



**A REVIEW ON TISSUE CULTURE OF AN IMPORTANT MEDICINAL HERB OF
FABACEAE- *MUCUNA PRURIENS* L**

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ABSTRACT

The current article presents a review on different aspects of cell and tissue culture of an important medicinal herb, *Mucuna pruriens* L. of Fabaceae. This plant contains different active compounds of which L-dopa, a neurotransmitter precursor is the most valuable one. L-dopa is used as an effective principle for symptomatic relief of Parkinson's disease and other mental disorders. The wild population of this plant is decreasing at an alarming rate for indiscriminate exploitation by pharmaceutical companies and local people. The alternative approaches are necessary for maintaining the elite populations of this plant species. In the present review, different approaches of regeneration including axillary bud culture and callus regeneration of both stable and variable plants through tissue culture have been discussed and compared with several studies. The principles and involvement of different factors including the role of different plant growth regulators on different regeneration methods have been highlighted.

Keywords: Axillary bud culture; Callus culture; *Mucuna pruriens* L.; Organogenesis; Somatic embryogenesis

INTRODUCTION

Mucuna, a genus of annual and perennial twining herbs or shrubs belonging to Fabaceae contains about 15 species in India [1]. *Mucuna pruriens* L., commonly known as velvet bean, is an

important medicinal plant growing in the bushes, hedges and dry deciduous forests throughout India [2, 3, 4]. The plant shows vigorous growth and has characteristic compound trifoliolate leaves. The leaflets are

broadly ovate and the terminal leaflet is symmetrical and smaller than the lateral ones, which are conspicuously asymmetrical at bases [5]. Purple flowers are borne in axillary racemes. Pods are oblong, more or less S-shaped. The two varieties of *M. pruriens* (vars *pruriens* and *utilis*) are available in India. *M. pruriens* var *pruriens* is found in wild whereas *M. pruriens* var *utilis* is a cultivated variety. These two varieties differ in seed and pod characteristics. *M. pruriens* L. (DC.) var *pruriens* possesses seeds which are black with brown spots and ovoid in shape. The mature pods contain reddish-brown irritating hairs that cause severe itching on contact with skin (pruritis) due to presence of mucunain. The seeds of *M. pruriens* var *utilis* Wall. ex Wight are oblong-ellipsoid and glossy with variable seed coat colour and sizes. The pods are covered with velvety non-irritant hairs [6]. In general, mature seeds of *M. pruriens* possess raised white hilum half the length of the seeds. The cytological study has not been done extensively so far. However, this plant species has been found to be diploid with $2n=22$ chromosomes and both the strains, *M. pruriens* var. *pruriens* and *M. pruriens* var. *utilis* are diploids with same chromosome number [7]. 2C nuclear DNA values revealed a moderate genome size [7].

As all parts of this plant possess valuable medicinal properties [8], there is an ever-increasing demand of *Mucuna* in the international drug market. The roots are bitter-sweet, thermogenic emollient, stimulant, purgative, aphrodisiac, diuretic, febrifuge and tonic. It is also considered useful to relieve constipation, strangury, dysmenorrhoea, amenorrhoea, elephantiasis, dropsy, neuropathy, ulcers, helminthiasis, fever and delirium. The leaves are aphrodisiac and are useful in ulcers, inflammation, cephalalgia and general debility. The seeds are astringent, laxative, antihelminthic, alexipharmic and tonic. The seed coat contains a number of bioactive substances such as tryptamine, alkylamines, steroids, flavonoids, coumarins and cardenolides [9, 10].

Mucuna extracts are used in the treatment of several diseases like cholera, diabetes, diarrhoea, cancer, tuberculosis and many other ailments, and also as a cure for snakebites and scorpion sting. This plant synthesizes a medicinally important compound, L-dopa, a neurotransmitter precursor, which is used as an effective principle for symptomatic relief of Parkinson's disease and other mental disorders [1, 5, 11]. Brain (1976) [12] first reported its synthesis and accumulation in cultures of *Mucuna pruriens*. The need for L-dopa is largely met by extracting the

compound from wild populations of this plant. In addition, this plant contains glutathione, lecithin, gallic acid, glucosides and a number of alkaloids including nicotine, prurieninine, prurienidine and others [5].

