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Regeneration mechanisms in plant tissue culture: A review

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Abstract
Plant regeneration occurs when plants repair or replace damaged structures based on the totipotency and pluripotency of their cells. One of the most popular regeneration technologies is tissue culture. Recent years have seen a number of innovations in the field of plant regeneration. Plant micropropagation and genetic modification are made possible by the environmental conditions controlling plant regeneration from explant sources, basal culture medium, plant growth regulators, light/dark therapy, and plant growth stimulating microorganisms. Plant growth-promoting microorganisms (PGPMs) are important for the fixation of atmospheric nitrogen, water intake, solubilization, and transport of minerals from the soil to the plant in a variety of ecosystems. To increase plant growth and productivity and hence support sustainable agriculture and food security, many PGPMs are recommended as biofertilizers, biostimulants, and biocontrol agents.

Keywords: Regeneration, mechanisms, culture, pluripotency, totipotency

Introduction
Plant tissue culture is the process of growing an entire plant from an explant or even just one plant cell under aseptic conditions. This aspect of plant biotechnology depends on the phenomenon known as cell totipotency, which describes any single cell's capacity to produce all the differentiated cells that are characteristic of organs and to regenerate into a full plant (Trigiano and Gray, 2016) [26]. The rapid mass multiplication of elite genotypes on huge sizes in a relatively short amount of time is made possible by micro propagation, which takes use of this essential characteristic of plant cells. Micro propagation now plays a significant role in agriculture, horticulture, and industry thanks to the year-round generation of healthy seedlings and the shortening of the vegetative cycle (Suman, 2017) [23]. It is also a key method for genetic engineering, agricultural enhancement, and the propagation of new kinds resulting from somaclonal variation. But practically at every stage of the growth and development process, the technique calls for the application of chemical disinfectants, varying quantities of the right phytohormones, and occasionally antibiotics, antifungals, and antivirals (Liang *et al*., 2019) [16]. Tissue culture was first proposed a century ago, and it called for the *in vitro* regeneration of entire plants from somatic cells (Haberlandt, 1902) [13]. Since the historical discovery that various auxin and cytokinin (CK) concentration ratios are essential for the regeneration of adventitious roots and shoots, the tissue culture system has advanced (Skoog and Miller, 1957) [21]. Using isolated phloem cells from carrot roots, Steward *et al*. (1958) [22] successfully created fresh somatic embryos and later formed roots and shoots, demonstrating the totipotency of plant cells. Since then, basic research, micropropagation, and transgenic breeding have all made substantial use of tissue culture techniques based on regenerative capacity. Several variables, such as the usage of a plant growth regulator (Gerdakaneh *et al*., 2020) [11], the makeup of the basic medium (Chimdessa, 2020) [6], and the type of explant, have an impact on a plant's capacity for regeneration (Minutolo *et al*., 2020) [17]. To increase plant regeneration rates and the effectiveness of genetic transformation, it is helpful to understand the regulatory network and genetic control of plant regeneration ability in tissue culture.
Therefore, this review explores how the factors affect plant regeneration from the aspects of plant growth regulators, explant sources, basal culture medium, light/dark treatment and plant growth promoting microbes.

Plant tissue culture technology

With the use of the *in vitro* culture technique known as micro propagation, plant material from an explant can be multiplied in large numbers. There are six phases to the micropropagation process:

Stage 0: Plant stock immobilization and pre-treatments, selection of the explant.
Stage I: Culture establishment.
Stage II: Elongation and multiplication.
Stage III: Rooting.
Stage IV: Weaning, hardening, and acclimatization.
Stage V: Transfer under natural conditions (to the field).

The first four stages of the micro propagation process often occur in an extremely safe setting without the chance of contact with bacteria typically present in nature (Orlikowska *et al*., 2017) [18].

