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Plant cell totipotency: Plant tissue culture applications-an updated review

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Abstract

Plant tissue culture is the fastest *in vitro* cloning method used in the production of secondary metabolites and phytoconstituents often difficult to regenerate and conserve the species saving them from extinction. Plant tissue culture can be used for a wide range of purposes with various applications in research and industry. The resulting clones are true to type of selected genotypes and used for the large scale plant multiplication. Plant *in vitro* propagation using tissue culture techniques have been exploited for the commercialization of ornamental plants (orchids), vegetable and fruit plants (papaya, mango, and grape), medicinal, woody plants, teak (*Tectona grandis* and sandalwood (*Santalum album*) and conifers with economically important products. There are many applications of plant tissue culture which is used in the production of somatic embryos, plant regeneration via organogenesis, germplasm conservation, synthetic seeds, protoplast culture for the production of somatic hybrids, anther culture for the production of haploids, synthesis of secondary metabolites of pharmaceutical interest, and plant cells used for the bioenergy. Since plant tissue culture is simple, low-cost and environment friendly, it is imperative to employ this technique for the development of sustainable agriculture in order to meet the food demand of increasing human population. However, the plant tissue culture technique also has limitations of somaclonal variation resulting in the chimeric plants with low yields. Reprogramming of plant somatic cells under *in vitro* conditions towards somatic embryogenesis is also a challenging process since many factors and signaling molecules govern and controlled the process.

Keywords: Cloning; *In Vitro*; India; Micropropagation; Somatic Plant Cells; Tissue Culture

1. Introduction

Plant tissue culture is an important biotechnological technique solving the problems of modern agricultural providing solutions to major food security issues [1-20]. Plant tissue culture techniques are the most frequently used biotechnology tools ranging from basic to applied investigation purposes in plant sciences [1-40]. Gottleib Haberlandt (1854–1945) proposed the concept of plant totipotency in 1902, based on the cell theory of Schleiden (1838) and Schwann (1839) [1-5, 110]. Gottlieb Haberlandt, working in Graz, Austria, was the first to culture isolated somatic cells of higher plants under *in vitro* conditions[1-6]. He began these investigations in 1898 and published the results in 1902 (Haberlandt, 1902) [4, 5]. At that time, Haberlandt hypothesized that entire plants could be generated by culturing isolated somatic cells [1-5, 110]. However, there was not any experimental evidence to support the hypothesis for more than half a century[1-5, 110]. Then in 1958, Steward showed that entire plants could be regenerated from segments of the differentiated secondary phloem of a carrot (Steward et al. 1958), thus demonstrating the remarkable totipotency of plant cells [1-5,110]. It was first in 1902; the first reports of tissue culture having success was that of Gottlieb Haberlandt who was able to develop and maintain mesophyll cells with totipotentiality in culture[1-5, 110]. Since then the tissue culture was developed constantly with reports suggesting its use in the application of breeding programs, biopharmaceutical production and genetic biodiversity conservation [1-5, 110]. German Botanist Golliob Haberlandt is

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regarded as the father of plant tissue culture[1-5, 110]. He later continued to work in the area and developed palisade tissue grew on knob's salt solution. Just in other years Hanning (1904) excised matured embryos and grew them *in-vitro* on a mineral salt sugar solution [1-5, 110]. This was a turning point when embryo culture was developed [1-3, 110]. It was then 1950s when tissue culture was used on a large scale by the orchid industry. After many years in 1972, Carlson et al. 1972 created the first somatic hybrid of *Nicotiana gluca* and *N. langschorffii* by fusion of their protoplast [1-5, 110].

Plant tissue culture has become the most appreciable method when it is used in the production of metabolites and phytoconstituents often difficult to regenerate and conserve the species saving them from extinction [1-5-30]. Under appropriately inductive conditions, differentiated somatic cells can be reprogrammed into totipotent cells and initiate SEs that roughly follow zygotic embryogenesis[1-3-30]. Tissue culture technique offers several advantages over plant propagation under natural conditions[1-30]. It is a rapid procedure as thousands of seedlings can be produced from small fragments (explants) of plants in a short period of time in contrast to conventionally propagated flora [1-20]. Plant *in vitro* propagation using tissue culture techniques have been exploited for the commercialization of ornamental plants (orchids), vegetable and fruit plants (papaya, mango, and grape), medicinal, woody plants, teak (*Tectona grandis* and sandalwood (*Santalum album*) and conifers with economically important products [1-30]. Large-scale plant tissue culture has been shown to be an appealing alternative approach to traditional plantation methods since it provides a regulated supply of defined nutrients (including carbohydrate) independent of plant availability [1-40]. The augmented use of plant culture is due to a superior perceptive of plant oriented compounds and secondary metabolites from economically important plants. Plant tissue culture has to lead to significant contributions in recent times and today they constitute an indispensable tool in the advancement of agricultural sciences and modern agriculture[1-3-50].

India is the number one world leader in the production of woody species like teak (*Tectona grandis*) (Motherbiotech Inc, Bengalore, Karnataka State, India), Sandal wood (*Santalum album*) at Vatican Shona Agrotech Pvt Ltd, MP, India (www.vsagreenwealth.com), and Sheel Biotech Limited, New Delhi, India, bamboo, eucalyptus, populus, pine and red sanders, ornamental species such as, orchids, gerbera, carnation, anthurium, lily, syngonium, cymbidium, limonium, dracena, philodendron, rose-miniature, caladium, gentiana and cactus, fruit crops such as banana, grapes, cashew, pineapple, strawberry, sapota, watermelon, mango papaya grapes, apple and citrus [121]. Cash crops like turmeric, sugarcane, ginger, vanilla, large cardamom, small cardamom, vanilla and clove. Medicinal plants aloe vera, geranium, stevia, patchouli, rosemary, gloriosa, tulsi, and *Costus speciosus*. Biofuel like Jatropha, and Pongamia has been produced under *in vitro* conditions[121]. In 2025, some ornamental plants that are exported from India under *in vitro* (tissue culture) conditions include orchids, anthuriums, and other plants like monstera, philodendron, aglaonema, alocasia, tillandsia, and those in the *Piperaceae* and *Araceae* families [1-3-25, 124]. These plants are exported as cut flowers, pot plants, or other forms, and *in vitro* propagation helps with their rapid and disease-free production. The first-ever export of anthurium flowers from Mizoram to Singapore follows the success of the International Conclave cum Buyer-Seller Meet (IBSM) organized by APEDA in collaboration with the Government of Mizoram, India on December 6, 2024, in Aizawl [121, 124]. The IBSM witnessed participation from nine international buyers from countries such as Singapore, UAE, Nepal, Jordan, Oman, Azerbaijan, Russia, and Ethiopia, along with 24 domestic exporters. The event established important trade connections and market opportunities for Mizoram's floriculture products. India's floriculture exports reached USD 86.62 million in FY 2023-2024 [124]. This first consignment of anthurium flowers from Mizoram, India to Singapore marks a significant step toward expanding floriculture exports, particularly from the North Eastern Region [121, 124]. NER holds immense potential for the export of horticultural and floricultural products. APEDA remains committed to supporting this potential through export promotional activities and collaborations with various stakeholders in the region[122, 124].

Major ornamental pot plants such as begonia, ficus, anthurium, chrysanthemum, rosa, saintpaulia, and spathiphyllum are being produced in the developed countries. About 212.5 million plants including 157 million ornamental plants amounting to 78% of the total production were reported. The Netherlands dominates the export of ornamental plants including pot plants like begonia, ficus, cyclamen, philodendron, saintpaulia, spathiphyllum and rhododendron. About 200 ornamental genera are propagated through tissue culture in different commercial laboratories worldwide. The Netherlands is worlds largest producer of *in vitro* derived ornamental plants including orchids. Second leading country in the world is China producing *in vitro* ornamentals, orchids, and woody species. The third leading countries are Japan, France, Spain, USA Taiwan, Singapore, Italy, South Korea, and Thailand. The leading exporters (The Netherlands, India, China, Japan, South Korea, Taiwan, France, Colombia, Italy Australia, and Israel) constitute about 80% of the world market. Planting material of ornamental plants is in great demand for commercial production as well as for domestic gardens and landscaping[1-3-20]. The better quality of planting material is a basic need for growers for boosting productivity[1-3]. The ornamental crops in India are grown for house gardens, landscape design, cut flowers, and potted flowering and ornamental plants, such as trees, shrubs, climbers, orchids, palms, ferns, grasses, bamboos, cacti, succulents, annuals, bulbs, and other flowering crops [121]. The ornamental crop cultivation for the international

markets has a bright future [1-3-20]. The export values are high and quite stable. Orchids will continue to dominate other ornamental crops due to better technology know-how in cultivation, suitable climatic conditions, experienced and skilful growers and exporters as well as their nationwide popularity [1-3-35, 121]. Orchid cultivation in India is a good example of biotechnology development for an ornamental crop, which does not fall in the category of staple food, to have become the major crop of this country (Figure-6) [1-3-15, 121].