Mucuna has several other uses. In many parts of the world it is used as an important forage, fallow and green manure crop. Since the plant belongs to the legume family, with the help of nitrogen fixing bacteria it takes nitrogen from the air and combines it with other chemical compounds producing fertilizer and thereby improving the soil fertility/quality. Large scale, unrestricted exploitation of the plant coupled with limited cultivation has led to sharp depletion of the wild stock of this valuable medicinal plant. The plant usually propagates through seeds, but the germination rate and viability of seeds are very poor. *In vitro* plant culture technique including organ culture and callus culture may be an effective alternative for propagation and conservation of plants of such an economic importance in which conventional methods show limitations.

Micropropagation through organ culture is an alternative to the conventional method of propagation. It is suitable for rapid mass propagation of the desired plant. Moreover, the genetic uniformity of the regenerates is maintained utilizing shoot

bud multiplication. There are reports of direct *in vitro* regeneration through shoot bud multiplication [13-16]. This plant is self-pollinated where genetic variation is rare. Plant tissue culture has also contributed to produce genetically variable plants through callus tissue regeneration. Therefore, callus regeneration would be very desirable to induce variability and would produce somaclonal variable clones. *In vitro* originated variability along with the naturally pre-existing variation among the wild varieties would lead to form a large pool of variation, and by providing suitable selection pressure on such pool, production of elite clone of this plant may be possible. Micropropagation through organ culture and regeneration from callus tissue have also proved to be a viable alternative for raising variable clones in addition to parental genotype with the objective of enhancing the rate of multiplication [9, 10, 16-19].

Axillary bud multiplication:

Micropropagation technique (shoot tip and meristem culture) is mainly used for clonal propagation of selected and pathogen eradicated (virus free) stock plants. It's an important technique for clonal propagation of horticultural and floricultural crops.

The main advantage of micropropagation is the extremely high

multiplication rates. A small piece of plant part can produce hundreds of new plants that can be identical to the mother plant, the production of which is impossible with conventional technique. During micropropagation, fungi and bacteria are usually eliminated so that the plants obtained are clean, while conventional methods propagate the disease as well. Plants can be maintained *in vitro* in a pathogen-free state. Such plants are easy to export since there is no quarantine problem, and their packaging is easier due to their smaller size. Micropropagation can be carried out throughout the year independent of seasons. Micropropagation also gives the plants a healthier start and a better chance of defense.

A micropropagation system must produce large number of uniform plants that are genotypically and phenotypically similar as the original plant from which they are produced. To satisfy this criterion, the most appropriate technique is the apical or axillary bud multiplication. A large number of genetically uniform and stable plants can be produced under sterile condition with minimum stress imposed on the growing plants. Environmental and nutritional conditions can be easily manipulated and strictly controlled. In most cases, a medium containing either cytokinin alone or high cytokinin with low

auxin favours multiplication of shoot bud [16].

The application of shoot-apex cultures for multiplication of plants was first described by Morel (1960) [20] in his studies on propagation of orchids. Conventional propagation methods and basal medium without any growth regulators have very low propagation efficiency [21, 22] and the vegetative propagation by fragmentation is possible on a small scale but practically impossible on a commercial basis [23]. Modified MS medium is more suitable for shoot-root induction and addition of ancymidol may contribute to the formation of shoot bud cluster by disrupting the apical dominance [24]. Sucrose concentration in culture medium also influences the formation of shoots and roots in many plants [25]. There are few reports of direct plant regeneration through shoot bud multiplication in *Mucuna pruriens* [13-16, 26].

The responses from shoot bud multiplication were analyzed using both cytokinin and auxin in combination and cytokinin alone. The presence of α -naphthaleneacetic acid (NAA) might have an adverse effect on shoot bud multiplication in presence of all the three cytokinins studied [16]. There are reports where NAA in association with cytokinins

like 2-isopentyl adenine (2iP), kinetin, 6-benzylaminopurine (BAP), did not induce shoot bud multiplication [16]. In other reports, NAA combined with BAP and 2iP showed higher efficiency on shoot bud multiplication [13-15]. It is generally noticed that auxin at very low concentration along with a high cytokinin level induces shoot bud multiplication [17, 27, 28]. However, the negative effect of auxin-cytokinin combination on shoot bud multiplication of *Mucuna pruriens* might indicate a high endogenous level of auxin in the strains studied [29]. This may be supported by the lavish and luxuriant growth of the field-grown plants of these strains [16].