Pathways of plant regeneration in tissue culture

The first pathway, which is frequently applied in plant-cutting propagation methods, deals with how immature plant tissues, such as root or leaf tips, repair damaged areas. Plants in tissue culture regenerate mostly by somatic embryogenesis (Hill and Schaller, 2013) [14].

Somatic embryogenesis

In somatic embryogenesis, plant somatic cells dedifferentiate into embryonic stem cells before developing into full-fledged plants. This process demonstrates that plant cells are totipotent due to the embryogenic callus (Verdeil *et al*., 2007) [27]. As a result of somatic embryogenesis, a somatic cell transforms back into an embryonic stem cell. Through this process, dedifferentiation typically occurs in response to stress, hormonal stimulation (such as auxin), or gene expression alteration (Horstman *et al*., 2017) [15]. Individual somatic cells can be used to directly induce somatic embryos or embryonic callus can be used to indirectly produce somatic embryos (Yang and Zhang, 2010) [29]. The embryonic callus is the first developmental stage in the most frequent pathway of indirect somatic embryogenesis, particularly in crop plants (an unorganised cell mass).Following the production of an embryonic callus, proembryonic masses emerge on the callus mass's exterior or within it, from which individual cells or clusters of cells differentiate into somatic embryos (Toonen *et al*., 1994) [25]. Somatic embryos have the potential to grow into shoots and roots in the right circumstances. Embryonic callus differentiates into shoots when placed in a shoot-inducing media (SIM) that has a high concentration of CK and a low concentration of auxin. Incubating embryonic callus in root-inducing media with some auxin but no CK is necessary for root regeneration. Direct somatic embryogenesis is less well understood and lacks the callus phase than the formal pathway. In this approach, the explant displays a less prolific and more regular compact cell division.Under the right circumstances, each somatic cell in one or more cell layers divides and bulges to form a new embryo with recognisable morphology that can grow into a whole plant (Fitch and Manshardt, 1990) [9]. Without going through the callus stage, these somatic embryos might then be instantly germinated into plants. Although both direct and indirect somatic embryogenesis processes can take place in the same explant, there are differences in the times required to regenerate plants (Zhang *et al*., 2021) [32]. Due to the callus-induction process, the indirect approach takes longer to rejuvenate plants than the direct somatic embryogenesis pathway. Consequently, the process of indirect somatic embryogenesis is usually linked to somaclonal variation (Bahmankar *et al*., 2017) [2]. However, because of the abundant callus growth, the indirect somatic embryogenesis pathway generates more regenerated plantlets than the direct pathway (Gaj, 2011) [10]. As a result, the direct method is more effective than the indirect pathway if the goal is quick plant regeneration. The indirect approach, however, is preferable for species for which explants are hard to come by or in circumstances where numerous regenerated plants are sought.

Molecular mechanisms of somatic embryogenesis

The pluripotent callus continues to divide after being cultured on somatic induction media rich in CK, and cell groups gradually emerge for future differentiation, indicating the creation of the stem cell niche. Two regulatory pathways—WUS-clavata 3 (CLV3) and shoot meristemless (STM)-cup-shaped cotyledon-maintain the homeostasis of shoot stem cells (CUC). WUS expression starts 2 to 3 days after SIM culture and is the key factor in the early stages of stem cell niche construction (Zhang *et al*., 2021) [32]. The initial expression of WUS signifies the establishment of shoot progenitor cells and is the most important molecular event in de novo shoot organogenesis. While WUS overexpression causes ectopic shoot production, the WUS mutant entirely loses its ability to regenerate, showing that WUS is required for de novo shoot regeneration (Gordon *et al*., 2007) [12]. WUS expression is also impacted by the auxin and CK signalling pathways. Type-B ARRs (ARR1, ARR2, ARR10, and ARR12) directly boost WUS expression after binding to its promoter as transcriptional activators of CK signalling, and they also block YUC-mediated auxin accumulation to further activate WUS expression (Zhang *et al*., 2021) [32]. Type-B ARRs control type-A ARRs directly, which results in a negative-feedback loop. Type-A ARRs (ARR5, ARR6, ARR7, and ARR15) act as negative regulators of CK signalling (Sugimoto *et al*., 2019). Additionally, miR-156's targeting of the squamosa promoter binding protein-like (SPL) mRNA reduces the age-dependent regulation of type-B ARR activity. MiR156 levels are higher in juvenile explants compared to adult explants and suppress SPL expression, enhancing type-B ARR activity and the capacity for shoot regeneration (Shin *et al*., 2020) [19].