The principle of totipotency, the ability of a single plant cell to regenerate into a whole plant, is the foundation of plant tissue culture [1-3-15, 121, 128]. Key to this process is the regulation of growth hormones, particularly auxins, which promote root formation, and cytokinins, which encourage shoot development[1-3-15, 121, 128]. The balance between these hormones determines the regenerative pathway, making them essential for tissue culture success [1-3-15, 121, 128]. A sterile environment is also crucial to prevent contamination, and the culture medium must be optimized with essential nutrients such as nitrogen, potassium, and key micronutrients like iron and zinc for proper plant cell growth and differentiation [1-3-15, 121, 128]. Recent advancements have improved the precision of hormone regulation and nutrient optimization, enhancing the efficiency of plant tissue culture method [1-3-15, 121, 128].

2. Plant Tissue Culture: Importance

Plant tissue culture is an *in vitro* aseptic culture of cells, tissues, organs or whole plant under controlled defined nutritional medium conditions often to produce the clones of plants (Figure- 1-9) [1-50, 132]. Plant tissue culture can be used for a wide range of purposes with various applications in research and industry [2-90]. The resulting clones are true to type of selected genotypes and used for the large scale plant multiplication [3-80]. Chemical and hormonal control of regeneration, basic and applied aspects of organogenesis, somatic embryogenesis, micropropagation and production of virus-free plants, haploid plants, production of secondary metabolites, and large-scale cell cultures in bioreactors are few landmarks and notable discoveries in plant tissue culture research [1-50]. It also helps in the development of pathogen-free micro-plants saved from various diseases and the new plants produced by tissue culture under aseptic conditions are also disease free plants [1-70]. *In vitro* micro-propagation is a valuable technology since many secondary plant metabolites cannot be manufactured chemically [1-90]. There are many applications of plant tissue culture which is used in the production of somatic embryos, plant regeneration *via* organogenesis, germplasm conservation, synthetic seeds, protoplast culture for the production of somatic hybrids, anther culture for the production of haploids, synthesis of secondary metabolites of pharmaceutical interest, and plant cells used for the bioenergy [1-100].

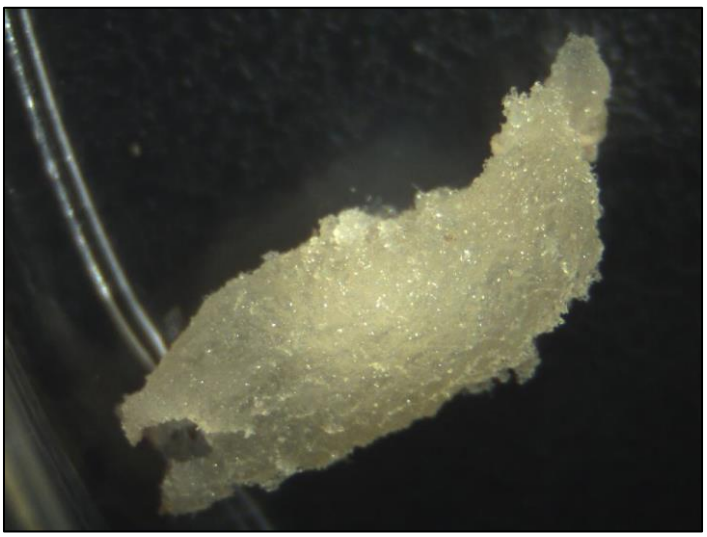

Figure 1 Proliferation of un-organized mass of callus in sugarcane with a mixture of embryogenic and non embryogenic cells

Various plant tissue culture systems have been extensively studied to improve and enhance the production and quality of plant metabolites produced by the medicinal plants[3-50]. *In vitro* plant micropropagation is a powerful technique in order to acquire plant extracts with various commercial applications than using whole plants [1-50]. Tissue culture can be used to propagate perennial woody species, orchids, endangered and threatened plant species irrespective of weather or season [6-90]. This also helps to accelerate the production process of new crop varieties with superior traits as tissue culture experiments required less time and space compared to *in-vivo* plant growth (Figure- 1-8) [1-90]. Callus

culture is a crucial technique in plant tissue culture, allowing for the formation of undifferentiated cell masses from plant explants through the manipulation of plant growth regulators, balance between auxins, such as 1-naphthaleneacetic acid (NAA), and cytokinins, such as 6-benzylaminopurine (6-BA), is essential for inducing callus formation and regenerating shoots, as demonstrated in white clover (*Trifolium repens*) [1-90, 128]. This hormone balance controls the developmental pathway of the callus, either leading to root or shoot formation depending on the relative concentrations of these regulators[1-90, 128, 132]. In addition to its role in regeneration, callus culture plays an essential part in genetic transformation, where callus serves as a platform for introducing foreign genes into plants through methods such as A*grobacterium*-mediated transformation[1-90, 128]. For example, in tobacco (*Nicotiana tabacum*), the overexpression of transcription factors like LEC2 has been shown to significantly enhance callus formation and shoot regeneration without the need for exogenous hormone [1-90, 128].

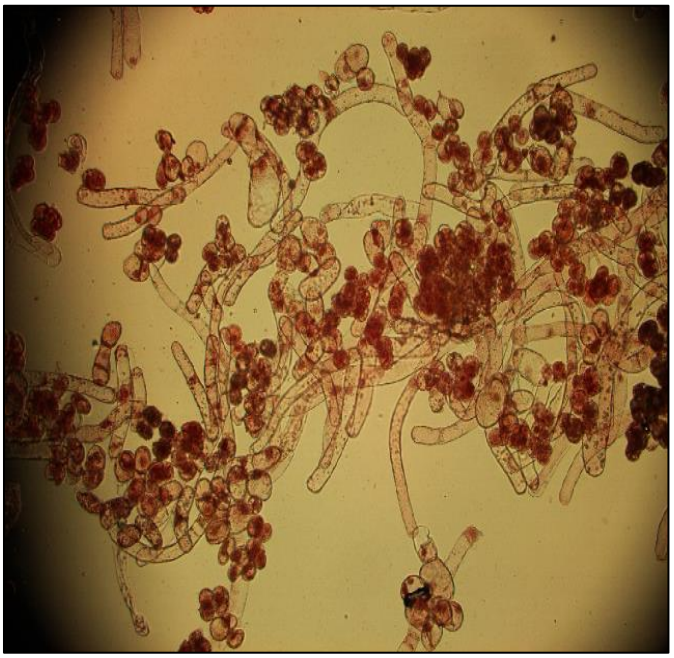

Figure 2 Microscopic observation of callus showing the mixture of embryogenic and non embryogenic cells in sugarcane

3. Plant Tissue Culture: Totipotency

Tissue culture technique depends mainly on the concept of totipotentiality of plant cells, which refers to the ability of single cell to express the full genome by cell division [1-50]. The totipotency of somatic plant cells is a specific and scientifically exciting phenomenon, which is based on the developmental program of plants [1-60]. It can be best demonstrated in an *in vitro* system where somatic plant cells can regain their totipotency and are capable of forming embryos through the developmental pathway of somatic embryogenesis [1-60]. This then triggers the reprogramming of plant cells into the pathway of embryogenic development (commitment) leading to somatic embryo formation [1-90]. These conditions include proper supply of nutrients, source of carbohydrate, pH of the medium, adequate temperature and proper gaseous and liquid environment [1-100]. The controlled conditions provide the culture of explants on a defined nutrient medium with the source of carbohydrate in an environment conducive for their growth and multiplication [1-60]. Under *in vitro* conditions, one or a few somatic cells of the plant or explants have to be competent to receive a signal (endogenous or exogenous) [1-60].