Due to the unfavourable effect of exogenous NAA, all the three cytokinins alone were used for shoot bud multiplication [16]. In some cases, 2iP alone did not support the process. Among the other two cytokinins, both BAP and kinetin showed different multiplication rates at different concentrations. Moreover, the responses in presence of BAP were more favourable than those of kinetin [16]. The efficiency of BAP in shoot bud multiplication has also been reported in this species [26] as well as other plant species [17, 18, 30]. The genotypic differences between the strains of the same species might have been responsible for

difference in responses using similar concentrations of a particular cytokinin. Moreover, the requirement of different levels of cytokinins might be correlated with the difference in the endogenous level of different genotypes [17].

Callus induction:

Different combinations of both auxin and cytokinin were used to induce callus tissue from nodal and internodal explants of *Mucuna pruriens* L. The morphology of the callus tissue differed and the growth rate also varied in the media containing various levels of auxin and cytokinin. Except 2,4-dichlorophenoxyacetic acid (2,4-D) and low-BAP combination, the other cytokinin-auxin combinations produced green callus tissue [31]. On the other hand, in the medium containing two auxins (2,4-D and NAA) and low cytokinin (BAP), creamish-white, nodular, embryogenic and highly proliferating callus was obtained. All these findings indicate that the nature of callus induced is solely dependent on the type of exogenous growth regulator [9, 31]. There are reports on production of active compounds from suspension culture of this species [11, 32, 33]. These reports have shown efficient continuous synthesis of such compound.

Plant regeneration from callus culture:

In plant cell culture, regeneration of a complete plant is the main objective. Complete regeneration of plants from isolated protoplasts or cell or tissue or organ clearly demonstrates the unique property of a plant cell – the cellular totipotency. The somatic tissue of a plant originates through the process of mitosis and each cell possesses the built-in potential for complete plant regeneration. The capacity of cells to regenerate via different morphogenic programmes is a result of cell dedifferentiating to become competent to the stimulus. This is then followed by induction for the developmental programme and eventual development into the organ [34]. Regeneration methods frequently depend on the type of tissue used to initiate cultures, with the generation or acquisition of starting material potentially becoming a limiting factor [35].

Depending on the composition of the callus culture medium, plant regeneration in culture can occur by either of the two possible routes, i.e. organogenesis and somatic embryogenesis. The methods may be useful in studies on somaclonal variation, cell selection for desirable traits, suspension cultures, genetic transformation, and protoplast isolation. The process of morphogenesis, *sensu lato*, involves shoot bud differentiation from

cultured explant and regeneration of complete plants by rooting the regenerated shoots. Plant regeneration through morphogenesis may occur by one of the three following modes: (i) Adventitious organ formation from explant derived callus. (ii) Emergence of adventitious organs without an intermediate callus phase and (iii) Production of plants from outgrowth of axillary buds. Root meristem initiation is also a type of organogenesis most frequently found in cultured cells. There should be a clear distinction between the rooting of the shoots under *in vitro* condition and rhizogenesis from meristemoids within callus tissue.

Skoog (1944) [36] suggested that organogenesis can be regulated by exogenous growth regulator concentration [37, 38]. According to him, root initiation is stimulated by exogenous auxin, whereas a cytokinin like adenine sulphate promotes shoot differentiation. The systematic study of Skoog and his associates led to the hypothesis that morphogenesis is regulated by the ratio of auxin and cytokinin. A medium containing only cytokinin may induce organogenesis, though in some cases a higher cytokinin level along with an auxin at a very low concentration has been effective. Plant regeneration through organogenesis will lead to the formation of

both stable and variable genotypes, if callus tissue contains cells of both types.

Somatic embryogenesis, on the other hand, refers to a process of embryo development from somatic cells following the pathway similar to zygotic embryo development. Unlike cells of other eukaryotes, almost all plant cells have the capacity to become embryogenic under defined culture conditions. The process of embryo development is characterized by a series of morphological changes leading to its maturation. Only a limited number of cells will form somatic embryos at one time and this fraction is highly variable among different plant species. It depends partly on the genotype of the plant and the source of the explant [19, 39, 40].

Morphogenesis, *in vitro*, is a complex developmental process by which plants are regenerated either by somatic embryogenesis or by organogenesis. Physical, physiological, cytological and chemical factors have been found to control morphogenesis. The use of appropriate plant growth regulator at a definite concentration and optimum culture conditions are important for successful plant regeneration in culture. The role of phytohormones is well established in both organogenesis and embryogenesis processes [41] and in some species the differentiation process is governed by

quantitative interactions between different hormones/growth regulators [23].

