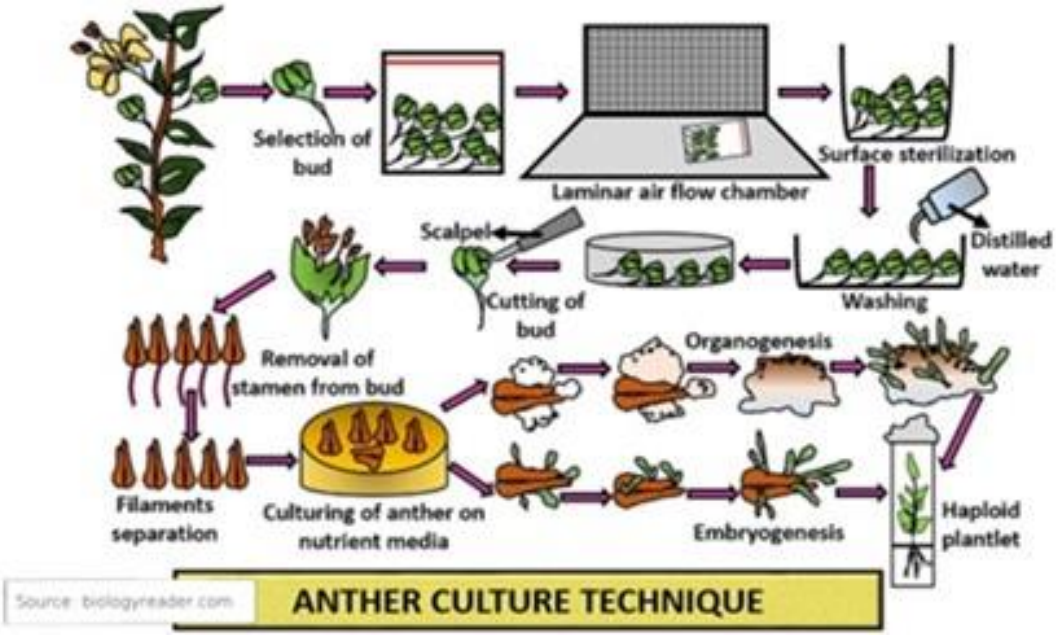

Figure 1. Anther culture technique [12]

2.2.2. Ovule culture is the process of cultivating ovules (female gametes) in order to generate haploid plants. Although less prevalent, this technique is applies to numerous plant species [18].

- Induced Parthenogenesis is the process of stimulating the formation of haploid embryos from unfertilized ovules [19].
- Genetic Engineering: The field of genetic engineering is now investigating technological advancements, like as CRISPR/Cas9, to enhance the efficiency of producing haploid plants [20].
- Applications in Breeding: Haploid plants are highly relevant in plant breeding initiatives due to their ability to expedite the formation of homozygous lines. This is especially advantageous in arable crops where the optimization of time and resources is of utmost importance [21]. Notwithstanding the progress made, there are still obstacles in the extensive implementation of haploid induction methods. These factors encompass the requirement for streamlined procedures tailored to various species and the possibility of unintended consequences in genetically modified plants. Further investigation is directed towards surmounting these obstacles to improve the feasibility of haploid technology in agriculture [22].
- Utilization and Advantages
  - Genetic Research: The straightforward genetic background of haploid plants makes them highly useful for investigating gene function and plant genetics. The utilization of haploid plants in crop improvement facilitates the rapid development of homozygous lines, therefore expediting the breeding process.
  - Haploid plants are frequently employed for screening for disease resistance and other advantageous characteristics [23].

2.3. Anther and Pollen Culture

Anther and pollen cultures are specific techniques used in plant tissue culture to produce haploid plants, which are of great benefit for breeding and genetic research. These methodologies involve the controlled cultivation of plant anthers or pollen grains to promote the development of embryos or plant lets [12].

2.3.1. Pollen Culture

- Pollen culture is the intentional cultivation of pollen grains in a medium that is abundant in chemicals, with the aim of promoting the development of haploid plants. This technique is similar to anther culture, but it explicitly employs direct methods to target the pollen grains [24, 25]. Pollen Extraction and sterilization: Pollen grains are collected from mature blooms and then undergo sterilization.