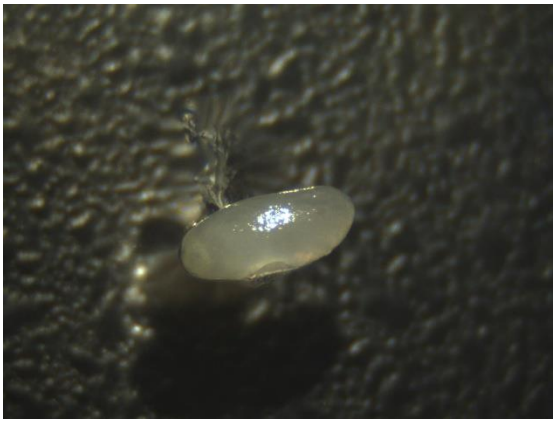

Figure 3 *In vitro* culture of immature zygotic embryo of *Pinus roxburghii*

Plant tissue culture is a technique in which fragments of tissues from a plant (explants) are developed *in vitro* in an artificial medium under aseptic conditions [1-50]. It involves culturing explants (such as shoot tip, root tip, callus, seed, embryo, pollen grain, ovule or even a single cell) isolated from mother plant on a sterile defined nutrient medium which leads to cell multiplication and plant regeneration [4-90]. Several methods are available for plant tissue culture[1-90]. In organogenesis, the commonly used method, organ formation can occur directly from meristems, or indirectly from dedifferentiated cells (callus) [6-90]. The resultant cultures can then be utilized to mass produce plants (micro propagation) or to develop specific organs (e.g., roots in hairy root culture) [1-80].

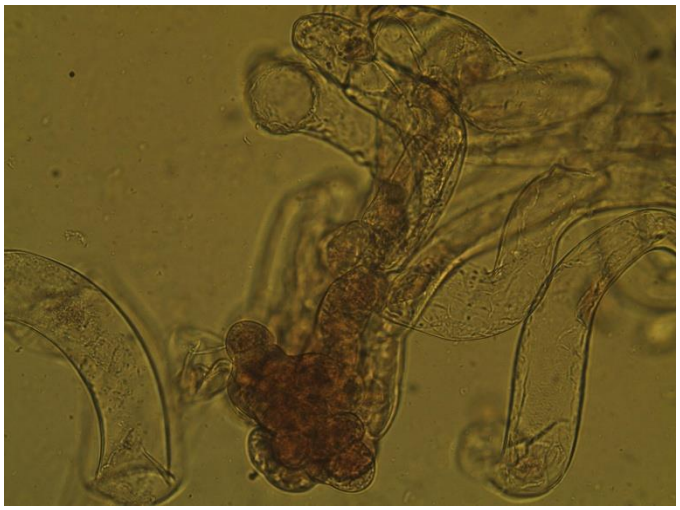

Figure 4 *In vitro* culture of immature zygotic embryo of *Pinus roxburghii* showing the sign of development of head and suspensor cells

Somatic embryogenesis (SE) is a critical process in plant tissue culture, enabling the regeneration of entire plants from somatic cells rather than through traditional sexual reproduction pathways [3-100-128]. This process involves inducing somatic cells to become totipotent, allowing them to differentiate into any cell type and ultimately form a complete plant. SE has significant applications in agriculture, particularly for the propagation of genetically identical plants at a large scale, which is especially valuable for crops that are difficult to reproduce through conventional methods, such as soybeans and bamboo[3-100-128].

The success of somatic embryogenesis (SE) is influenced by several factors, including the plant's genotype, the type of explant used, and the composition of the growth medium[3-100-128]. For instance, in soybean cultivars like 'Snowy', SE induction from immature cotyledons has been optimized, resulting in high regeneration rates and making them suitable candidates for genetic transformation [3-100-128]. Plant growth regulators, particularly auxins such as 2,4-dichlorophenoxyacetic acid (2,4-D), play a pivotal role in promoting callus formation and embryo initiation during SE. [3-100-128]. Additionally, transcription factors like WUSCHEL-RELATED HOMEOBOX (WOX2:) are crucial for maintaining cellular totipotency and regulating the developmental pathways of somatic embryo [3-100-128].

Plant tissue culture plays a significant role in basic research in the areas of plant pathology, plant physiology, plant metabolites and conservation [1-80]. Due to advancement in contemporary techniques, several protocols have been developed for the production of a wide variety of plants secondary metabolites on a commercial scale [1-80]. Apart from their use as a tool of research, plant tissue culture techniques have in recent years, become the major commercial importance in the area of plant propagation system, disease elimination, plant improvement, secondary metabolite production and genetic transformation [3-70].

Figure 5 Proliferation of un-organized mass of callus in *Carica papaya* with a mixture of embryogenic and non embryogenic cells

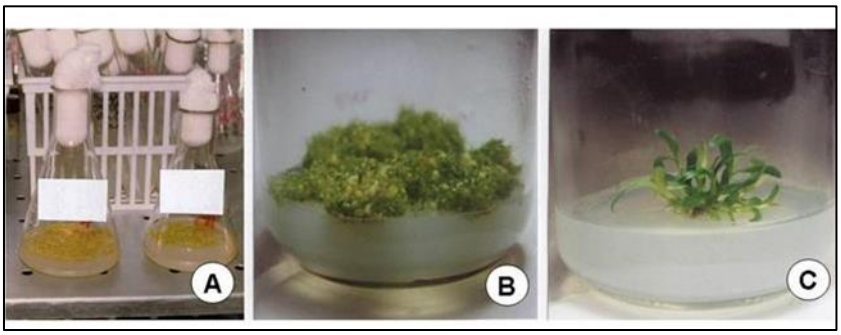

Figure 6 *In vitro* regeneration of orchid, *Phollidota pallida*

4. Plant tissue culture: Sustainable agricultural

Plant tissue culture coupled with molecular biological approaches leads towards sustainable agricultural development providing solutions to major food security issues [11-110]. Plant micropropagation with different explants like seeds, embryos, calli, anthers, protoplasts, and meristemetic tissues of root/shoot tips is used for large-scale production of industrial products [3-100]. Several endangered, rare, threatened, and commercially important plant species have been tissue cultured and successfully micropropagated for large scale production [3- 94]. In addition to this plant tissue culture is an efficient technology for the production of somaclonal and gameto clonal variants [3-94]. Plant tissue culture plays an important role in plant biotechnology due to its potential for massive production of improved crop varieties and high yield of important secondary metabolites [6-45]. Several efforts have been made to ameliorate the effectiveness and production of plant tissue culture using biotic and abiotic factors[4-90]. The micropropagation technology has a vast potential to produce plants of disease resistant and plants of superior quality, well adapted high yielding genotypes with better stress tolerance capacities [4-110]. Plant micropropagation, also known as plant tissue culture, is a technique that isolates, sterilizes, and incubates cells, tissues, or organs of chosen plants in a growth-promoting aseptic environment to create a large number of plantlets [3-94]. The isolated cloning technique revealed that, given the right conditions, somatic cells may develop into a complete plant [3-94]. The sterile or endangered flora can also be conserved by plant micropropagation methods [3-94]. Hence, plant tissue culture is an extremely efficient and cost-effective technique for biosynthetic studies and bio-production, biotransformation, or bioconversion of plant derived compounds [3-98]. However, there are certain limitations of *in-vitro* plant regeneration system including

difficulties with continuous operation, product removal, and aseptic conditions[8-99]. For sustainable industrial applications of *in vitro* regenerated plants on a large scale, these constraints need to be addressed in future studies [13-110].

Figure 7 *In vitro* regeneration via callus in *Ocimum tenuiflorum*, commonly known as tulasi or holy basil

In vitro micropropagation process is a rapid process that can lead to the production of plants of virus free[1-100]. In plant cell culture, plant tissues or organs are grown under *in vitro* fully defined nutrient medium under aseptic conditions and controlled environment [10-100]. Plant tissue culture medium contains all the nutrients required for the normal growth and development of plants[10-100]. It is mainly composed of macronutrients, micronutrients, vitamins, other organic compounds and plant growth regulators, carbon source and some gelling agents of solid medium [10-100]. There are many mediums used for the *in vitro* culture and one common medium is the MS medium [1-90]. The Murashige and Skoog (MS) basal medium supplemented with the required amounts of plant hormones which include auxins, cytokinins, abscisic acid, gibberellins, smoke saturated water, ethylene, and growth regulators with similar metabolic effects [4-90].

Figure 8 *In vitro* regeneration of *Ocimum tenuiflorum*, commonly known as tulasi or holy basil

Figure 9 *In vitro* cloning and plant regeneration of Sugar maple (*Acer saccharum*)

5. Plant Tissue Culture: Factors influencing *in vitro* Cultures

The pH of the nutrient medium (5.8) is also important that affects both the growth and activity of plant growth regulators [4-100]. Plant growth regulators (PGRs) plays an important role as signal molecules and regulators of growth and development in plants. An endogenous auxin pulse is one of the first signals leading to the induction of somatic embryogenesis [3-100]. Auxin (IAA, IBA, NAA and 2,4-D) and cytokinins (BA, TDZ, 24-Epibrassinolide, Melatonin, Smoke saturated water, BAP, Kinetin) are the main PGRs in plants involved in the regulation of cell division and differentiation [3-100]. The higher concentrations of auxins generally favours root formation, where as higher concentration of cytokinin favors shoot formation [3-100]. Endogenous PGR levels, however, can be considered as major factors in determining the specificity of cellular responses to these rather general stress stimuli[3-100]. In addition to the absolute requirement of exogenous auxins for sustained growth under *in vitro* cultures, plant cells may produce substantial amounts of the native auxin, indole-3- acetic acid (IAA) [3-100]. A balance of both auxin and cytokinin some times leads to the formation of unorganized mass of cells known as callus [3-100].