Plant growth regulators

Auxin, CK, and other exogenous hormones, in particular, are crucial for plant somatic embryogenesis and de novo organogenesis. The administration of exogenous hormones and the response to these hormones during tissue culture are necessary for plant regeneration *in vitro* (Bernula *et al*., 2020) [4]. Explants typically respond to PGRs in three stages: (1) cultured explant cells detect plant hormone signalling to cause subsequent dedifferentiation; (2) under the influence of plant hormone balance, specific cells in plant tissue receive differentiation instructions, laying the groundwork for later differentiation of specific organs; and (3) plant morphogenesis occurs without the aid of exogenous hormones (Ye *et al*., 2012) [30]. Even while exogenous auxin triggers somatic embryogenesis, auxin is not necessary for the process to continue. In tissue culture, auxin is the main factor influencing somatic embryogenesis in many species.

Exogenous auxin induces the development of endogenous precursors of ethylene synthesis, such as 1-aminocyclopropane-1-carboxylic acid, which in turn increases callus formation from cultured materials (Singla *et al*., 2007) [20]. Many species use the synthetic auxin 2,4-Dichlorophenoxyacetic acid (2,4-D), particularly cereal crops and medicinal plants. The ideal 2,4-D concentration differs for various species or tissues, which has an impact on callus formation. The general rule is that a low dosage encourages the production of embryonic calluses, whereas a high quantity inhibits it.It appears that the action of 2,4-D is boosted during embryogenic callus induction and suppressed during embryogenic callus development into a full plant because there is no need to add 2,4-D to the medium once the embryonic callus transforms into an embryoid and regenerates seedlings (Singla *et al*., 2007) [20]. Furthermore, a variety of auxin concentrations, including indole-3-acetic acid (IAA) and -naphthalene acetic acid, are crucial for encouraging the differentiation of adventitious roots in tissue culture (El-Sherif, 2018) [8]. The most popular PGR for inducing adventitious shoots and starting somatic embryogenesis in tissue culture is CK. Cell proliferation involving cell mitotic activation is necessary for de novo shoot regeneration. Competent cells in the process of shoot regeneration are impacted by CK, which results in cell-mass generation and fate alteration. CK alone can stimulate adventitious shoots, and it works in conjunction with auxin to boost cell proliferation in specific cell types (Cortleven *et al*., 2019) [7]. According to Skoog and Miller's (1957) [21] theory, roots form when the CK-to-auxin ratio is low, whereas a high ratio promotes the growth of shoots.

Plant growth promoting microbes

Numerous soil microorganisms from a variety of taxa have been found to be effective PGPMs. Rhizospheric PGPMs are soil-borne organisms that live on plant roots or invade plant tissues internally (referred to as PGP endophytes). They perform a variety of tasks including mineral solubilization (Zn, P, and K), iron chelation, nitrogen fixation, phytohormone production, and biocontrol of plant pathogens. They are divided into three major groups according to their activities, which correspond to three growth promotion strategies:

1. Biofertilizers, they increase the availability of nutrients and their utilization by plants.
2. Biostimulants or phytostimulants, produce beneficial substances such as PGRs, which are not nutrients, pesticides, or soil improvers.
3. Through the generation of antimicrobial metabolites or competition for resources and space, biocontrol agents prevent the growth of infections. Some PGPMs have two or three pathways that encourage plant growth. All facets of plant life, including seed germination, nutrition, growth, and reaction to biotic and/or abiotic challenges, are influenced by PGPMs through their multifunctional activities (Sunita *et al*., 2020) [24].