- Culture media: Pollen is placed onto a specialist medium specifically formulated to facilitate the process of germination and development into micro spores or embryos.
- Induction and Development: The pollen grains undergo regulated developmental processes to initiate the formation of micro spores, which subsequently grow into plant lets or callus.
- Regeneration: The resulting structures are then induced to undergo growth into mature plants [26]. Haploid induction is a method used to generate haploids in species where traditional methods may have restricted effectiveness.
- Genetic Studies: It provides a controlled environment for studying genetic traits and mutations.
- Hybridization: Pollen culture facilitates the fusion of different species or variants, developing new cultivars with enhanced desirable traits.

Cultivation varies and ovules is a crucial method in plant biotechnology, namely for the generation of haploid plants and for comprehending reproductive biology [27] and [28]. According to Kumar and Gupta [29], ovary and ovule culture are significant methodologies in plant breeding that enable the generation of haploid plants and the examination of reproductive development.

- Protocols for Ovary and Ovule Culturing Procedure: This operation entails the removal of ovaries from flowers and their placement onto an appropriate culture medium. The ovaries can undergo embryonic development, subsequently leading to the regeneration of entire plants.
- Applicability: Employed for the generation of haploid plants by hybrid crossings, especially in species where fertilization is challenging or unattainable [30].

3. Ovule Culture

- **Experimental Protocol**: Ovules are extracted from the ovary and cultivated on a nutrient-rich medium. This process can result in the formation of embryos specifically from the ovules. Application: Beneficial for generating haploid offspring and for saving embryos from interspecific or intergeneric crossings that would otherwise result in termination.
- **Rescue of embryos:** Methodology: Entails cultivating undeveloped embryos from ovules that are not capable of surviving in their original location. The embryos are positioned on a growing medium containing nutrients to facilitate their development. Application: Facilitates the retrieval of hybrid embryos that may not survive in their original plant species.

2.4. Fertilization conducted in a test tube

Plant in vitro fertilization (IVF) is a method employed to expedite the fertilization process in a controlled setting, apart from the natural environment. Implementing this technique is crucial for the production of hybrids, preservation of plant species, and investigation of plant reproductive biology. Pollination is the process of fertilizing ovules or egg cells with pollen in a specifically regulated laboratory environment. The process of plant in vitro fertilization Methodology: Ovules and pollen are obtained from plants and subjected to serialization in order to remove any potential contamination. Culture Medium: Ovules and pollen are deposited on a nutrient-dense medium that facilitates fertilization and the growth of embryos. Fertilization is the regulated introduction of pollen to ovules in order to achieve biological reproduction. The process of embryo development involves the cultivation of embryos after fertilization until they mature into plant lets [31]. Regeneration: The plant lets are subsequently propagated to a growth medium in order to undergo development into fully grown plants.

- **Application**: Hybrid Production: In vitro fertilization (IVF) is generates hybrids that may not successfully develop in natural environments because of obstacles between distinct species or types [32].

Genetic research facilitates the examination of plant reproduction and the creation of novel cultivars with certain desirable characteristics.

- **Conservation:** Facilitates the reproduction of endangered plant species via precisely regulated fertilization.
- **Analysis:** Low success rates: Fertilization and embryo development in vitro may exhibit lower efficiency when compared to natural systems [33]. Protocol optimization necessitates meticulous regulation of culture conditions and medium composition in order to attain ideal results.

2.5. Culture of endosper

1. Definition and Significance: endosperm is a tissue that is synthesized during the fertilization process in angiosperms, serving as a source of nourishment for the growing embryo. It plays a vital role in seed development and has significant economic value in crops such as rice, wheat, and maize [34].