It has been found that the differentiation of organs derived from unorganized mass of cells has different biochemical requisition and metabolisms [42]. Both differentiation and morphogenesis are accompanied by the formation of organ-specific proteins and enzymes. The intermediate regulatory control of protein synthesis is sought at the gene level and is believed to be mediated by messengers.

The formation of meristemoids leading to differentiation of shoot meristems in callus tissue was observed at variable rates in different strains of *Mucuna pruriens*. There are few hypotheses regarding origin of growth centers. Torrey (1966) [43] suggested that all the organized structures developed from the callus tissue have a common origin in “activated” single cells, called the meristemoids. The meristemoids reported in callus tissues in specific nutrient media can be regarded as growth centers [9]. The similar structures in callus culture of *Rauwolfia serpentina* were observed that gave rise to shoot buds [38, 44]. Halperin (1966) [45] observed three modes of plant origin from callus culture. The first one showed shoot bud regeneration followed by adventitious root formation [36] or regeneration of root is

followed by the formation of shoot buds [46]. The second pathway involves independent origin of root and shoot buds in callus culture followed by their integration into one axis [47] and the third one is plant formation through embryogenesis. In different strains of *Mucuna pruriens*, plant regeneration was observed following the first method, where shoot buds originated from the callus tissue grown in medium containing only either BAP or kinetin and roots were induced from the regenerated shoots in half-strength MS medium supplemented with NAA [9].

In *Mucuna*, BAP was found to be more effective than kinetin in both shoot bud multiplication [14, 16, 26] and callus organogenesis [9]. Similar reports on efficacy of BAP over kinetin are also available for other plant species [48, 49]. In successive days, the regeneration potentiality was found to increase upto 150 days. This may indicate the genetic stability of the callus and favourable condition (both physical and chemical) of the culture environment [48].

Micropropagation of *M. pruriens*, by direct shoot bud multiplication, was reported [13-16, 26]. Also, plant regeneration through development of somatic embryos from callus tissues in this plant was observed [10, 50]. Harini and Sathyanarayana (2009) [51] also reported

somatic embryo development which failed to germinate. The establishment of highly efficient, reproducible and continuous plant regeneration of *M. pruriens*, var. *pruriens* through somatic embryogenesis was also reported by the present author [10].

However, callus tissue induced from nodal explants of 7 days-old seedlings showed earliest response and proliferated very fast. Moreover, the callus tissue was nodular, friable and creamish-white in colour. On the other hand, internodal segments produced green, compact and hard callus tissue with relatively slow growth. The nodal segments of 7 days-old seedlings were more favourable than internodal segments in inducing embryogenic callus culture [10]. The induction of embryogenic callus tissue in culture in presence of 2,4-D has been well established in different plant species [16, 19, 39]. The role of different auxin and cytokinin in the induction of somatic embryo formation is well known [52, 53]. The determination of right combination and concentration of growth regulators that will induce somatic embryogenesis is important. Moreover, differences exist even within a particular type of tissue of same species. There are earlier reports of somatic embryogenesis in other species of Fabaceae to study the ontogeny stages of somatic embryo differentiation [54-56]. Different

factors like type, combination and concentration of growth regulators, reduced nitrogen, level of carbon source, addition of additives including specific amino acids have effects on somatic embryo differentiation and its maturation [57]. Lahiri *et al.* (2012b) [10] indicated that BAP in association with NAA was suitable to induce somatic embryos in large numbers. The efficiency of kinetin in somatic embryo induction was also demonstrated [50]. It was also observed that the efficiency of somatic embryo differentiation was largely dependent on the age of embryogenic callus tissue and that the callus tissue growing in presence of 2,4-D more than 2 months differentiated embryos in much reduced frequency [10]. It is well known that 2,4-D being herbicide may introduce some stress responses in plant cells [58] which ultimately leads to the embryo differentiation. Earlier studies have also revealed that longer exposure to 2,4-D leads to a change in differential gene expression probably by an increased demethylation of DNA [52, 53].

CONCLUSION

The present article has presented a short review on tissue culture of *M. pruriens* L. where regeneration of plants has been reported through axillary bud multiplication, organogenesis and somatic embryogenesis from callus tissues. All the

information is very important for further researches on exploitation of these techniques for propagation and conservation of this important medicinal plant and also for continuous production of medicinally important active compound, L-DOPA.

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