By direct or indirect mechanisms of action, PGPMs may improve plant development and protection. Direct mechanisms help plants grow by giving them nutrients or creating growth regulators, while indirect mechanisms support healthy plant growth in the face of abiotic stress or defend plants from diseases, parasites, or some predators (Arora *et al*., 2020) [1]. Plant growth-promoting fungi (PGPF) and plant growth-promoting bacteria are the two primary categories of PGPMs (PGPB). There are numerous relationships that plants develop with soil fungus. many taxa that are members of arbuscular mycorrhizal fungus, such as Rhizophagus, Gigaspora, and Funneliformis. Over 90% of all plant species form symbioses with arbuscular mycorrhizal fungus (AMF), which have an impact on hosts at different growth stages (Begum *et al*., 2019) [3]. Plants are infected by PGPF without showing any symptoms, and depending on the genotype, age, and physiology of the host, different lifestyles (mutualistic, latent pathogen, and latent saprophyte) are expressed. A limited percentage of fungus are latent pathogens, though. They fall under one of the four categories of bacteria: free-living bacteria, associative bacteria, endophytic bacteria, and bacteria that form nodules (symbiotic). Similar to PGPF, they can function as biocontrol, biostimulants, and/or biofertilizers. The genera of Pseudomonas, Bacillus, Azospirillum, Azotobacter, Rhizobium, Bradyrhizobium, Frankia, Burkholderia, Thiobacillus, Serratia, and Streptomyces are among the most commonly used groups of PGPB (Verma *et al*., 2019) [28].

Basal culture medium

Murashige and Skoog (MS), N6, Woody Plant Medium (WPM), and B5 are some of the culture media that are utilised for callus induction and shoot differentiation and have a big impact on plant regeneration in tissue culture. However, various species or tissues could also call for various basal media. In several medicinal plants, a half strength MS medium outperformed other media during the transformation of somatic embryos into plantlets.

Light/Dark treatment

Polyphenol oxidases will oxidise phenolic substances in explants under light circumstances, causing the tissue to turn brown. The oxidation products may darken tissues and impede the action of a number of proteins, which may have a negative impact on how somatic embryos develop (Bhatia and Bera, 2015) [5]. As a result, for many species of plants, dark conditions are necessary for callus initiation, maintenance, and development. According to a prior study, light causes auxins to degrade in plants, lowering endogenous CK and auxin levels (Zenser *et al*., 2001) [31]. In this manner, darkness may support callus development in explants by maintaining a high auxin-to-CK ratio. Darkness can also result in thinner cell walls and lower cell-wall deposits, which makes it easier for PGRs to enter cells.

Conclusion and future perspectives

The process of plant tissue culture is a crucial tool in horticulture, forestry, and contemporary agriculture. Plant regeneration from explant sources, baseline culture media, PGRs, PGMs, and light/dark therapy are significantly impacted by environmental conditions. This information will help to clarify the fundamental ideas behind plant regeneration from precursor cells and provide a strong basis for the use of plant micropropagation and genetic engineering. PGPMs can be thought of as prospective biofactories because they naturally possess the ability to create PGRs. Despite the breadth of study and the significant advancements produced as a result, more research is still needed to fully understand the mechanisms that control plant regeneration. Only a

portion of the intricate developmental process of plant regeneration *in vitro* is now understood, and more research is necessary to gain a complete and integrative understanding. First, although the initial regulatory network governing plant regeneration has been identified, it is still unknown how these participants and signalling molecules coordinate the many stages of regeneration. The interaction between external and internal signals to achieve the dynamic balance of growth and development needs more research, despite the fact that we are aware that complex networks of genes regulate plant regeneration and are affected by external environmental stimulation.

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