6. Plant Tissue Culture: Commercial Applications

Plant tissue culture has many commercial applications and can be applied for the production of disease resistant plants either via organogenesis or somatic embryogenesis (Figure- 1-8) [3-100]. The greatest value of plant cell/ tissue culture rests not so much on their application to mass clonal propagation (micro propagation), but also in their involvement in plant improvement and bio-processing [5-113]. In case of organogenesis, the explants of selected plant produces plants via callus formation [5-113]. Organogenesis allows for the effective regeneration of new plants from callus (Figure- 1-8) [5-113]. Organogenesis is the production of plant organs from a specific tissue in order to develop complete plants [5-115]. It is characterized by being polar, which means that just one aerial organ or root is released and a new complete plant is generated [5-113]. Simultaneously, organogenesis can be direct in which the organogenic shoot is produced directly from the explants, or indirect, in which the organogenic process happens from previously created callus in the original explants [5-113]. Callus is an undifferentiated mass of tissue that forms explants after a few weeks on growth medium with appropriate hormones [5-113]. Callus development is the result of a well-known process known as de-differentiation or re-differentiation [5-113]. To stimulate callus induction and development, several growth hormones are employed [5-113]. Callus induction and development were increased by 2,4-D, NAA, and kinetin. Somatic embryogenesis is the process of producing embryos from somatic plant cells (any non-sexual cell) in order to produce a whole plant. Organogenesis allows for the effective regeneration of new plants from callus [5-113]. In contrast to organogenesis, this is a polar process in which the aerial structures and roots of plants develop from the somatic embryo [5-113]. It can either be direct or indirect, depending on whether the process begins with the original explants or with previously produced callus[5-113]. On the other hand, in case of somatic embryogenesis, the explants cultured under *in vitro* conditions produces callus tissue [5-113]. The cells of callus tissue are programmed towards somatic embryogenesis and produce somatic embryos [5-113]. There are few specific examples where explants produced somatic embryos directly without callus formation [5-113]. The somatic embryos on germination medium germinate to produce shoot and roots. This can be applied in the conservation of endangered/rare threatened plant species [5-113]. Clonal propagation of high-value forest trees, medicinal plants, endangered /threatened plant species through somatic

embryogenesis has the potential to rapidly capture the benefits of breeding or genetic engineering programs and to improve the uniformity and quality of the nursery stock [5-113].

Embryo culture has fertilized or unfertilized zygotic (seed) embryos dissected from maturing seeds or fruits and cultivated in vitro until seedling formation [5-113]. Embryos are genetically programmed to form the embryos. Embryo culture is not the same as somatic embryogenesis. In case of conifers, the use of zygotic embryos as explants has several disadvantages including heterogeneity as a result of cross pollination, which may result in the new generation having characteristics inferior to those of the parent (seed-bearing) tree [5-113].

The disadvantages associated with zygotic explants may be overcome if mass propagation of elite, mature trees can be achieved from vegetative tissue explants, such as secondary needles or apical shoots, because the regenerated plantlets will be uniform and possess elite characteristics from clearly defined parents [5-113]. The induction of somatic embryogenesis using shoot apical thin layers has been successful in few conifers (*Pinus roxburghii, Pinus kesiya, Pinus wallichiana, Pinus patula,* Scots pine) [5-113]. Here the somatic cells of are not genetically programmed towards somatic embryogenesis. The somatic cells of the plants cultured under *in vitro* conditions are reprogrammed towards somatic embryogenesis. The concept of reprogramming somatic cells is only possible due to some of the stress related factors, then interaction of growth hormones, selection of proper explants, the particular stage growth explants. and role of signalling molecules [5-113].

Tissue culture has been extensively utilized in breeding programs and the hybrids of such crosses are often sterile due to embryo abortion but can be 'rescued' by means of culturing or transplanting the embryos [5-113]. As a result, plant tissue culture is a viable technology for producing desirable bioactive chemicals from plants [5-113]. In some cases, inter-specific and inter-generic hybrids can be obtained using embryo rescue technique which is not possible through conventional methods [5-113]. Plant tissue culture is also used to help save endangered species, as many therapeutic plants are on the verge of extinction due to overuse [5-113]. The development of plant tissue culture techniques will expand the long-term use of therapeutic plants in the future [5-113]. Plant tissue culture coupled with biotechnological approaches is applicable to the development of genetically modified plants as well as embryo rescue procedures [5-113]. The medium utilized for cell culture can be optimized for the production of desirable products As a result, plant tissue culture is a viable technology for producing desirable bioactive chemicals from plants [5-113]. As a result, plant tissue culture is a viable technology for producing desirable bioactive chemicals from plants [5-113]. Tissue culture has been extensively utilized in breeding programs and the hybrids of such crosses are often sterile due to embryo abortion but can be 'rescued' by means of culturing or transplanting the embryos [5-113].

Lateral bud node culture is carried out on a short piece of stem tissue where stem portions carrying single or many nodes may be cultivated [5-113]. Each bud is cultivated to produce a single shoot. In isolated root culture, a branching root system can be generated by growing roots that are not attached to shoots [5-113]. Shoot tip culture is developed from excised shoot tips/buds larger than the shoot apices (used for meristems cultures), and had several leaf primordia [5-113]. These shoot apices are often cultivated such that each one generates several shoots [5-113].

The second application of plant tissue culture is the cryopreservation [5-113]. Cryopreservation is the successful storage of biological materials at ultra low termperatures -196^0 C [5-113]. The risk of loosing the plant material due to human or accidental error or undesired genetic changes is always present [5-113]. To reduce the costs and risk, cryopreservation is a valuable method for long term preservation of plant material [5-125]. Cryopreservation of embryogenic tissue has generally been considered as a means of avoiding the loss of embryo maturation potential during long-term *in vitro* culture of evading possible somaclonal variation caused by the long-term maintenance of actively growing embryogenic cultures, and of storing a large number of genotypes [5-113-125]. Similarly, cryopreservation provides a means of storing genetically altered material while field tests are conducted [5-113-120]. It may also limit the amount of contamination and somaclonal variation resulting from routine subculturing of callus tissue[5-113-125]. Furthermore, several plant species generate resistant seeds that cannot be retained for extended periods of time [5-113-125]. Hence in this situation, tissue culture can be utilized for plant conservation in vegetative state, generally under slow growth conditions or for cryopreservation [5-113-125].

A third powerful tool in plant biotechnology is genetic transformation in order to transfer relevant genes from bacteria, fungi, animals or plants into plants of interest [5-113]. The most recent feature of plant cell and tissue culture is genetic transformation, which allows the transfer of genes with desirable traits into host plants and the recovery of transgenic plants[5-113]. This approach offers a high potential for the development of agricultural plants with novel traits. The genetically modified plants exhibit agronomically significant features such as greater yield, improved nutritional quality, improved pest and disease resistance [5-113].

Plant tissue culture plays a pivotal role in vector mediated or vector-independent gene-delivery into plant genome for the production of transgenic plants with improved traits [5-113]. One important aim of genetic manipulation of plants is the increase of resistance against various fungal pathogens. The recent development of molecular tools for genomic analysis of plant species makes it possible to transfer or identify genes controlling agronomic traits [5-113]. However, both the mechanisms for DNA transfer to a plant cell and targeting of the DNA to a complex tissue or organ competent for regeneration is another major issue to be considered for effective and successful transformation [5-113]. Now-a-days there are many genes available for use in plant transformation experiments. However, most of these genes have been used as reporter genes for establishing a model transformation system, and very few have been used for novel phenotypes or for tolerance to various stresses [5-113]. Selectable marker genes have been pivotal to the development of plant transformation technologies because the marker genes allow scientists to identify or isolate the cells that are expressing the cloned DNA, and to select for the transformed progeny [5-113-125]. Different transcription factors which regulate nutrient assimilation pathways have been over expressed in staple crops that may improve crop yield. There are three methods of genetic transformation, 1) Biolistic method and 2) Agrobacterium method, 3) Protoplast method [5-113-125].

