



## An assessment of *In vitro* propagation of some important medicinal plants

Aditi Mishra<sup>1</sup>, Ayesha<sup>2</sup>, Mayank Singhal<sup>1</sup>, Arzoo Khan<sup>1</sup>, Ghazala Shaheen<sup>1</sup>, Naveen Gaurav<sup>3\*</sup>

<sup>1</sup> Research Scholars, Department of Biotechnology, Shri Guru Ram Rai University, Dehradun, Uttarakhand, India

<sup>2</sup> Research Scholars, Department of Biotechnology, Graphic Era Deemed To Be University, Dehradun, Uttarakhand, India

<sup>3</sup> Faculties, Department of Biotechnology, Shri Guru Ram Rai University, Dehradun, Uttarakhand, India

### Abstract

Liberation of diseases, is one of the oldest human practices and several traditional clinical procedures have been deputed using many plant species. In the recent years, several hurdles related to the search for new drugs from natural products have been overcome through the use of new technologies, from the performance of chemical pharmaceuticals to pharmacological studies including the integration of metabolomics and genomics approaches to develop traditional methods of studying. The plant uses for several therapeutic purpose followed by isolation of natural and medicinal products. This has allowed the development of herbal medicines with safety, efficacy and quality which stand out in the international pharmaceutical market.

**Keywords:** traditional, clinical, therapeutic purpose, pharmaceutical market, etc

### Introduction

Between 1981 and 2010, 34% of new medicines approved by US Food and Drugs Administration were based on molecules or direct derivatives from natural products, including statins, anticancer, antimicrobial and immunosuppressant [1]. Globally, the herbal medicines market trades around \$20 billion every year. The use of the plant for therapeutic purpose, whether in the treatment or obstruction of diseases, is one of the oldest human practices and several traditional clinical procedures have been registered using many plant species. In the last years, several hurdles related to the search for new drugs from natural products have been overcome through the use of new technologies, from the performance of chemical, pharmaceuticals to pharmacological studies, including the combination of metabolomics and genomics approaches to improve traditional methods of studying natural products [2]. This has allowed the development of herbal medicines with safety, efficacy and quality which stand out in the international pharmaceutical market [3, 4]. Between 1981 and 2010, 34% of new medicines approved by US Food and Drugs Administration were based on molecules or direct derivatives from natural products, including statins, anticancer antimicrobial and immunosuppressant [1]. Globally, the herbal medicines market trades around \$20 billion every year [5]. The plant's range includes Sri Lanka, Nepal, Bangladesh, India, Pakistan, Indonesia, Malesia and China as well as Panama where it is thought to have been introduced.

### Review

The term tissue culture also referred to as cell, *in vitro* or sterile culture is an important tool in both basic and applied studies. It's usually used in a very wide sense to include *in vitro* culture of plant cells, tissues as well as organs. generally used media for tissue culture derived its name from the work of Skoog and co-workers during 1950s and 19th owes its origin to the ideas of the German scientist and plant physiologist Haberlandt, at the beginning of the 20th

century [6]. Early as 1902, Haberlandt, was the first to envision the idea of growing plant cells in an artificial media develop to a tissue and for his contribution, this field of plant regeneration is regarded as "father of plant tissue culture.

Rapid advances in diverse aspect of plant culture have been made during the last few years and plant tissue culture techniques have been broadly applied to agriculture and industry. Plant tissue culture broadly refers to the *in vitro* cultivation of all plant parts, whether a single cell, tissue or an organ under aseptic conditions. The underline principle involve in plant tissue culture are very simple. Firstly, a plant part from the plant with an appropriate environment in which it can express its intrinsic or induced potential. This means that a suitable culture medium and proper culture condition must be provided. Finally, the above procedures must be carried out aseptically [7, 8].