2. Methodologies: *In vitro* culture of endosperm allows for scientific investigation of its growth and the production of haploid plants. The procedure

entails extracting the endosperm tissue and immersing it in a nutrient-rich solution conducive to development. The process of somatic embryo-genesis enables the regeneration of plants from endosperm tissue, so offering potential use in breeding initiatives.[35]

- Utilization: In plant breeding, endosperm culture is a valuable technique for generating hybrid plants and enhancing agricultural productivity [36].
- Genomics studies involve the use of endosperm culture to investigate gene expression and the genetic foundation of characteristics associated with seed growth and nutrition. Analysis of endosperm development in food production can result in enhancements in the nutritional value of essential crops [37].
- Soma clonal variation :Genomics diversity in plants regenerated from tissue culture refers to the genetic variation that can arise. This phenomenon holds significance in the field of plant breeding and biotechnology, since it has the potential to facilitate the emergence of novel characteristics and enhanced cultivars. The phenomenon of soma clonal variation occurs when plant cells, tissues, or organs are cultured in vitro. This phenomenon can arise due to a multitude of reasons, encompassing genetic alterations, epigenetic modifications, and environmental impacts that occur during the culture process. This diversity may appear as variations in the physical structure, physiological processes, or chemical composition of regenerated plants. Soma clonal diversity is frequently utilized by researchers to augment characteristics such as disease resistance, stress tolerance, and yield [38].

2.5.1. Mechanisms through which somaclonal variation occurs there are various processes by which somaclonal variation can occur, including:

- Alterations in Chromosome Number: This can encompass aneuploidy or polyploidy resulting from mistakes during cellular division.
- Mutations: Tissue culture can give rise to several forms of mutations, such as point mutations, insertions, deletions, and transposable element activity.
- Epigenetic alterations refer to modifications, such as changes in DNA methylation, that can modify gene expression without changing the DNA sequence itself.
- Environmental variables, including the media type, light exposure, temperature, and growth regulators, can impact the extent of variance in tissue culture [39].
- Applications in the field of plant breeding.
- The use of somaclonal diversity in plant breeding enables the development of novel varieties with enhanced characteristics. For instance, it has been implemented with great success in agricultural produce such as rice, wheat, and potato [40].

2.6 Synthetic Seeds

- Definition and Structure: Synthetic seeds refer to artificially produced seeds that are utilized for the purpose of plant propagation. Plant tissues or cells, including somatic embryos, give rise to these structures, which are then enclosed in a protective covering to aid in their manipulation and seeding [41].
- Benefits: Synthetic seeds have various advantages, including as consistency in plant characteristics, enhanced storage and transportation capacities, and possible financial savings in plant cultivation [42].
- Utilization; Artificial seeds find application in the fields of agriculture, horticulture, and conservation. They facilitate the large-scale reproduction of crops, decorative plants, and native species at risk of extinction [43].
- Encapsulation of Biopolymers: Biopolymer encapsulation of synthetic seeds serves to safeguard the plant material and improve its survival during storage and transportation [44].
- Applications in Forestry and Horticulture Synthetic seeds are highly beneficial in the fields of forestry and horticulture as they enhance the productivity of plant growth and contribute to the preservation of indigenous species [45].

2.7. Improvement of medicinal plants' genetic systems.

In human civilization, secondary metabolites are utilized to make a variety of goods, such as medications, colors, and flavorings. Plant tissue culture is a simple and affordable method that can enhance the secondary metabolites of plants to satisfy the huge demand of these sectors [46]. Mutation breeding is a crucial technique that has produced plant species with traits including chromosome doubling, polyploidy induction, and increased callus size and friability depending on the culture medium that contains nutrients and plant growth hormones. This technology is vital for the commercial production of secondary metabolites, which boosts many areas of biology and biochemistry research. Likewise this kind of callus can be employed for secondary metabolite subsequent ones, cell suspension culture creation, and propagation

