



TISSUE CULTURE AS A PLANT PRODUCTION TECHNIQUE FOR MEDICINAL PLANTS: A REVIEW

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ABSTRACT

Haberlandt envisioned the concept of plant tissue culture and provided the basic work for the cultivation of plant cells, tissues and organs culture. In recent years, a number of plants has been investigated and reported possessing medicinal values. Over-utilization and continuous depletion of traditional medicinal plants have affected their supply and loss of genetic biodiversity. Many Endangered plants having medicinal value are tissue cultured by callus growth and somatic embryogenesis. In the present days many medicinal plants are reproduce by in vitro propagation. By this technique desired characteristics are also attain. Tissue culturing of medicinal plants is widely used to produce active secondary metabolites for herbal and pharmaceutical industries. So tissue culture is a best way to conserve the medicinal plant and also the mass production of these valuable plants. This paper highlights some of the applications of plant tissue culture for medicinal plants and limitations of tissue culture. The effect of media formulations and culturing techniques on the growth and multiplication of medicinal plants is also reviewed.

Key Words: *Endangered plants, In-vitro Propagation, Medicinal Plants, Secondary Metabolites, Tissue culture,*

1. INTRODUCTION

The term medicinal plants include a various types of plants used in herbalism and some of these plants have a potential medicinal activities. Since the ancient time, medicinal plants have been used virtually as a source of medicine. It is estimated that 70-80% people worldwide relay chiefly on traditional, largely herbal, medicines to meet their primary health care needs [1]. In the past two decades, there has been an increasing global inclination towards herbal medicine, followed by a belated growth in international awareness about the dwindling supply of the



world's medicinal plants [2]. The WHO Almost 85% of traditional medicine preparations involve the use of plants or plant extracts [3]. The pharmaceutical industry has made massive investments on extensively used in the pharmaceutical, clinical and chemical researches all over the world in an effort to discover and still more potent drugs by these medicinal plants. Exploitation of medicinal plants as pharmaceuticals, herbal remedies, cosmetics and other natural products has greatly increased in recent years. The degradation of genetic diversity of medicinal plants in the world has reached dangerously at alarming rate because of continuous harvesting practices and over-harvesting for production of medicines. Also, extensive destruction of the plant-rich habitat as a result of forest degradation, agricultural encroachment, urbanization etc. are other factors, thus challenging their existence [4]. Due to this, plant species in nature and the number of threatened plant species is increasing gradually [5]. According to the need list (IUCN 2000) of threatened species 44 plant species are critically endangered, 113 endangered and 87 vulnerable [6]. Hence, there is a need to conserve the plant resources on the ground level for the benefit of human beings and sustainable development of environment. One of the possible methods of protection of endangered taxon is multiplying and conservation of plants by *in vitro* cultures. Tissue culture has superiority over conventional method of plant propagation because of high multiplication rate. Like other technologies, it has gone through different stages of evolution; scientific curiosity, research tool, novel applications and mass exploitation. Initially, plant tissue culture was used as a research tool and focused on attempts to culture and study the development of small, isolated segments of plant tissues or isolated cells. Biotechnological approaches, specifically plant tissue culture plays a vital role in search for alternatives to production of desirable medicinal compounds from plants.

AIMS AND OBJECTIVES

This study carried out some of the applications of plant tissue culture for medicinal plant and limitations of tissue culture. The effect of media formulations and culturing techniques on the growth and multiplication of medicinal plants is also reviewed.

MATERIAL AND METHODS

This study is carried out by critical reviews. Searching various medical database like PubMed, Google scholar, Google Search, etc. related to plant tissue culture in Herbal medicinal plant, endangered medicinal plants.

CONCEPT OF PLANT TISSUE CULTURE

Gottlieb Haberlandt (1854-1945), a German botanist is considered as the father of plant tissue culture, was the first to separate and culture plant cells on Knop's salt solution in 1898[7]. Plant tissue culture is the technique of maintaining and growing plant cells, tissues or organs or any plant part on artificial nutrient solid or liquid media in suitable containers under controlled environmental conditions. This practice is used to propagate plant clones under sterile conditions. *In vitro* culture is one of the key tools of plant biotechnology that exploits the totipotency nature



of plant cells [8]. and this was demonstrated for the first time in plants by [9]. After the discovery of kinetin [10], the major work on in vitro regeneration has been done on tobacco (*Nicotiana tabacum* L.) tissue culture, culminating in the first convincing demonstration of the control of differentiation of shoots or roots or both by the kinetin-auxin ratio [11] followed by carrot (*Daucus carota* L.) tissue culture and birth of the concept of totipotency of plant cell with the regeneration of complete flowering plants of carrot from its phloem cells [9].