Plant tissue culture involves the following of plant tissue from plant materials taken from a source plant. It has been found the plant can reproduce whole plant from fragments of plant materials when given a nutrient media (murshid and Skoog) capable of supporting growth and appropriate hormone control. The nutrient media used in plant tissue culture is an agar media with macro and micro nutrients dissolved in it. Unlike plants growing from a seed, tissue culture requires a supply of carbon in an organic form such as sugars. They also require amino acids, vitamins and growth hormones. The constituents of the media will vary with the plant material being cultured [9].

Plant tissue culture also used in research for geneticists, biochemist plant breeders and plant pathologists. Plant tissue culture has also proved more efficient in the production of secondary metabolites than the use of the parent plants in various instances and has been used in the commercial production of the naphthoquinone pigment shikonin. Plant tissue culture has also been used in the production of sweeteners, flavors, natural colorants and pharmaceuticals. With the advent of gene insertion, plant cells with gene material inserted can be regenerated using

tissue culture than other but most plants can be cultivated with time and practice <sup>[10]</sup>.

The term tissue culture was composed by American Pathologist Montrose Thomas Burrows. Tissue Culture is an important tool for the study of the biology of the cells from multicellular organisms. Plant tissue culture is a laboratory technique abundantly refers to the in- vitro cultivation of the plant whether a single cell, tissue or an artificial nutrient media under aseptic conditions. It is known as in- vitro culture or micro propagation. Micro propagation generally involves four distinct stages:

- a. Initiation of cultures
- b. Shoot multiplication
- c. Rooting of *in vitro* grown
- d. Acclimatization

It is based on the principle of cellular totipotency. The use of tissue culture technique was originated by German plant physiologist and in an attempt to cultivate Mengkultarkan hair cells of the mesophyll tissue of the leaf of monocot plant through plant tissue culture *in vitro*. He known as the father of tissue culture. Plant tissue culture involves the growing of tissue or a cell from plant materials taken from a source plant. It has been found that plant can reproduce whole plant fragments of plant material when give a nutrient media capable of supporting growth and appropriate hormone control. The nutrient media used in plant tissue is an agar media with macro and micro nutrients dissolved in it. Unlike plants growing from a seed, tissue cultures require a supply of carbon in an organic form such as sugars. They also require amino, vitamins and growth hormones or phytohormones. The constituents of the media will vary with the plant materials being cultured <sup>[11]</sup>.

Plant tissue culture has also proved more efficient in the secondary metabolites with the advent of gene insertion; plant cells with gene material inserted can be regenerated using tissue culture to produce a whole new plant. Plant tissue culture becomes a thrust area during the last few decades due to renewed emphasis it has received in all areas of crop improvement programmers <sup>[12, 13]</sup>.

Tissue culture is mostly used in obtaining disease free plants, rapid propagation of plants those are difficult to propagate, somatic hybridization, genetic improvement of commercial plants, obtaining haploid plants from pollen cultures or anther culture for breeding programs.

Fractionally new techniques for cultivation of plant cells under defined conditions were learned through demonstration of totipotency by R.J. Gautheret and P.R. White. Progressively novel techniques were soon available that lead to application of tissue culture to five broad areas namely,

1. Cell behaviour (including cytology, nutrition, metabolism, morphogenesis,
2. Pathogen-free plants and germplasm storage,
3. Clonal propagation, and
4. Product (mainly secondary metabolite) formation, starting in the mid-1960s. Currently the various applications of genetic engineering are implemented in medicinal plants to increase the production of secondary metabolites embryogenesis and pathology) <sup>[7]</sup>.

Over 100 years ago, Haberlandt imagine the concept of plant tissue culture and provided the ground work for the

cultivation of plant cells, tissues and organs in culture. Initially plant tissue cultures arose as a research tool and focused on attempts to culture and study the development of small, isolated cells and segments of plant tissues. At the peak of the plant tissue culture era in the 1980s, in a relatively short time, many commercial laboratories were established around the world to capitalize on the potential of micropropagation for mass production of clonal plants for the horticulture industry.