[47]. High levels of harmine and harmaline have been observed in callus *P. harmalain*, and other secondary metabolites have been successfully produced from callus culture as alkaloid production in induction experiments using sodium chloride [48]. Additionally, chitosan and titanium dioxide nanoparticles from *Fusarium oxporum* are utilized in different amounts as catalysts in flavonoid production to promote the generation of active chemicals from Salvadora persica callus. High-performance liquid chromatography (HPLC) analysis showed notable variations in the elevated synthesis of flavonoids (catechin, luteolin, apigenin, kaempferol, quercetin, and rutin), and the results were superior when flavonoids were present in the samples than when alkaloids were present [49]. Abed and Jassim [50], Moreover, it was demonstrated that callus cultures had greater concentrations of the tropane alkaloids (hyoscyamine, scopolamine, and atropine) than the mother plant's leaves. Biostimulation with chitosan, yeast extract, and fungal extract of *Trichoderma asperlum* resulted in an apparent increase in tropane alkaloids, especially CHT stimulation, which produced the highest levels of these three stimuli. In a study on Lavandula angustifolia callus culture, it was discovered that callus tissues with competing volatile oil in the leaves had a bigger volatile oil content [51]. Saleh and Al-Dabbagh [52] demonstrated that callus activated with varying doses of SA as a stimulant produced more kaempferol and gallic acid in Shea plants. Plant tissue culture (PTC) is an essential breeding method that enables the manipulation of medicinal plant genomes and the production of pharmacological secondary metabolites through artificial polyploidy and Agrobacterium-mediated gene transformation. Plant varieties have been developed as a result of the crucial tactic of mutant breeding. Chromosome doubling, or polyploidy induction, can result in larger, more valuable plant portions that have both commercial and therapeutic benefits [53]. The concept of "mutation" describes a heritable alteration in an organism's genetic composition that produces people with differences in certain features that can be passed down to the following generation. Similarly, Bhaskaran etal. [54] noted the beneficial benefits of "dead leaves," nerium leaves, and mutants generated by ethyl methanesulfonate (EMS). While their leaf and root yields were much lower than those of the original cultivar, these mutants exhibited higher amounts of root and leaf alkaloids as well as the anticancer leaf alkaloids vinblastine and vincristine. In order to select for improved shape and performance across several generations, fenugreek seeds were also exposed to a variety of mutagens, including ethylene imine, gamma rays, and ethyl methane sulfonate. [55].

2.8. Plant Protoplast :Isolation and fusion, purification and culture

Isolation, Fusion, Purification, and Culture of Plant Protoplasts Plant protoplasts, or "naked" cells, are a distinctive single-cell system that underpins numerous domains of contemporary biotechnology. Somatic hybridization through protoplast fusion facilitates the amalgamation of nuclear and cytoplasmic genomes, either entirely or partially, at interspecific and intergeneric levels, hence surmounting inherent sexual incompatibility barriers in plant genetic manipulation [56].

2.8.1. Sources of protoplasts

Protoplasts are isolated from tissues and organs. The components encompass leaves, petioles, petals, roots, tips, microspores, and pollen grains. They produce a large quantity of homogeneous cells; therefore, it is advisable to separate them from the leaves. Callus cells, albeit sterile, are predominantly influenced by the age of the culture. Factors affecting protoplast production encompass: a) Pre-enzyme treatment: The leaf tissues are superficially sterilized to prevent contamination. Prior to plasmolysis, the donor tissues are lysed with plasmol, causing the protoplasts to shrink and retract from the cell wall for 10 minutes, provided that the osmotic pressure of the plasmolysis solution is equivalent to that of the enzyme solution [57]. The lower epidermis of the donor tissues must be either peeled or diced and immersed in the enzyme solution, ensuring that the exposed surface contacts the solution. B- Enzymatic treatment: Concentrating the enzyme is essential during protoplast isolation. The three enzymes employed to decompose the cell wall are cellulase, pectinase, and hemicellulase [58].

2.8.2. Purification of protoplasts, their viability and density

To get intact protoplasts for fusion, isolated protoplasts must be purified from damaged cells, vascular components, and remnant cell walls. Filtering, centrifuging, and washing isolate protoplasts. Check isolated protoplast viability. 1. FDA fluorescein diacetate technique. Non-polar fluorescein diacetate reaches living cells through plasma membranes. Blue Evans dye. Healthy cells do not absorb Evans blue (0.025%), while damaged cells do. The density of protoplasts per unit volume of media determines protoplast culture efficacy. Minimum plating density (mpd) is required [57].

2.8.3. Protoplast fusion

It was the work of Withers and Quiking [59] that established the protoplast fusion method. This can happen in various ways: A. Enzymatic digestion of the wall followed by the spontaneous fusing of individual protoplasts. B-Fusion agents promote induced fusion. Typical approaches include:

1. NaNO3 Is Used.
2. A high-calcium, high-pH treatment.
3. The polyethylene glycol (PEG) technique is a popular choice; nevertheless, fusion involving PEG is typically linked to acidic conditions and high calcium concentrations. [60].
4. Electrofusion: Because isolated protoplasts have a negative charge on their surfaces, a pearl chain arrangement can be achieved by applying alternating electric current that generates a dipole inside the protoplast. Because it is manageable and doesn't compromise protoplast viability, it's a popular choice number nine. Fusion of the cytoplasm of distinct protoplasts always occurs when two or more protoplasts are separated together (Figure 2).

