Journal of Pharmacognosy and Phytochemistry

Available online at www.phytojournal.com

E-ISSN: 2278-4136
P-ISSN: 2349-8234
JPP 2018; 7(6): 2228-2231
Received: 19-09-2018
Accepted: 21-10-2018

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A review on *in vitro* propagation of medicinal plants

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Abstract
Justicia adhatoda, usually known as malabar nut, Adulsa, Adhatoda, Vasa, Vasaka. In tribal name of *Justicia adhatoda* known as KALA BASA (Encyclopedia, 2013). It is a medicinal plant native to Asia widely used in ayurveda, homeopathy, unani and siddha medicines. Curing of diseases through medicinal plants is one of the oldest human practices and several traditional clinical procedures have been deputed using many plant species. From the last few decades, several hurdles related to the research for novel drugs from natural products have been overcome through the use of new technologies, from pharmaceutical approaches, including the integration of meta-bolomics and genomics approaches to develop traditional methods of studying. The use of the plant for therapeutic purpose, whether in the treatment or natural nutritional products. This has allowed the development of herbal medicines with safety, efficacy and quality which stand out in the international pharmaceutical market. In the mid of 1981 and 2010, 34% of novel herbal drugs and medicines approved by US Food and Drugs Administration were based on molecules or direct derivatives from natural products, including statins, anticancer, antimicrobial and immunosuppressant. Globally, the herbal medicines market trades spreading around $20 billion every year. The plant's range includes Sri-Lanka, Nepal, Bangladesh, India, Pakistan, Indonesia, Malaysia and China as well as Panama where it is thought to have been introduced.

Keywords: Propagation, medicinal plants

Introduction
The term tissue culture also referred to as cell grown *in vitro* or sterile culture media, has great significance in both basic and applied studies. Primarily it is widely used in a wide sense to include *in vitro* culture of plant cells, tissues as well as organs. Generally MS media is used for tissue culture derived its name from the researcher Murashige and Skoog and their co-workers (1962) (Gaurav, *et al.,* 2015) [6]. In 1902, Haberlandt, was the first to invented cell culture and for the idea of growing plant cells in an artificial media he is now famous as "father of plant tissue culture". Rapid advances in diverse aspect of plant tissue culture have been made during the last few years and plant tissue culture techniques have been broadly used in 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 and optimal conditions (Ahmad, 2013) [2].

The underline principle involve in plant tissue culture in which a plant part from the mother plant with an appropriate environment in which it can express its intrinsic or induced potential, must be carried out aseptically. It has been found the plant can reproduce whole plant from fragments of plant materials when given a MS media capable of supporting growth and appropriate hormone control. This nutrient MS media used in plant tissue culture is an agar media with macro and micro nutrients mixed in it. Unlike those plants which is growing from a seed, tissue culture require a supply of carbon in an organic form such as sugars. They also require amino acids, vitamins and growth hormones. The composition of the media will vary with the plant material being cultured and some plants also successfully propagated *in vitro* in B5 medium *(*Gaurav, *et al.,* 2018) [8].

Plant tissue culture also used in research for geneticist, biochemist plant breeders and plant pathologists and other researchers. Plant tissue culture has also proved more efficient in the production of secondary metabolites and has been used in the commercial production of the napthoquinones, pigments and shikonin. Plant tissue culture has also played a significant role in the production of sweeteners, flavours, natural colorants and pharmaceuticals (Gaurav, *et al*., 2016) [7].

The term tissue culture was compose by American Pathologist Montrose Thomas Burrows. Tissue Culture is an important and very effective 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 micropropagation which generally involves five distinct stages:

a) Selection of mother plant
b) Initiation of cultures
c) Shoot multiplication
d) Rooting of *in vitro* grown
e) Acclimatization

It is based on the principle of tot potency of the cell. The use of tissue culture technique was originated by German plant physiologist in an attempt to cultivated Mengkultarkan hair cells of the mesophyll tissue of the leaf of monocot 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 (Shrivastava and Rajani, 1999*)* [15].

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 programmes.