Today plant tissue culture applications encompass much more than clonal propagation. The range of routine technologies has expanded to include somatic embryogenesis, somatic hybridization, virus elimination as well as the application of bioreactors to mass propagation. Perhaps the greatest value of these tissue culture technologies lies not so much in their application to mass clonal propagation but rather in their role underpinning developments and applications in plant improvement, molecular biology and bio processing, as well as being a basic research tool. Plant tissue culture technique though an underutilized tool in Nigeria, it can be extensively applied in horticulture to increase crop production. This paper highlights some of the applications of plant tissue culture to horticulture, the achievements and limitations of tissue culture and some insights into current and possible future developments. With rapid population growth, the total acreages of fruits, vegetable and various ornamental plants have not been able to meet the needs of people in the developing countries <sup>[14]</sup>.

A mass *in vitro* propagation system devoid of growth regulators has been developed. MS medium supplemented with an antibiotic (trimethoprim) or a fungicide (Bavistin) supports direct shoot bud induction on inter-node and leaf explants. Bavistin showed a marked cytokinin like activity thus optimum adventitious shoot buds induction occurred on MS medium supplemented with 1569.14µM Bavistin. MS medium supplemented with 0.44µM BAP and 1.14µM IAA (indole-3- acetic acid) found most suitable for shoot elongation. *In vitro* derived shoots exhibited better rooting response on MS medium containing 4.9µM IBA. Regenerated rooted plantlets of *Buddleja davidii franch* were successfully acclimatized in soil rite.

Shashikant Behera *et al*, (May-June, 2015) did work on an efficient plant regeneration protocol through two-stage culture of nodal segment has been reported here for a valuable medicinal plant, *Buddleja davidii Franch*. Multiple shoots with large number of shoot buds were induced from nodal explant on Murashige and Skoog's (1962) (MS) medium fortified with (1.0-5.0 mg L<sup>-1</sup>) N6 Pennell through two-stage culture of nodal segments and *ex vitro* acclimatization <sup>[15]</sup>.

#### Surface Sterilization

Surface sterilization is most important step before inoculation of explant. Different steps have been employed for treatment of ex-plant. Scientists, have described sterilization treatment of *B. davidii*, which includes use of 0.1% Mercuric chloride (w/v) for 2 mins followed by rinsing thoroughly with sterile distilled water. Different sterilization treatment was followed by Mathur and Kumar, 1998, in which leaves and stem explants were shaken for 10 minutes in Tween-20 (Ranbaxy) and Savlon (Johnson & Johnson) in water for 10 minutes, rinsed in running water for 30 minutes, treated with 0.1% Mercuric chloride for 3-4 minutes and washed several times with sterile water <sup>[16]</sup>.

Tiwari *et al.*, (2000), suggested that for micro-propagation of *Centella asiatica*, plants were washed thoroughly for 30 minutes under running tap water followed by removal of leaves, which was followed by soaking in the mixture of 1% cetrimide solution containing 150 mg/l Bavistin (fungicide) and 50mg/l Trimethoprim for 25-30 minutes. The explants were finally treated with 0.1% mercuric chloride for 3-4 minutes followed by rinsing in sterile distilled water for 4-5 times [17].

Rani *et al.*, (2003) reported that callus induction in *Withania somnifera* (L.) Dunal was observed from hypocotyl, root, and cotyledonary leaf segments, grown on Murashige and Skoog (MS) medium supplemented with various concentrations and combinations of 2, 4-dichlorophenoxyacetic acid (2, 4-D) and kinetin (Kn). Maximum callusing (100%) was obtained from root and cotyledonary leaf segments grown on MS medium supplemented with a combination of 2 mg/l 2, 4-D and 0.2 mg/l Kn. When hypocotyl segments were used as explants, callus induction was noticed in 91% of cultures, which showed shoot regeneration on MS medium supplemented with 2 mg/l 2, 4-D and 0.2 mg/l Kn (18).