Biolistic or particle bombardment is used as a direct gene transfer method without using a vector for plant transformation, and relies entirely on physical or chemical principles to deliver foreign DNA into the plant cells. In this method, there is no dependence on bacteria, so the limitations inherent in organisms such as *A*. *tumefaciens* do no apply [5-113-125]. Plant transformation method by *A*. *tumefaciens*, soil pathogenic bacterium, has become the most used method for the introduction of foreign genes into plant cells and the subsequent regeneration of transgenic plants under in vitro conditions. Various transgenic plants including *Arabidopsis*, wheat and tobacco are developed through genetic engineering and plant tissue culture conferring resistance against different environmental stresses [5-113-125].

Plant tissue culture and genetic transformation have emerged as cornerstone technologies in agricultural biotechnology, significantly influencing the trajectory of crop improvement [5-113-128]. Continuous advancements in molecular biology and biotechnology, including the development of new genome editing tools and improved tissue culture techniques, hold promise for overcoming barriers and enhancing the capacity for crop improvement[5-113-128].

Plant tissue culture and genetic transformation enable the precise manipulation of plant genetics, allowing for the rapid propagation of disease-free plants and the introduction of traits such as higher yield, improved nutrition, and greater stress resilience[5-113-128]. As the global population nears 10 billion by 2050 and climatic conditions worsen, these technologies are critical for enhancing crop performance and ensuring food security. However, challenges such as maintaining genetic stability during tissue culture and achieving high transformation efficiency still limit their broader application across diverse crops [5-113-128].

The agricultural biotechnology market was valued at USD 116 billion in 2023 and is expected to grow to USD 293.35 billion by 2034, with a compound annual growth rate (CAGR) of 8.8% [113-128]. Biotechnology encompasses a wide range of innovative tools, such as genetic engineering, tissue culture, and marker-assisted breeding, which collectively enable the development of high-yield, pest-resistant, and climate-resilient crop [128]. According the recent review by Koti and Bill (2025) [128], in the United States, genetically modified (GM) crops like herbicide-resistant soybeans and insect- resistant corn have significantly enhanced agricultural productivity and efficiency[128]. The Asia-Pacific region is also emerging as a major force in agricultural biotechnology, with countries such as China and India investing heavily in research and swiftly adopting biotech solutions to improve food security and agricultural output[128]. China, in particular, has implemented government-backed initiatives to develop pest-resistant cotton and nutrient-enriched rice through extensive funding and supportive policies[128]. Additionally, crop production remains a critical segment in the agricultural biotechnology market, accounting for approximately 30% of total market revenue in 2023, with genetic engineering technologies playing a transformative role in enhancing both crop yield and quality [128]. Moreover, in Asia-Pacific countries like China and India, marker-assisted breeding and tissue culture methods are being widely adopted to develop disease-resistant and drought- tolerant crops, helping these nations to meet the agricultural demands of large and growing populations[128].

In the last two decades, the technique based on *Agrobacterium rhizogenes* inoculation has gained popularity as a means of creating secondary metabolites generated in plant roots[5-113-123]. Organized root cultures can contribute significantly to the generation of secondary metabolites. Hairy root disease is caused by *Agrobacterium rhizogenes* in plants [5-113=124]. The neoplastic (malignant) roots, generated at rapid growth rate, by *A. rhizogenes* infection are genetically stable and are developed in hormone-free conditions [5-113-120]. Hairy roots high stability and productivity allow them to be used as a powerful instrument for the recovery of important secondary metabolites. Hairy

roots have high stability and productivity allow them to be used as a powerful instrument for the recovery of important secondary metabolites [5-113].

Protoplasts are plant cells with cell walls removed by enzymatic or mechanical methods [5-113]. Protoplasts are obtained by immersing plant cells in a hypertonic solution, which causes the plasma membrane to shrink off the cell wall due to water efflux Cell wall may be removed using either enzymatic digestion with pectinase and cellulose, or by mechanical techniques Plant protoplasts are the important ideal tools for genetic manipulations such as gene transfer, mutation breeding and somatic hybridization [5-113]. Protoplast culture when coupled with an efficient protocol for gene delivery and plantlet regeneration serves as an excellent system for the recovery of transgenic plants [5-113]. Various crops with superior traits have been developed using this technology with enhanced nutritional value and biotic/ abiotic stress resistance that leads to increased crop yield [5-113]. Conventionally, somatic hybridization, which produces interspecific and intergeneric hybrids, was commonly used as an essential method for plant breeding. The procedure entails the fusion of two somatic protoplasts followed by the selection of desirable hybrid cells and then regeneration of hybrid plants [5-113].

Protoplast fusion is an effective method of transferring genes with desirable traits from one species to another with a great impact on crop development [5-113]. Somatic hybrids created from rice and ditch reed by electrofusion exhibited better results against salt stress [5-113].

The fourth application of plant tissue culture is the synthesis of synthetic seeds under *in vitro* conditions [5-113]. Synthetic seed production is a well documented applied technology that capitalizes on the capacity for rapid plant multiplication via somatic embryogenesis. Synthetic seed is also defined as a somatic embryo, or any other vegetative propagule, with an artificial, biodegradable coating entrapped in a nutrient medium supplying carbon sources, mineral nutrients, vitamins and growth regulators [5-113]. The entrapping matrix or coating should not have any adverse effect on the embryo or propagule and should allow germination under both in vitro or *ex vitro* conditions. The term therefore, reflects the artificial nature of the seed coat as well as somatic origin of the encapsulated propagule [5-113].

The fifth application of plant tissue culture is the anther culture. Anther culture technique is the most viable and efficient method of producing homozygous doubled haploid plants within a short period [5-113]. Agricultural activity demands improved crop varieties with desirable traits such as quality, crop yield, and resistance to environmental stresses. However, the practical application of this technology in plant improvement is still limited by various factors that influence culture efficiency. Therefore, the improved anther culture method can produce doubled haploid plants for which can be useful in different breeding programs that will enable the speedy development of commercially important plant varieties for resource-poor farmers. Although conventional breeding approaches have substantially enhanced cereal productivity, and their progress rate is slow. Thus, there is a need to respond quickly and make rapid changes to drastically reduce breeding cycles to sustain the crop under unpredictable environmental conditions [5-113]. The application of anther culture along with advanced biotechnological approaches could hasten the process to develop superior breeding materials or varieties by decreasing the number of breeding cycles dramatically. Anther culture technique is a biotechnology tool that has found importance and a niche in expediting many crop breeding processes [5-113]. Therefore, in the future, the prospects for anther culture and the integration of genomics tools provide novel opportunities for improving selection efficiency, maximizing genetic grain, and improving varietal development, leading to the early release of rice varieties with desirable traits [5-113].

Use of haploids has emerged as a key strategy for crop improvement. Haploids having a single set of chromosomes in the sporophytic phase have become a valuable source to screen for desired traits or to introduce a mutation in their genetic content[112]. Furthermore, doubled haploids (DHs) can be obtained by spontaneous or induced chromosome doubling[112]. Double haploids are homozygous at all loci, and they can be propagated through seed[112]. Doubled haploids (DHs) achieve complete homozygosity in a single generation [112]. On the contrary, the conventional breeding method requires six to seven generations of self-crossing [112]. *In vitro* production of haploids for crop improvement has been successfully achieved in many crops such as rice, wheat, barley, maize, tomato, potato, brassicas, grapes, sunflower and so on [112]. In addition to crop improvement, doubled haploids (DHs) are an excellent source for gene mapping, cytogenetic research, and evolutionary studies [112]. Various agronomic crops i.e., cereals, fruits, vegetables, ornamental plants and forest trees are currently being used for in vitro propagation [112].

The sixth application of plant tissue culture is the mass in vitro propagation of medicinal plants for the isolation of secondary metabolites of pharmaceutical interest [5-110]. *In vitro* cell culture has the inherent advantage of producing therapeutic proteins such as monoclonal antibodies, antigenic proteins that act as immunogens, human serum albumin, interferon, immuno-contraceptive proteins, antihypertensive drug angiotensins, and human haemoglobin in certain situations [5-110]. The manufacturing of pharmaceuticals using culture systems of plants can provide remarkable

benefits including cost reduction, quick production, and scalability [5-114]. Biotechnological approaches associated with plant tissue culture have increased the scope of medicinal plants along with traditional agriculture used for the industrial production of bioactive metabolites[83-194].