The cultured cells and tissue can take several pathways to produce a complete plant. Among these are organogenesis and somatic embryogenesis are common. In organogenesis, groups of cells of the apical meristem in the shoot apex, axillary buds, root tips etc are stimulated to differentiate and grow into shoots and ultimately into complete plants. In somatic embryogenesis, groups of somatic cells/tissues lead to the formation of somatic embryos which resemble the zygotic embryos of intact seeds and can grow into seedlings on suitable medium

II MICROPROPAGATION

The technique was heralded as the universal mass clonal plant propagation system for the future and the term 'micropropagation' was introduced to describe more accurately the processes. In micropropagation rapid proliferation is achieved from tiny plant part such as stem cuttings, auxiliary buds, and to a limited extent from somatic embryos. The process of micropropagation is usually divided into several stages i.e., propagation, initiation of explants, subculture of explants for proliferation, shooting and rooting, and hardening. These stages are universally applicable in large-scale multiplication of plants. The in vitro propagated medicinal plants are genetically pure elite. Micropropagation techniques are must for conservation of an endangered medicinally important species within short period and limited space. Today plant tissue culture applications encompass much more than clonal propagation and micropropagation. The range of routine technologies has expanded to include somatic embryogenesis, somatic hybridization etc.

Micropropagation offers several distinct advantages not possible with conventional propagation technique of medicinal plants;

1. Rapid multiplication of genetically uniform plants (clones) that possess desirable traits.
2. The production of multiples of plants in the absence of seeds or necessary pollinators to produce seeds.
3. The regeneration of whole plants from plant cells.
4. Production of secondary metabolites.
5. *In vitro* conservation of germplasm.
6. New and genetically engineered plants can be produced.
7. Conservation of threatened plant species.
8. Plants are obtained throughout the year under controlled conditions, independent of seasons.

2.1 Explants source: Micropropagation start with the selection of plant tissue (explants) from a young, healthy mother plant. For tissue culture, any part of the plant (leaf, root etc.) can be used as explants. Tissue culture



success mainly depends on the age, types and position of explants [12]. Sometimes Large size explants can increase chances of contamination and small explants (meristems) can show less growth [13] [14]. To increase the probability of success, the mother plant should be ex-vitro cultivated under optimal condition to minimize contamination in the *in-vitro* culture.

2.2 Sterilization : Microbial contamination is a very common problem in plant tissue culture [13][15]. For this purpose explants is surface sterilized by different reagents then transferred into nutrient medium generally. Explants are cleaned by distilled water and sterilized using mercuric chloride, ethyl alcohol, and liquid bleach [16]. Sterilization of laboratory instruments is carried out by autoclaving, alcohol washing, baking, radiations, flaming and fumigation [17]. The conserved application and bactericide and fungicide products is suggested. The selection of products depends on type of explants to be introduced. Surface sterilization is used to remove contaminants with minimal damage to plant cells.

2.3 Tissue culture Media and Plant Growth Hormones : Media for tissue culture contains vital nutrients and elements for *in vitro* growth of plant tissues. Selection of the right media composition is important for successful tissue culturing. A wide range of media are available for plant tissue culture, but MS [18] medium is commonly[12]. Other media used are Linsmaier- Skoog (LS) [19], Schenk and Hilderbrandt (SH) [20], WPM (Woody plant medium [21]and the Nitsch and Nitsch (NN) [22]. Agar is not essential media component but is used as gelling agent [23]. The *pH* of culture media is normally between pH 5.7 to 5.8. Optimization of regeneration protocol supporting the action of growth additives as a supplement of growth regulators will be useful in the establish of tissue cultures. Growth hormones regulate various physiological and morphological processes in plants and are also known as plant growth regulators (PGRs) or phytohormones [23] [1]. PGRs are synthesized by plants; therefore many plant species can grow successfully without external medium supplements [[24] [25]. Hormones can also be added into cultures to improve plant growth and to enhance metabolite synthesis. Growth and morphogenesis of cultured plants depends on the selection of the initial material, media composition, plant growth regulators, cultivar and environmental factors [26]. The effects of auxins and cytokinins on shoot multiplication of various medicinal plants have been reported by [27] [28]. has indicated that the production of multiple shoots is higher in the medium having kinetin along with NAA. *in vitro* growth and shoot formation was not achieved without adequate concentrations exogenous hormones [10] Category of plant growth hormones and their functions.