The nuclei of the protoplasts that have joined together could stay separate or mix. These are cells that have nuclei that are not the same as each other. When its nuclei join together, a heterokaryote with two nuclei can change into a synkaryote or a true mixed protoplast. When two protoplasts from the same culture join together, you get a homokaryote. A lot of the time, genetic information from one nucleus gets lost. A cytoplasmic hybrid is what you get when two protoplasts fuse together if one nucleus is missing totally, but the cytoplasm of both can still be hybrid [59].

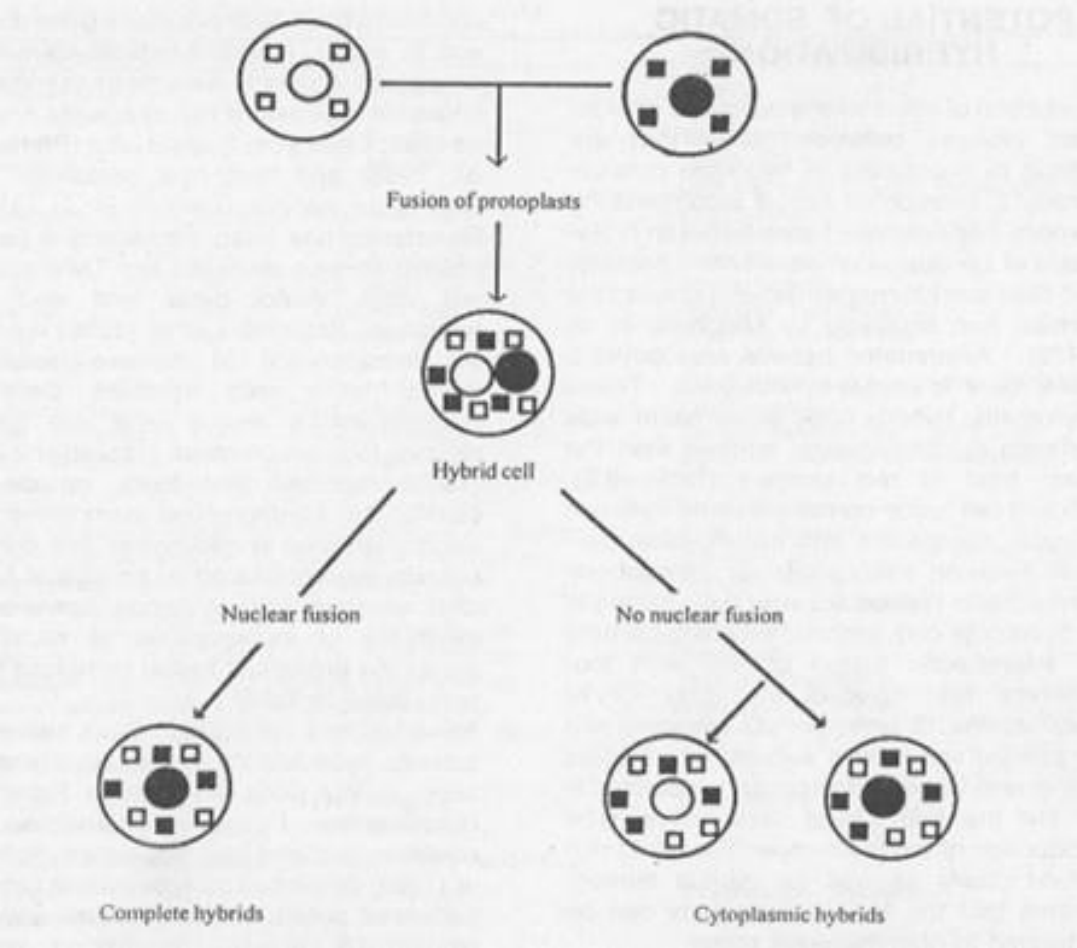

Figure 2. A cytoplasmic hybrid [59]