Abdelmageed *et al.*, (2012) did work on callus of *M. champaca* could be induced on MS media supplemented with 0.0 - 2.0 mg/l IAA, 0.0 - 2.0 mg/l 2, 4-D, and 0.0 - 2.0 mg/l BAP, respectively. All growth regulators that were used in this study are capable of inducing callus from explants. 0.10 mg/l IAA and 0.10 mg/l 2, 4-D were found to be the suitable concentrations for the induction of callus. The survival rate of explants in the media supplemented with IAA was found to be 81%. MS Media supplemented with different concentrations of BAP combined with IAA and 2, 4-D were tested for callus proliferation. The callus growth showed good response to all growth regulators combinations tested in this study, except the combination BAP/IAA with 0.2/0.10 mg/l which exhibited low response with dark brown callus [19].

Dharishini, *et al.*, (2014) did work on callus and shoot induction was observed from leaf, node and internode explants of *Buddleja davidii* Franch when grown in MS media amended with varying concentrations of auxin and cytokinin. Concentration at 0.2 mg and 0.4 mg of 2, 4-D showed 20 and 33% of callus formation from leaf ex-plant. *In vitro* callus was induced from nodal explants in media amended with naphthyl acetic acid (NAA) (0.2 mg) and 6-benzyl amino purine (BAP) (0.1 mg) wherein the growth response was 33.3%. A 50% callus induction was observed in BAP (0.1 mg) and Kinetin (KIN) (0.2 mg). Scientists did work on Leaves as well as Cotyledonary excised small explants of Ashwagandha were responsible and introduced to evaluate the effect of various growth regulators upon the *in vitro* micropropagation processes [20].

Tiwari *et al.*, (1998) work on Calli derived from nodal explants cultured on MS medium containing 0.5 mg/l 2,4-dichlorophenoxyacetic acid (2, 4-D), when sub-cultured on MS medium containing 0.1 or 0.5 mg/l BAP or 0.2 mg/l 2,4-D + 0.1 or 0.5 mg/l kinetin, developed somatic embryos. The somatic embryos germinated either on the same media or on MS basal medium, and the resulting plantlets were successfully transplanted to soil (16, 21). Explants were applied to generate callus, shoot and root regeneration. In the first experiment small segments of leaf were cultured on MS basal medium added with mixture of 2, 4 - Dichlorophenoxyacetic acid (2, 4-D), naphthalene acetic

acid (NAA) and indole butyric acid (IBA). The agile pharmacological components of *Withania somnifera* components are withanolides a Steroidal lactones ring with ergostane skeleton framework and alkaloids [22, 20]. The active contents or substances of Indian *Withania somnifera* or Ashwagandha are normally withaferin-A and withanolide-D, both are generally present in leaves, stems and roots of the plant in high quantity, are used as a source of drugs. This procedure was standardized and established for very easy large scale mass propagation of ashwagandha medicinal plant. Callus formation was seen best in Murashige and Skoog media supplemented with NAA (1.0-3.0mg/L) after 16-20 days [23].

Sharma, S., (2003) did work on *Michelia champaca* and reported that callus was induced from axillary bud explants cultured onto Murashige and Skoog (MS) basal medium supplemented with different concentrations of auxins (IAA and 2,4-D) and cytokinin (BAP) as plant growth regulators (24). It was observed that callus of *M. champaca* could be induced on MS media supplemented with 0.0 - 2.0 mg/l IAA, 0.0 - 2.0 mg/l 2, 4-D, and 0.0 - 2.0 mg/l BAP, respectively. All growth regulators that were used in this study are capable of inducing callus from explants. 0.10 mg/l IAA and 0.10 mg/l 2, 4-D were found to be the suitable concentrations for the induction of callus. The survival rate of explants in the media supplemented with IAA was found to be 81%. MS Media supplemented with different concentrations of BAP combined with IAA and 2, 4-D were tested for callus proliferation. The callus growth showed good response to all growth regulators combinations tested in this study, except the combination BAP/IAA with 0.2/0.10 mg/l which exhibited low response with dark brown callus. The combination 2.00 mg/l BAP with 0.50 mg/l IAA and all the combinations of BAP with 2, 4-D showed the highest rate of callus proliferation with cream colour. This study suggests that auxins along with cytokinin have a critical role on the callus and shoot proliferation of this plant [19].