- 1) Auxins :** Mainly play role in cell division, elongation and root differentiation. Some are - Indole -3-acetic acid (IAA) , indole -3- butyric acid (IBA) , 2,4- dichlorophenoxyacetic acid naphthalene acetic acid (NAA) , 2,4,5- trichl orphenoxy acetic acid naphthoxyacetic acid (NOA) [23].
- 2) Cytokinins :** main functions are cell division, shoot induction, development and proliferation. Some are - benzyl amino purine (BAP) , isopentenyl adenine (IPA or 2i -p), kinetin (furfurylamino purine) , 4-hydroxy -3-methyl -trans -2- butenylaminopurine (zeatin) [23].
- 3) Gibberellins:** Play role in growth, elongation and flowering such asGA3 [23].



2.4 In vitro Plant tissue culture stages and techniques: Any part of plant can be used as explants. The process of tissue culturing, from explants to mature stage plants involves some basic steps:

- 1) Initiation stage or culture establishment--In this, surface sterilized explants transferred into nutrient medium. The conserved application and bactericide and fungicide products is suggested. The selection of products depends on type of explants to be introduced.
- 2) Multiplication stage- The aim of this phase is to increase the number of propagules [29]. The number of propagules is multiplied by repeated sub cultures until the desired (or planned) number of plant is attained.
- 3) Rooting stage- The rooting stage may occur simultaneously in the same culture media used for multiplication of the explants. After changes the nutritional modification and growth regulator composition to induce rooting and the development of stage root growth. For rooting half strength MS medium supplemented with 1.0mg/l auxin was used.
- 4) Stage of Hardening and Acclimatization of Tissue Culture Plantlets - In this stage in vitro cultured plants hardened and acclimatized. Hardening is done gradually from high to low humidity and from low light intensity to high light intensity. The plantlets were removed from artificial medium and rinsed with sterile water to remove excess of agar on roots, to be then placed into the cup, introducing the roots into the soil sand mixture. The newly transferred plantlets were spray-irrigated while they stilled under transparent plastic cover on the top to prevent quick dehydration. The regenerated plantlets were placed in growth chamber (room) for one week, before they were transferred to green house for several weeks, with gradual release of plastic covers.

2.5 Callus Cultures: Primary callus culture derived from tissues with high contents of parenchyma or meristematic cells. Callus is an undifferentiated mass of tissue which appears on explants within a few weeks of transfer onto growth medium with suitable hormones[23].

2.6 Cell Suspensions culture : Suspension culture is a type of culture in which single cell or small aggregates of cell multiply while suspended in agitated liquid medium. Suspension cultures are of two types batch and continuous. These cultures are formed *in vitro* when friable calli are grown on liquid media in suitable container and constantly agitated to provide suspension of free cells [23]. Suspension cultures are widely used in large scale production of secondary metabolites [30].

2.7 Protoplast Cultures: Protoplasts are plant cells in which do not possess cell wall and wall has been removed by enzyme digestion or mechanical process [23]. Plant Cell wall can be removed through enzymatic digestion (pectinase and cellulose) or by mechanical methods [31]. The main aim in using this approach in the past however



has been interspecies hybridizations. Plant regeneration was carried out successfully in *A. judaica* and *E. spinosissimus* by protoplast culture [32].

2.8 Embryo culture: Embryo culture or Somatic embryogenesis is process by which a non zygotic embryo is produced in glass vials from plant tissue or cell, which can develop into a new plant [33] [34]. *In vitro* embryo culture represents a potential tool for improved recovery of hybrid germplasm [35]. Successful plant regeneration has been studied in *Aloe vera* and *Vitis vinifera* by somatic embryogenesis [36][37].