Tissue culture technology advancements showed that transcription factors are effective new molecular tools for plant metabolic engineering to boost the synthesis of important chemicals [74-92]. The *in vitro* plant propagation has not only made a significant contribution in the knowledge of basic research, but it also offers potential applications as it guarantees a sustainable industry that relies on commercial production of plant-derived compounds [74-92]. Alkaloids are the structurally diverse group of secondary metabolites which possess significant biological activities [74-92]. Plants make them as a defence mechanism in response to biotic and abiotic stressors. Plant tissue culture is a viable technology for producing desirable bioactive chemicals from plants. Plant tissue culture is also used to help and save the endangered species, as many therapeutic plants are on the verge of extinction due to overuse [2-110].

The seventh application of plant tissue culture is the production of nanoparticles. Nowadays, the addition of nanoparticles as elicitors has gained worldwide interest because of its success in microbial decontamination and enhancement of secondary metabolites [60-62, 107]. Nanoparticles are entities in the nanometric dimension range [60-62, 107]. They possess unique physicochemical properties. Among all the nanoparticles, silver-nanoparticles (AgNPs) are well-known for their antimicrobial and hormetic effects, which in appropriate doses, led to the improvement of plant biomass as well as secondary metabolite accumulation [60-62, 107]. Therefore, the evaluation of the integration of nanotechnology with plant tissue culture is a new advancement of plant biotechnology [60-62, 107]. The highlight is especially conveyed on secondary metabolite enhancement, effects on plant growth and biomass accumulation as well as their possible mechanism of action [60-62, 107]. In addition, the use of nanomaterials as potential therapeutic agents is gaining interest worldwide. Elicitation of silver-nanoparticles, as well as nanomaterials, function as therapeutic agents for animal well-being is expected to play a major role in the process [60-62, 107].

7. Advantages of Plant Tissue Culture

One of the main advantage of plant tissue culture technique is used for the micro propagation of medicinal plants in pharmaceutical industry for the isolation of bioactive phytocompounds which has numerous industrial applications[5-113]. It provides potential benefits for different industries which include food, pharmaceutical and cosmetics [5-113]. Plants are the rich source of phytochemicals with medicinal properties rendering them useful for the industrial production of pharmaceuticals and nutraceuticals [5-113]. The culture of plant tissues is an effective instrument for the isolation and processing of active compounds, including secondary substances and engineered molecules, from economically important plants [5-110]. Furthermore, there are numerous plant compounds with application in the cosmetics industry [5-113]. Plant cell cultures are now being used for the production of cosmeceuticals, products having cosmetic as well as therapeutic (medical or drug-like) effects that exert beneficial effects on skin health [5-113]. Plant cell culture extracts with several particular actions for skin care, make-up, and hair care as supplement components are gaining popularity in the cosmetics sector [5-116]. Cosmetic extracts derived from plant cell cultures suit the markets increasingly stringent safety requirements[3-115]. Plant cell culture cosmetic production is not dependent on appropriate seasonal conditions. Hence, it requires less time and energy. In addition to being free of pathogens, pollutants, and pesticide residues, plant cells generated under aseptic laboratory conditions rarely include any malignant compound or potential allergen, which would otherwise destroy the majority of the plant extracts obtained [5-113]. Plant tissue cultures are a perfect source of safe and pure components for cosmetic goods since they can be cultivated under controlled conditions with minimum possibility of pathogen or environmental contamination. Utilizing various extraction techniques and solvents while taking advantage of the chemical makeup of plant cell components, plant tissue culture technology allows the isolation of many active ingredients from a single cell culture [5-113]. Applications of plants/flowers extracts in cosmetics are significant which include skin moisturizing, whitening or tanning products, sunscreens, radical-scavenging antioxidants, immune stimulants, and skin thickeners etc [77].

Most of the phytochemicals such as polyphenols, phenolics acids, triterpenes, flavonoids, stilbenes, steroids, carotenoids, steroidal saponins, sterols, fatty acids, polysaccharides, sugars, and peptides are extracted with relevant solvents and utilized as an active constituents in cosmetic preparations [5-115]. The extensive research on plant cell culture has caused a surge in the use of this technique in the pharmaceutical industry. Plants are abundant sources of pharmaceutically significant compounds. However, there is a need to manufacture these compounds within stringent laboratory conditions. Various secondary metabolites having medicinal values can be obtained from plant cell culture. An extensive investigation is being done to investigate the plant sources producing active ingredients, such as antioxidants, ingredients with antimicrobial, anti-viral, anti-cancerous, antifungal, anti-inflammatory, and anti-allergy properties along with moisturizing, anti-ageing, anti-wrinkle and UV protective properties, which are crucial to cosmetics industry [5-115].

In addition to having moisturizing, anti-ageing, anti-wrinkle effects; plant-derived compounds also possess pharmacological properties such as antiviral, antimicrobial, antifungal, anticancer, antioxidant, anti-inflammatory, and anti-allergy characteristics [5-115]. The *in vitro* propagation of industrially significant flora is gaining attention because of its several advantages over conventional plant propagation methods. One of the major advantages of this technique is the quick availability of food throughout the year, irrespective of the growing season, thus opening new opportunities to the producers and farmers[5-116].

In vitro suspension cultures are created when friable calli are grown on liquid medium in a suitable container and regularly agitated to provide free cell suspension. Conical flasks are utilized because of its enormous surface area, which aids in the retention of liquid medium and the constant exchange of gases. Suspension cultures are classified as batch or continuous cultures [5-116]. At regular intervals, a part of the original cell suspension is collected and sub-cultured on to fresh medium in batch cultures. In continuous cultures, new media is introduced to the same culture on regular basis, and surplus cell suspensions are discarded. Suspension cultures are commonly utilized in large-scale synthesis of secondary metabolites. Cell suspension culture methods are currently being employed for large-scale plant cell culture from which secondary metabolites are extracted [10-116]. A suspension culture is created by moving the comparatively friable component of the callus into liquid media and maintaining it under appropriate physical conditions of aeration, agitation, light, temperature, and other physical factors. Cell cultures not only produce defined standard phytochemicals in huge quantities, but they also reduce the presence of interfering substances found in field-grown plants [20-113]. The primary benefit of cell cultures is the production of bioactive secondary metabolites in a controlled environment that is independent of climate and soil conditions [10-115].

Chemostat bioreactors are the devices particularly built for large-scale continuous culturing [77]. Recent breakthroughs in plant cell culture, molecular biology, enzymology, and fermentation technology indicated that these systems are a viable source of synthesis of important secondary metabolites [77]. Plants infected with an engineered virus generate relatively significant amounts of desired chemicals, and these plants can sustain steady levels of protein synthesis without extra intervention[3-77]. Plants infected with an engineered virus generate relatively significant amounts of desired chemicals, and these plants can sustain steady levels of protein synthesis without extra intervention[77]. Plants are used to treat and prevent particular illnesses and diseases in human beings since long time ago. Plants which possess healing metabolites with useful pharmacological effects are referred to as medicinal plants. They are rich in phytochemicals which have the spectacular capability to treat diseases and may be used for the industrial production of pharmaceuticals and nutraceuticals.

Plant micro-propagation has extensively been used to have an insight into plant pathology studies which include factors influencing penetration, infection and multiplication of pathogens, the nature of irregular cell division or growth, and the morphogenetic potential of the diseased cell [3-120]. The employment of plant tissue culture in plant pathology is not only restricted to use plant tissues as a substrate for the pathogens, but it provides the basic understanding of various characteristics of pathological growth, pathogens' attack weapons, and the host response to an infection caused by the invading organism [3-114]. Plant physiology and plant morphogenesis requires the capability to grow plants in vitro that might be best accomplished with plant tissue culture procedures [3-114-128].

Furthermore, the conservation of plant biodiversity is indispensable for future crops safety due to increasing challenges of biotic and abiotic stresses [3-114]. In this regard, in vitro techniques permit improvement in various traits associated with plant growth and yield that can later be used for ex-situ conservation [3-114]. The active plant compounds obtained from rare or endangered species can be manufactured by in vitro techniques without adverse environmental effects and in agreement with the bio- sustainability matters that the market demands [3-114].

Plant tissue culture is a powerful tool of agricultural improvement and offers tangible solutions to major crop problems that arise due to constant threat of biotic and abiotic stresses minimizing the crop yield [3-114-126]. Since plant tissue culture is simple, low-cost and environment friendly, it is imperative to employ this technique for the development of sustainable agriculture in order to meet the food demand of increasing human population [3-114]. Agricultural diversification is to satisfy our future demands necessitates the implementation of innovative agricultural technology. The finest cultural methods, excessive fertilizers, and pest control procedures will not yield the desired results unless the best planting material is used [3-114]. Although somaclonal variation is deleterious to quick clonal multiplication, some off-types have been discovered that have significant agronomic utility [3-114]. In the future, plant tissue cultures might not only be a source of novel chemicals with uncharted biological actions, but they might also work as alternative recombinant protein bio-factories, particularly for those whose expression might be problematic or constrained in fermenting microbes[3-114]. Biotechnological approaches associated with plant tissue culture have increased the scope of medicinal plants along with traditional agriculture used for the industrial production of bioactive metabolites [3-114].