Minal *et al.*, (2011) reported the objective of this study was to optimize the concentration of different plant growth regulators or hormones for callus induction of cotton (*Gossypium hirsutum* L.). Different types and concentrations of growth regulators were tested in order to obtain the best callus formation [25]. Four growth hormones such as 1-naphthalene acetic acid (NAP), 6-Benzyl aminopurine (BAP), Kinetin and 2, 4 - dichloro phenoxy acetic acid (2, 4 - D) were used in this study. It was found that growth regulator type and concentration had as significant effect on the callus induction, the increment of callus index and callus physical appearance. The higher frequency of callus growth (95-100%) were observed on both epicotyls and cotyledon explants cultured on basal medium supplemented with 0.1 mg/l NAA + 0.5 mg/l Bap and various concentrations such as 0.2 + 0.1, 0.5 + 0.1, 1.0 + 0.2 mg/l of NAA + BAP also shows good callus response but at higher concentration of the same hormones shuts the callus growth [5]. The concentration of BAP and 2, 4- D also shows good callus response in higher concentration whereas low concentrations of these hormone combination show nil effect. The morphology of callus differs upon the hormonal concentration from green to white and green to brown with various textures. This protocol paves the way for the development of *in vitro* regeneration for cotton and

consequently will promote the application of plant tissue culture.

### Discussion

Medicinal plants are potential renewable natural resources and are generally considered to play a beneficial role in human health care. The medicinal value of these plants contains phytochemicals such as alkaloids tannins, saponins, flavonoids, and phenolic compounds. The most important is vasicine, a quinazoline alkaloids. In India, north part is under strategic geographical location and possesses an invaluable treasure of medicinal plants holding a major share in cultivation and export. *Adhatoda vasica* (Kala Basa), Giloe, Ashwagandha, Tulsi, Turmeric etc., are the most important medicinal plants belong to India having high activity as immune-modulator. Micropropagation techniques offer new avenues for the improvement of this important medicinal plant.

### Acknowledgment

We would like to express our scincere gratitude to Department of Biotechnology, Shri Guru Ram Rai University, Dehradun, Uttarakhand, India for their guidance and constant inspiration and valuable suggestion during our article.