2.9 Anther or Microspore Cultures: Anther culture is the *in vitro* development of haploid plants originating from potent pollen grains through a series of cell division and differentiation. Androgenesis has become a powerful tool for the rapid production of haploid and inbred lines used for obtaining hybrid cultivars [38]. An auxin has been reported to be absolutely required to initiate and promote microspore embryogenesis and a small amount of cytokinin in addition to auxin improves the yield of embryoids in anther culture of oilseed rape [39]

2.10 Pollen culture: Pollen culture is the *in vitro* technique by which the pollen grains (preferably at the microscope stages) are squeezed from the intact anther and then cultured on nutrient medium where the microspores without producing male gametes.

2.11 Shoot tip and Meristem culture: The shoot tips (shoot apical meristems) can be cultured *in vitro* producing clumps of shoots from auxiliary and adventitious buds. Shoot meristem multiplication is generally used for producing virus free material and maintaining germplasm via cryopreservation [40]. Several micropropagation protocols of medicinal plants such as Ashwaganda have been reported from shoot tip explants [41] .

III SECONDARY METABOLITES

Plants produce two types of metabolites -primary and secondary. Primary metabolites are essential for the growth and development of the plants. Plants are attacked by pests and predators. To overcome this problem, during metabolism plants produce enormous number of compounds as part of defense Mechanism[42] [43]. These compounds do not play essential role like primary metabolites, they are called secondary metabolites. . Secondary metabolites are used as pharmaceutical, agrochemicals, aromatics and food additives [42] [44]. *In vitro* tissue culture offers an effective and potential alternative of production of bioactive compound because the amount of secondary metabolites produced in this technique can be even higher than in parent plants [44] [45]. Plant derived compounds include many terpenes, polyphenols, cardenolides, steroids, alkaloids and glycosides [30] [46].

Figure-1 shows different groups of chemical compounds produced found plants.

Phenylpropanoids

- 1) Anthocyanins
- 2) Coumarins
- 3) Flavonoids
- 4) Isoflavonoids
- 5) Hydroxycinnamoyl Derivatives
- 6) Lignanins
- 7) Phenolenons
- 8) Proanthocyanidins
- 9) Stilbenes
- 10) Tanins

Medicinal Plants

Alkaloids

- 1) Acridines
- 2) Quinolizidines
- 3) Betalanes
- 4) Furonoquinones
- 5) Harringtonines
- 6) Isoquinolines
- 7) Indoles
- 8) Purines
- 9) Pyridines
- 10) Tropane alkaloids

Quinones

- 1) Anthroquinones
- 2) Benzoquinones
- 3) Naphthoquinon

Terpenoids

- 1) Carotenes
- 2) Monoterpenes
- 3) Sesquiterpenes
- 4) Diterpenes
- 5) Triterpenes

Steroids

- 1) Cardiac glycosides
- 2) Pregnenolone derivative

Figure -1 : Adapted schematic showing the classification of plant derived compounds [44]

In vitro grown plant cells and tissues have been used extensively for the production of secondary metabolites. Depending on the objectives, biotechnological techniques are used for understanding metabolic pathways and improvement of plants for the production of secondary metabolites. Plants are potential source of various pharmaceutical and industrial products. Nearly 30% of the drugs produced are of plant origin. Tissue culture of medicinal plants provides a continuous and reliable source of these bioactive compounds round the year without the destruction of the entire plant. Hashimoto *et al.* [47] reported increased production of tropane alkaloids in genetically engineered root cultures. There are several strategies that can be used to enhance the production of desired pharmaceuticals by genetic engineering [48]. Oksman-Caldentey and [42] have reviewed the work on the production of designer metabolites in the post-genomic domain.

IV DISADVANTAGES OF MICROPROPAGATION

Micropropagation is not always the perfect means of multiplying plants, conditions that limits its use include: —

- It is very expensive, and can have a labour cost of more than 70%.
- A monoculture is produced after micropropagation, leading to a lack of overall disease resilience, as all progeny plants may be vulnerable to the same infections.
- An infected plant sample can produce infected progeny. This is uncommon if the stock plants are carefully screened and vetted to prevent culturing plants infected with virus or fungus.