In vitro culture is a method applied for the growth and development of plant cells, tissues, and organs that uses a nutritive culture medium under controlled sterilized conditions [3-114]. Tissue culture is now widely being used as a viable horticultural propagation. technology, and it has changed the horticultural business [3-114]. This approach is used to achieve mass proliferation and the creation of disease-free stock material. Commercial laboratories are now using tissue culture protocols that minimize somaclonal variations, and working on creating accurate screening and selection approaches for early detection of off-types [3-114]. This method is considered as one of the most promising and environmentally friendly biotechnological practices for the sustainable supply of biofuels [3-114]. In this regard, plant tissue culture methods hold enormous promise [3-114]. There are three main *in vitro* culture systems including organogenesis (e.g., embryogenesis, direct and indirect shoot regeneration), rhizogenesis, and callogenesis [3-114]. Among these methods, callogenesis can be considered as a robust method for biofuel production. The main advantages of callus culture are- the callus is generally defined as an irregular bulk of parenchymatous tissue with meristematic cells that are broadly used for the production of different bioactive plant molecules [3-114].

Genetic manipulation of callus for lignin engineering through transient gene transformation is much easier than other methods due to the lack of need for transgenic plant regeneration [3-114]. Somaclonal variations, which are usually seen in callus culture, can result in changes to metabolic pathways and even allows the production of new metabolites [3-114]. Any phenotypic variation during callus culture is referred to as somaclonal variation that can be a result of RNA interference, histone modification, chromatin remodeling, DNA methylation, and spontaneous mutation. Callus culture can be easily scaled up in different bioreactor systems [77]. Callus culture is considered as sustainable and eco-friendly process [3-114]. These callus cultures of many medicinal plants were also used for the production of thin films as biodegradable **biopolymer composite** in food packaging industries [3-114-126].

Tissue culture is a fastest *in vitro* cloning technique. Additionally, plant cell and organ cultures are of interest for the production of secondary metabolites of industrial and pharmaceutical interest [1-114]. New technologies, such as the genome editing ones combined with tissue culture and *Agrobacterium tumefaciens* infection, are currently promising alternatives for the highly specific genetic manipulation of interesting agronomical or industrial traits in crop plants [1-114]. Application of omics (genomics, transcriptomics, and proteomics) to plant tissue culture will certainly help to unravel complex developmental processes such as organogenesis and somatic embryogenesis, which will probably enable to improve the efficiency of regeneration protocols for recalcitrant species [114]. Additionally, metabolomics applied to tissue culture will facilitate the extraction and characterization of complex mixtures of natural plant products of industrial interest [1-114]. Plant tissue culture has developed widely incorporated into biotechnology, the agricultural systems being a key factor to support many pharmaceutical and industrial outcomes [5-114]. Plant tissue culture allowed using its several traits through transgenic breeds for the benefit of farmers and companies helped in the reduction of pesticide application having better nutritional quality [5-114].

Advancements in plant tissue culture and genetic transformation are pivotal in advancing agricultural biotechnology, enhancing crop yields, nutritional value, and environmental resilience [1-114-128-131]. Integration of sophisticated synthetic biology and precise genome editing techniques has significantly refined the accuracy of genetic modifications, enhancing crop yields, nutritional value, and resilience to environmental stresses [1-114-128-131]. The burgeoning role of automation and artificial intelligence revolutionizes tissue culture processes by significantly improving both efficiency and scalability [114-128-131]. Key challenges such as public skepticism, regulatory barriers, and technical issues like genetic stability and transformation efficacy are the major challenges[1-114-128-131]. It also explores ethical concerns and diverse global regulatory landscapes, highlighting factors that influence the adoption of these technologies [1-114-128-131]. Case studies confirmed the substantial benefits of these biotechnological advances, affirming their potential to dramatically transform agricultural productivity [1-114-128-131].

8. Application of Artificial Intelligence (AI) in Plant Tissue Culture

Artificial neural networks (ANNs) are widely used in science and technology, and have been successfully applied in *Cannabis sativa* plant tissue cultures [125-127]. Furthermore, Artificial neural networks (ANNs) can also simulate the growth of plants under different *in vitro* conditions [125-127]. *Cannabis sativa* micropropagation has largely been an underground effort with few peer reviewed studies. This lack of insight concerning *in vitro* cannabis techniques has limited the biotechnological utility of cannabis crop [125-127]. This is mainly due to the fact that *Cannabis sativa* found to be recalcitrant under *in vitro* conditions, restrictions, long legacy of prohibition and stigmatization surrounding this Indian origin medicinal plant [125-127]. Machine Learning (ML) and Deep Learning (DL) are two of the most exciting technological areas of Artificial Intelligence (AI). Data is a power today, and artificial intelligence (AI) can help cannabis businesses to gather and analyze data in a wide variety of ways. Artificial Intelligence (AI) technology has enhanced cannabis crop production and improved real-time monitoring, harvesting, processing and marketing [125-127]. These technologies saves the excess use of water, pesticides, herbicides, maintains the fertility of the soil, and also helps in the

efficient use of man power and elevated the productivity and improved the quality of cannabis products [125-127]. However, very few and limited *in vitro* regeneration protocols have been developed in cannabis and existing protocols highlights only organogenesis [125-127]. Therefore, there is a golden opportunity for the development of new *in vitro* regeneration protocols particularly induction of somatic embryogenesis, cryopreservation, protoplast isolation and culture, genetic transformation, production of synthetic seeds, and anther culture for the production of haploids in *Cannabis sativa* [125-127].

Automation and artificial intelligence (AI) are transforming plant tissue culture, markedly improving efficiency, precision, and scalability [125-128]. These technologies enable precise control over growth conditions and automate routine tasks such as micropropagation and explant handling, reducing labor requirements and minimizing human error[125-128]. Moreover, AI algorithms analyze extensive datasets to predict optimal growth outcomes and dynamically adjust protocols, enhancing the adaptability and efficiency of tissue culture practices. For instance, robotic systems now perform delicate operations such as cutting and transplanting tissue cultures, ensuring consistent handling and reducing contamination risks [125-128]. These innovations not only streamline production but also significantly reduce costs, making advanced tissue culture techniques more accessible and economically viable [125-128]. As AI and automation continue to evolve, they are setting new industry standards for producing high-quality, genetically uniform plantlets, and supporting scalable and sustainable agricultural operations[125-128].

9. What is New in Plant Tissue Culture

In another major development in plant tissue culture, a study by Ruiz-Solaní et al., (2025) [115] has elucidated the mechanism by which bacterial cellulose mediates plant tissue regeneration [108, 115, 116]. The work has been published in the journal *Science Advances in* 2025 and includes collaborations with researchers of the Institute of Materials Science of Barcelona (ICMAB-CSIC) and Colorado State University [108, 115, 116]. Bacterial cellulose (BC), synthesized by certain bacteria as a biofilm, consists of highly pure cellulose fibres [108, 115, 116]. Bacterial cellulose (BC), has been widely used in human biomedical applications showing a high degree of biocompatibility, but its potential healing effects in plants were unknown [108, 115, 116]. In this work, scientists have demonstrated that BC patches induce plant tissue regeneration and have identified for the first time the precise molecular mechanism underlying the process [108, 115, 116]. Wounded leaves of the model plants *Nicotiana benthamiana* and *Arabidopsis thaliana* were covered with BC patches and formation of new cells on both sides of the cut was observed two days post-wounding, reaching complete wound closure after seven days [108, 115, 116]. This wound healing process was promoted by bacterial cellulose (BC) but not by other structurally similar matrixes such as plant cellulose, indicating that bacterial cellulose (BC) had specific features beyond preventing dehydration or promoting physical contact [108, 115, 116].

10. Limitations of Plant Tissue Culture

The limitations of plant tissue culture methods include difficulties with continuous operation, product removal, and aseptic conditions [1-125]. A few culture systems appear to have the potential to become commercially viable because of these limitations. Plant cell development under *in vitro* conditions and regeneration into full plants is an asexual process that involves just mitotic division of the cell and, ideally, should not result in variation. Clonal multiplication of genetically homogenous plants is the ideal scenario. Uncontrolled and unpredictable spontaneous variation throughout the cultural process is thus an unanticipated and largely undesirable phenomenon. This is attributed to somaclonal variation in production of clones and low secondary metabolite titers. In contrast to these detrimental consequences, its use in crop improvement through the development of new variations is widely recognized. The expense of culture material, electricity, and labor are other issues with *in vitro* tissue culture cultivation [1-125]. Somaclonal differences that occur during *in vitro* propagation, commercial phytochemical synthesis, or genetically modified plants can have significant economic consequences are the major impediment to the practical application of plant tissue culture techniques for the production of active metabolites [1-125]. Reprogramming plant somatic cells under *in vitro* conditions towards somatic embryogenesis is also a challenging process since many factors and signalling molecules govern and controlled the process [1-125].