Techniques for cultivation of plant cells under defined conditions were learned through demonstration of tot potency by R.J. Gautheret and P.R. White. Progressively novel techniques were soon available that leads to the application of tissue culture to five broad areas, namely:-

1. Cell behavior (including cytology, nutrition, metabolism, morphogenesis
2. Pathogen-free plants and germplasm storage
3. Clonal propagation
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)
5. Plant modification and improvement,".60s.

Akin-Idowu, P. E, Ibitoye, D. O and Ademoyegun, OT (2009) [3]. Over century 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 method for mass production of clonal plants for the horticulture industry.

Today plant tissue culture applications encompass much more in 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 of plants. 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, vegetables and various ornamental plants have not been able to meet the needs of people in the developing countries

K Tiwari, *et al.,* (2000; 2010) [16, 17] 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 14µM bavistin. MS medium supplemented with 0.44µM BAP and 1.14µM IAA (indole-3- acitic 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.

Shashikanta Behera, *et al., (*2015) did work on an efficient plant regeneration protocol through two-stage culture process of nodal segment has been reported for a significant medicinal plant, *Buddleja davidii Franch*. Multiple shoots with large number of shoot buds were induced from nodal explant on Murashige and Skoog's medium fortified with (1.0-5.0 mg L-1) N6 Pennell through two-stage culture of nodal segments and *ex vitro* acclimatization. Surface sterilization is most important step before inoculation of explant. Different steps have been employed for treatment of ex-plant for sterilization. Scientists, have described sterilization treatment *of B. davidii*, which includes use of 0.1% Mercuric chloride (w/v) for 2 min followed by rinsing thoroughly with 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 distilled water.

Tiwari *et al. (*2000) [16], suggested that for micropropagation 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.

Tiwari, *et al.,* (1998) [16] did work on callus 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 MS basal medium, and the resulting plantlets were successfully transplanted to soil.

Rani, *et al., (*2003) [13] reported that callus induction in *Withania somnifera* (L.) Dunal was observed from hypocotyl, root, and Cotyledonary leaf segments, grown on Murashige and Skoog 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/_*M*) 2,4-D and 0.2 mg/l*M*) 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.

A.H. A. Abdelmageed, *et al*., (2012) [1] 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 positive response to all growth regulators combinations tested in this study, except the combination BAP/IAA with 0.2/0.10 mg/I which exhibited low response with dark brown callus.

M. Priya Dharishini1, *et al.,* (2014) did work on Callus and shoot induction was observed from leaf, node and internode explants of *Buddlejadavidii 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 napthyl 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).

Gaurav, *et al.,* (2016) [7] did work on Withania and proved that *in vitro* shoot differentiation and micropropagation of *Withania somnifera* from various different small excised explants such as hypocotyl and cotyledonary leaf, shoot tip, node, internode. Optimal normal growth, multiplication and development of *Withania somnifera* through morphogenesis *in vitro* processes of many tissues may vary for several different plants based on important nutritional requirements of plants. Basic nutrient basal media that are most frequently applicable and used include MS (Murashige and Skoog) medium, Gamborg (B5) medium, Linsmaier and Skoog (LS) medium and Nitsch and Nitsch (NN) medium. The traditional or conventional and old micro-propagation technique cannot be applicable in increasing and maintaining of demand of this herb which is used as very important raw substance for the preparation of therapeutic and pharmaceutical products in medicinal industries because of these reason the application and cultivation of *in vitro* micro-propagation methods of ashwagandha plants can be the another most effective method for the regular and continuous possibility of maintaining demand and supply or distribution of several plantlet which is in stock of *Withania* for large scale field mass cultivation i.e. mass propagation *in vitro* culture room.

Mitra, *et al., (*2011) [10] 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. Four growth hormones such as 1-naphthalene acetic acid NAP), 6-Benzylaminopurine (BAP), Kinetin and 2, 4 - dichlorophenoxyacetic acid (2, 4 – D) were used in this study. It was found that growth regulator type and concentration had a 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. The concentration of BAP and 2, 4- D also shows good callus response in higher concentration whereas low concentrations of these hormone combination shows 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 as a secondary metabolites. The most important is vasicine, a quinazoline alkaloids are produced through PTC methods. 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.

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