2.8.4. Plant regeneration

The round shape of protoplasts is lost as a result of the accumulation of cellulose as microfibrils on the plasma membrane or between the plasma membrane and the loosely arranged multi-lamellar cell wall. This behavior indicates the formation of a cell wall. A typical cell wall will be formed once the microfibrils are in order. In typical tissue culture conditions, the cell wall of normal cells undergoes division to generate progeny cells, multinucleated cells proliferate, and the cell wall expands. After some time, these cells change into stem cells, and then they turn into young plants. Improving plants by somatic hybridization. Among the many plants researched for their protoplast fusion capabilities, cybernetic cells derived from the black eggplant genome and the eggplant tubular plastome allowed researchers to examine nuclear/chloroplast interactions in eggplants. The resistance of diploid aubergine fusion products to the Colorado beetle was investigated [58]. Other crop types have also reaped the benefits of somatic hybridization. Four types of rice: *Oryza sativa, Hordeum vulgare*, *Helianthus annuus*, and *Heliotropium maximiliani*. The successful protoplast fusion of the domestic potato (*Solanum tuberosum*) and the wild potato (*Solanum brevidens*) is the finest example of a related but sexually incompatible species. The goal of crossing cultivated potatoes with their wild relatives was to make domestic potatoes more resistant to viruses like potato leaf roll and potato virus [60].

2.9. Harry roots

Hairy root cultures are frequently used in hybrid plant development as a potent tool to investigate hybrid vigor (heterosis) and create hybrids with superior features, such as higher yields and better disease resistance. Rapid growth, genetic stability, and the capacity to produce secondary metabolites are characteristics of hairy roots, which are produced when *Agrobacterium rhizogenes* infects plant tissues. This approach opens the door for the creation of hybrids that are more resilient to illness and environmental stressors by enabling researchers to study genetic interactions and physiological traits linked to hybrid vigor in a controlled setting. According to research, hybrid plants created from hairy root cultures, for instance, may grow more quickly and be more resilient to stress than their ancestors additionally, using hairy roots helps speed up breeding while preserving genetic variety by making it easier to produce transgenic plants that express desired features from many parent species [61].

3. Conclusions

The application of plant tissue culture methods in hybrid plant cultivation has greatly revolutionized contemporary agriculture and horticulture. The implementation of these techniques has facilitated the effective generation of hybrid plants, which are crucial for augmenting agricultural productivity, upgrading quality, and advancing the development of novel plant cultivars. The following are significant findings derived from the implementation of these methodologies:

a. Improved Efficiency and Scale: The development of plant tissue culture methods such as micropropagation, somatic embryogenesis, and callus culture has significantly enhanced the efficiency and scale of hybrid plant production. These techniques facilitate the fast and extensive replication of hybrid plants from small tissue samples, satisfying the commercial requirements for consistent and superior yields.
b. Uniformity and Quality: Tissue culture methods provide genetic homogeneity among hybrid plants, which is essential for preserving constant crop quality and performance. Uniform production of plant material facilitates the attainment of predictable results in agricultural and horticultural contexts.
c. Overcoming Hybridization Barriers: Embryo rescue and somatic embryogenesis techniques are employed to overcome obstacles to hybridization, including differences in compatibility between parent plants. This facilitates the effective generation of hybrids that may not arise spontaneously or be practical to produce using conventional breeding techniques.
d. Conservation and Research: Plant tissue culture has uses that extend beyond commercial production, such as the preservation of uncommon or threatened plant species and the investigation of plant reproductive patterns. These methodologies enable the conservation of genetic diversity and the creation of novel plant cultivars with favorable characteristics.
e. Technical Advancements: The effectiveness and application of plant tissue culture techniques are continuously being improved by advancements in technology. Advancements in culture media, genetic engineering, and optimization of protocols are enhancing success rates and broadening the scope of plant species that can be cultivated with high efficiency.

Challenges and Future Directions: Despite the advantages, obstacles such as exorbitant expenses, the requirement for specially equipped facilities, and

the optimization of protocols persist. Further investigation and scientific advancement are necessary to tackle these obstacles, enhance methodologies, and increase their availability and cost-efficiency for wider implementation.