CRISPR/Cas9 genome editing represents a revolutionary advance in plant genetic transformation, offering unparalleled precision in making targeted genetic modifications[128-132]. Utilizing a simple guide RNA to direct the Cas9 nuclease to specific genomic locations, this method allows for precise cuts and edits within the plant genome, enabling the deletion, insertion, or modification of plant genes [128-132]. This technology has been rapidly adopted across various plant species due to its efficiency and simplicity compared to earlier genetic engineering techniques [128-132]. CRISPR/Cas9 not only enhances the toolkit for basic plant science research but also holds promise for rapidly introducing traits such as disease resistance, drought tolerance, and enhanced nutritional content in crops [128-131].

However, the technique does face challenges such as potential off-target effects and regulatory hurdles for genetically edited crop [128-131]. Despite these challenges, CRISPR/Cas9 continues to evolve, with new variants being developed that expand its potential applications and improve specificity and efficiency[128-131]. Gene editing tools such as CRISPR/Cas, TALENs, and ZFNs have markedly advanced genetic engineering in agriculture, yet they encounter significant technical hurdles that limit their application [128-131]. These challenges primarily involve variability in editing efficiency, which is often low and inconsistent across different species due to complex tool assembly and the risk of unintended off-target mutations [128-131]. For example, ZFNs generally showed an editing efficiency of only 1% to 10%, compounded by their potential to induce non- specific genetic changes [128-131]. TALENs, though more specific, are hindered by their large size which complicates cellular delivery and expression [128-131]. The CRISPR/Cas system, despite its relative simplicity and higher efficiency, also struggles with issues like stringent protospacer adjacent motif (PAM) requirements and off-target effects, which can lead to undesirable genetic alterations [128-131]. Additionally, the delivery mechanisms for these tools, such as *Agrobacterium*-mediated transformation or biolistics, often lead to random DNA integration or can damage the plant tissue, posing further challenges to precise and safe genetic modifications[128-131].

Scalability and reproducibility are crucial challenges in the genetic transformation of crops using CRISPR/Cas systems, especially in the context of cereal crop improvement [128-132]. The CRISPR technology while transformative, encounters significant hurdles when scaled for agricultural applications across diverse environment [128-132]. These challenges stem from the variability in gene-editing outcomes influenced by environmental factors and the genetic diversity among crop species, which often result in inconsistent phenotypic expressions[128-131]. Additionally, technical issues such as off-target effects and the specificity required in the protospacer adjacent motif (PAM) sequences limit the predictability and uniformity of the desired genetic modifications [128-131]. Furthermore, the scalability of CRISPR applications is constrained by the extensive regulatory frameworks governing genetically modified organisms, which vary significantly across regions and impact public acceptance and adoption rates[128-132]. Effective implementation on a larger scale requires overcoming these reproducibility and regulatory challenges to ensure that genetically edited crops perform reliably in different agricultural setting [128-132].

Techniques such as CRISPR/Cas9 genome editing and synthetic biology have revolutionized traditional farming methods by enabling precise genetic modifications that improve crop yields, enhance nutritional profiles, and increase resistance to environmental stresses[128-132]. These innovations not only facilitate the rapid propagation of high-yield, disease-resistant plants but also promote sustainable agricultural practices by reducing reliance on chemical inputs such as fertilizers and pesticide [128-132].

Genetically modified (GM) crops present significant biological and ecological challenges, particularly due to gene flow which can lead to unintended spread of transgenic traits to wild or weedy relatives, increasing their invasiveness and disrupting local ecosystems [1-128-131]. This gene flow can reduce biodiversity by altering competition dynamics and displacing native species, while herbicide-resistant crops have led to the evolution of resistant weeds that necessitate higher herbicide use, impacting non-target species and reducing agricultural biodiversity [1-128-132]. Furthermore, the persistence of GM traits in the environment can lead to genetic homogenization, diminishing the genetic diversity and resilience of natural ecosystems, making them less adaptable to environmental changes and pest pressures[1-128-131]. These ecological impacts necessitate careful management and monitoring of GM crops to mitigate their potential negative effects on biodiversity and ecosystem health [1-128-131].

Advanced genome editing techniques such as CRISPR/Cas9, prime editing, and base editing are rapidly transforming crop improvement efforts by enabling precise genetic modifications[1-128-132]. CRISPR/Cas9, a foundational tool in genome editing, introduces targeted DNA breaks, facilitating gene knockout or the introduction of desired traits [128-131]. Recent advancements have led to prime editing, which avoids double-strand breaks and allows for accurate insertion, deletion, and base-to-base conversions, significantly reducing off-target effects and increasing specificity in plants like rice and Arabidopsis [1-128-132]. Additionally, base editing enables single-base alterations without inducing double-strand breaks, expanding the potential for multiplex genetic changes critical for crop enhancement[1-128-131].

Koti and Bill [128] are of the opinion that synthetic biology is revolutionizing crop design by enabling the development of plants with enhanced resilience, higher productivity, and improved nutritional value [128-132]. This approach integrates engineering principles into biological systems, allowing for precise genetic modifications that extend beyond the capabilities of conventional breeding [128-132]. Through the design of genetic circuits and metabolic pathways, synthetic biology makes it possible to create “super crops” that can withstand environmental stresses, increase photosynthetic efficiency, and reduce reliance on fertilizers [128-132]. Advanced computational tools and gene-editing technologies, such as CRISPR, play a crucial role by enabling scientists to incorporate complex, multi-gene traits into plants, tailoring crops to specific agricultural needs and environmental conditions[128-131]. Additionally, synthetic

biology facilitates targeted modifications of metabolic pathways to produce health-enhancing compounds, expanding the potential of crops to address nutritional challenges and promote sustainable agriculture [128-131].

Developing international regulatory frameworks that can adapt to rapid technological advancements while ensuring environmental safety and public health is essential[128-132]. Moreover, fostering constructive dialogue with the public to enhance understanding and clarify the benefits and risks associated with GMOs is crucial for building trust and achieving broader acceptance[128-132]. By effectively navigating these issues, plant tissue culture and genetic transformation can realize their full potential, significantly contributing to global food security and promoting sustainable agricultural practices [128-132].

11. Conclusion

The beauty of plant tissue culture lies in the culture of small piece of plant material (explant) on a defined nutrient medium to produce large number of plantlets or clones within a limited time in a continuous process, irrespective of season, and weather on year round basis. Gottlieb Haberlandt (1902), who has been studying plant tissues, was the very first student in that field who did the establishment of plant tissue culture. German Botanist Gottlieb Haberlandt is regarded as the father of plant tissue culture. Scientists reported the use of biotechnological approaches to improve horticultural crop production. Micropropagation via *in vitro* culture technique has several advantages over the traditional methods of propagation through seed, cuttings, grafting and air-layering etc. Plant tissue culture techniques are the most frequently used biotechnological tools for basic and applied purposes ranging from investigation on plant developmental processes, functional gene studies, commercial plant micropropagation, generation of transgenic plants with specific industrial and agronomical traits, plant breeding and crop improvement, virus elimination from infected materials to render high-quality healthy plant material, preservation and conservation of germplasm of vegetative propagated plant crops, and rescue of threatened or endangered plant species. However, there are few limitations of plant tissue culture are somaclonal variations of *in vitro* regenerated plants with low yields. Uncontrolled and unpredictable spontaneous variation throughout the cultural process is thus an unanticipated and largely undesirable phenomenon. Furthermore, tissue culture can not be applied to all the plants particularly monocotyledons since most of the plants are recalcitrant and can not be regenerated under *in vitro* condition. In spite of limitations, plant tissue culture also plays an important role in genetic transformation experiments and new traits have been successfully introduced. Furthermore, the scalability of *CRISPR* applications is constrained by the extensive regulatory frameworks governing genetically modified organisms, which vary significantly across regions and impact public acceptance and adoption rates. Therefore, future trends that are likely to further revolutionize this field, emphasizing the necessity for ongoing innovation, ethical vigilance, and unified regulatory frameworks to fully leverage these advancements for global food security.

Compliance with ethical standards

Disclosure of conflict of interest

No conflict of interest to be disclosed.

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