Not all plants can be successfully tissue cultured, often because the proper medium for growth is not known or the

**V IMPORTANCE OF MEDICINAL PLANTS.**

Medicinal plants contain inherent many bioactive compounds which are used to cure disease or relieve [49]. . Only about 10% of the known medicinal plants of India are restricted to non-forest habitat [50]. Medicinal plants are an integral component of ethno- veterinary medicine also. Approximately 80% of the people in the world's developing countries rely on traditional medicine for their primary health care, and about 85% of traditional medicine involves the use of plant extracts [3]. The World Health Organization (WHO) has estimated that the present demand for medicinal plants is approximately US \$14 billion per year [51]. The demand for medicinal plants based raw material is growing at the rate 15 – 25% annually, and according to an estimate of WHO, the demand of medicinal plants is likely to increase more than US \$ 5 trillion in 2050. In India the medicinal plant related trade is estimated to be approximately US \$ 4 billion/year. India has 2.4% worlds area with 8% of global biodiversity and it is one of the 12th mega-diversity hot spot countries of the world's with rich diversity of biotic resources [51]. Out of 34 hot spot India has two major hotspot the Eastern Himalayas and Western Ghats. India is also rich in medicinal plant diversity with all the three level of biodiversity such as species diversity, genetic diversity and habitat diversity. Across the country, the forest is estimated to harbor 90% of India's total medicinal plants diversity. It is estimated that in India 75,000 species of medicinal plants are present as compared to other countries[51]. IUCN recognizes the different categories: extinct, extinct in the wild, critically endangered, endangered, vulnerable, near threatened, least concern, data deficient and not evaluated. Species with small populations that are not at present endangered or vulnerable but are at risk are called rare. [52].

The diversity of medicinal plants is becoming endangered at alarming rate because of ruinous harvesting and overharvesting practices for the production of a large number of pharmaceutical products of interest, with little or no regard to the future. Also, extensive destruction plant rich habitat as a result of urbanization, forest degradation, agricultural encroachment etc is other factors, thus challenging their existence [4]. Some of these endangered medicinal plants are *Saussuorea lappa*, *Barleria prionitis*, *Aegel marmelos*, *Acorus calamus*, *Celestrus paniculatus*, *Chomifora mukul*, *Bacopa moneria*, *Glycerrhiza glabra*, *Picrorrhiza kurro*, *Rawalfia serpentine*, *Woodfordia fruticosa*.

Table 1: Some important medicinal plants of India [53].

Name	Common Name	Uses
Aegle marmelos	Beal tree	Diarrhea, dysentery, malaria, fever, jaundice.
Acorus calamus	Sweet flag, Bach	Anti-spasmodic, anti-helminthic properties also used for treatment of epilepsy, mental aliment, diarrhea, dysentery
Celestrus paniculatus	Malkangani	Memory booster, depression, paralysis.
Bacopa moneria	Brahmi	Mental function logativity, disease fatigue and depression, energise the CNS.
Glycerrhiza glabra	Brahmi	Ulcer, anti-spasmodic, asthma, cough.

**Table 2:- : In vitro cultured endangered and medicinally important medicinal plants of Indi [51].**

Name	Family	Explants
Aegle marmelos	Rutaceae	Nodal segments and
Commiphora mukul	Burseraceae	Leaf segments, apical and nodal segments
Stevia rebaudiana	Asteraceae	Apical and nodal segments
Bacopa moneria	Scrophulariaceae	Leaf explants and nodal segments
Ginkgo biloba	Ginkgoaceae	Apical and nodal segments
Oroxylum indicum	Bignoniaceae	Nodal segments
Tinospora cordifolia	Menispermaceae	Nodal segments

VI CONCLUSION

Medicinal plants are potential source of therapeutics aids play a significant role in health system all over the world for both humans and animals not only for diseased case but also for maintaining health. The over exploration and wide use of these life saving plants, causing long term negative impact on environment and availability. Efficient plant mass propagation is the main objective of plant tissue. Tissue culture technique is considered to be the best efficient technology for production of somaclonal and gametoclonal variants for large scale revegetation and for genetic manipulation studies. This biotechnological tool has a vast potential to produce plants of superior quality, high yielding with better disease resistance and enriched with bioactive ingredients of plants. During the past few decades a noticeable progress resulted in plant biotechnology has been witnessed with a flow of improved transformation regeneration protocols for many medicinal plants. In vitro techniques help to maintain the eco-balance of endangered, substituent and commonly used important medicinal plants.

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