### References

- Mishra BB, Tiwari VK. Natural products: an evolving role in future drug discovery. *European journal of medicinal chemistry*,2011;46(10):4769-4807.
- Harvey A, Edrada-Ebel R, Quinn RJ. The re-emergence of natural products for drug discovery in the genomics era. *Nature Reviews Drug Discovery*, 14, 111-129. ISSN 1474-1784, <http://dx.doi.org/10.1038/nrd4510>.
- Kumar V, Kumar V. An overview of herbal medicine. *Int J Ph Sci*,2009;1(1):1-20.
- Newman DJ, Cragg GM, Snader KM. Natural products as sources of new drugs over the period 1981–2002. *Journal of natural products*,2003;66(7):1022-1037.
- Gaurav N, Komal PJ, Tyagi M, Chauhan N, Kumar A. A review on *in vitro* propagation of medicinal plants. *Journal of Pharmacognosy and Phytochemistry*,2018;7(6):2228-2231.
- Maheshwari SC. Tissue Culture, Molecular Biology and Plant Biotechnology—A Historical Overview. In *The Impact of Biotechnology on Agriculture* Springer, Dordrecht, 1990, 1-12.
- Idowu PE, Ibitoye DO, Ademoyegun OT. Tissue culture as a plant production technique for horticultural crops. *African Journal of Biotechnology*, 2009, 8(16).
- Trigiano RN, Gray DJ. (Eds.). *Plant tissue culture concepts and laboratory exercises*. CRC press, 1999.
- Gamborg OL, Murashige T, Thorpe TA, Vasil IK. Plant tissue culture media. *In vitro*,1976;12(7):473-478.
- Chawla HS. *A Handbook of Introduction to Plant Biotechnology*, 2002.
- Mederos-Molina S. *In vitro* callus induction and plants from stem and petiole explants of *Salvia canariensis* L. *Plant tissue cult*,2004;14(2):167-172.
- Srivastava P, Singh RM. Optimization of cropping pattern in a canal command area using fuzzy programming approach. *Water Resources Management*,2015;29(12):4481-4500.
- Nidumolu UB, Van Keulen H, Lubbers M, Mapfumo A. Combining interactive multiple goal linear programming with an inter-stakeholder communication matrix to generate land use options. *Environmental Modelling & Software*,2007;22(1):73-83.
- Singh J, Tiwari KN. High-frequency *in vitro* multiplication system for commercial propagation of pharmaceutically important *Clitoria ternatea* L.—a valuable medicinal plant. *Industrial Crops and Products*,2010;32(3):534-538.
- Behera S, Nayak N, Shasmita BD, Naik SK. An efficient micropropagation protocol of *Bacopa monnieri* (L.) Pennell through two-stage culture of nodal segments and ex vitro acclimatization. *J Appl Biol Biotechnol*,2015;3(3):16-21.
- Gaurav N, Komal PJ, Tyagi M, Chauhan N, Kumar A. A review on *in vitro* propagation of medicinal plants. *Journal of Pharmacognosy and Phytochemistry*,2018;7(6):2228-2231.
- Sharma N. *Micropropagation of Bacopa monnieri L. Penn.-an important medicinal plant* (Doctoral dissertation, M. Phil. Thesis. Thapar Institute of Engineering and Technology Patiala, India), 2005.
- Rani G, Virk GS, Nagpal A. Callus induction and plantlet regeneration in *Withania somnifera* (L.) Dunal. *In vitro Cellular & Developmental Biology-Plant*,2003;39(5):468-474.
- Abdelmageed AHA, Faridah QZ, Julia AA. Callus induction and plant regeneration of *Michelia champaca* (Magnoliaceae): A multipurpose tree. *Journal of Medicinal Plants Research*,2012;6(17):3338-3344.
- Gaurav MN, Kumar A, Juyal MP, Kumar MD, Chauhan UK, Singh AP *et al.* *In vitro* callusing and effect of growth regulators on *in vitro* propagated withania (cultivated & wild) through cotyledonary leaves. *IN VITRO*, 2015, 1(5).
- Tiwari V, Singh BD, Tiwari KN. Shoot regeneration and somatic embryogenesis from different explants of Brahmi [*Bacopa monniera* (L.) Wettst.]. *Plant Cell Reports*, 17(6), 1998, 538-543.
- Elsakka M, Grigorescu E, Stănescu U, Dorneanu V. New data referring to chemistry of *Withania somnifera* species. *Revista medico-chirurgicala a Societatii de Medici si Naturalisti din Iasi*,1990;94(2):385-387.
- Maitha MJ. *Determination of withaferin a content of root, stem and leaf extracts of withania somnifera (l) dunal collected in Kenya* (Doctoral dissertation), 2014.
- Yee TF, Rao AN. A Brief Perspective Of Plant Tissue Culture Research and Recent Studies On Woody Medicinal Plant Species. *Journal of Tropical Medicinal Plants*, 2012, 13(1).
- Vaibhav B, Dipika S, Deelip D, Minal N, Jitendra N. Stavudine sintered matrix tablet: formulation and evaluation. *Research Journal of Pharmacy and Technology*,2011;4(9):1455-1460.