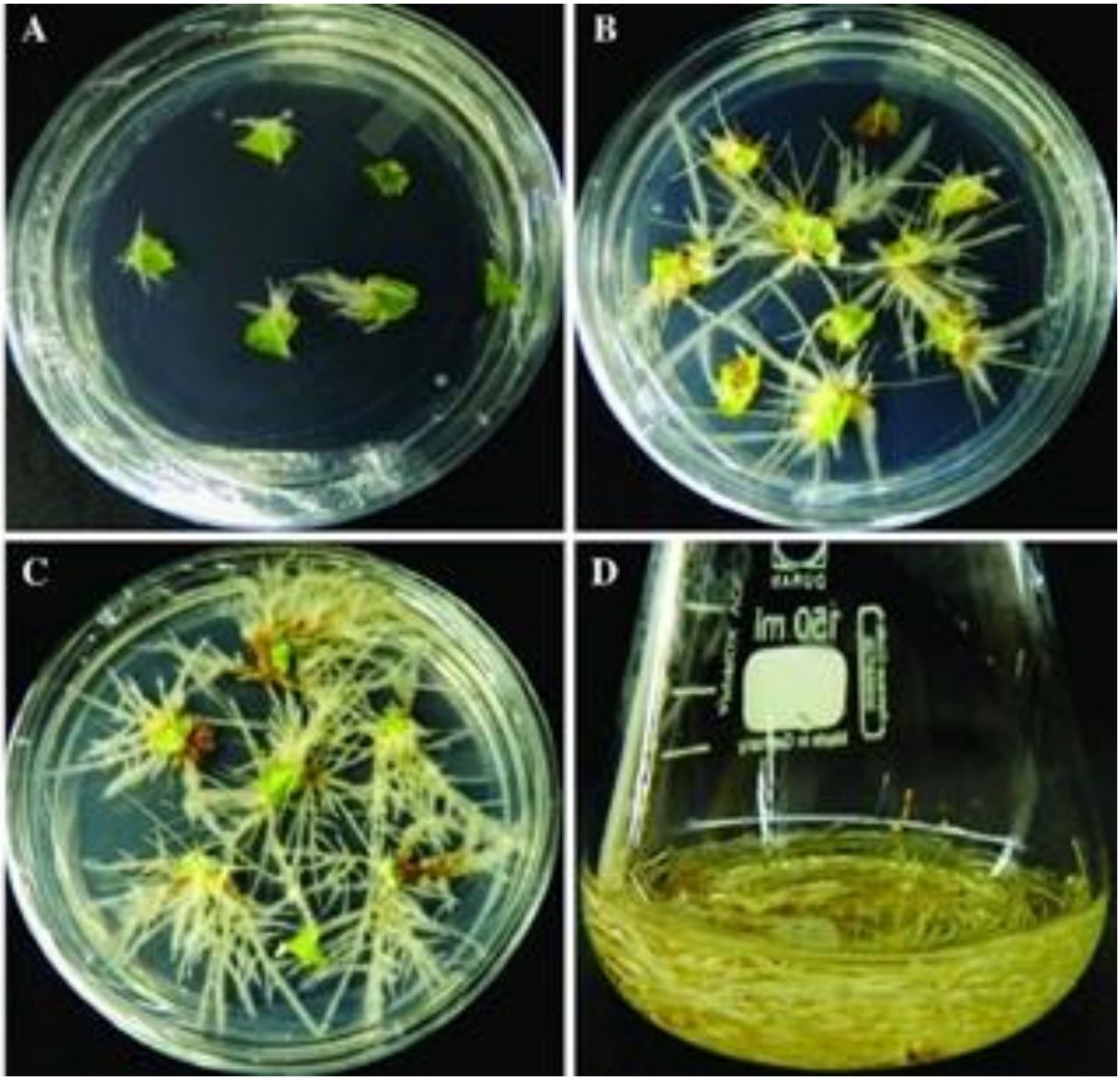

Figure 3. Hairy roots grown in hormone-free MS basal liquid medium showed a proliferation of bulk after four weeks of growth [62].

Conflicts of Interest: The authors declare no conflict of interest related to this work.

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