In Vitro Multiplication of Mucuna pruriens (L.) DC: A Potent Medicinal Plant

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***Abstract*—A rapid and efficient protocol for in vitro multiplication of *Mucuna pruriens* (L.) DC., a medicinally valuable plant, was established using seed and shoot tip explants cultured on Murashige and Skoog (MS) medium. Optimal concentrations of 6-Benzylaminopurine (BA) and Naphthalene Acetic Acid (NAA) facilitated multiple shoot induction and rooting, respectively. BA at 5.0 mg/L induced a maximum of 25 shoots per explant with an average length of 6 cm after six weeks. NAA at 1.0 mg/L was most effective for root induction. This study addresses the growing demand for *Mucuna pruriens* and demonstrates the potential for large-scale production using tissue culture techniques.**

I. INTRODUCTION

Mucuna pruriens (L.) DC., commonly referred to as Velvet Bean, Cowitch, or Alkushi, is a tropical legume belonging to the Fabaceae family. The plant is characterized by its climbing shrub habit, with vines reaching lengths of over 15 meters. It is predominantly found in the plains of India but is also cultivated in tropical regions worldwide. The plant's notoriety stems from the extreme itchiness caused by contact with its young foliage and seed pods, which are covered in fine hairs.

Mucuna pruriens holds immense significance in traditional and modern medicine. It is a key ingredient in over 200 indigenous drug formulations in India. The plant gained widespread recognition for its seeds, which are rich in L-DOPA (3,4-dihydroxyphenylalanine), a vital precursor to the neurotransmitter dopamine. L-DOPA is a cornerstone in the treatment of Parkinson's disease, leading to a surge in demand in both domestic and international pharmaceutical markets. Additionally, the seeds have dietary and nutritional value as a source of plant proteins.

Despite its therapeutic importance, the wild population of Mucuna pruriens has significantly declined due to overexploitation and limited cultivation efforts. The plant propagates naturally through seeds, but this method is inadequate for large-scale production. Low germination rates, coupled with the susceptibility of seeds to environmental stress, further exacerbate the challenge.

To address these limitations, tissue culture techniques have emerged as a promising alternative. This approach facilitates rapid and large-scale propagation, ensures genetic uniformity, and allows for the conservation of this medicinally valuable species. Moreover, in vitro techniques enable the production of disease-free plants and offer potential for germplasm preservation. This study aims to develop a robust tissue culture protocol for Mucuna pruriens, providing a sustainable solution to meet the increasing demand while conserving its natural populations.

II. MATERIALS AND METHODS

A. Explant Collection and Preparation

Healthy nodes and cotyledons of *M. pruriens* were collected from the Gopeshwar College campus in Hathwa. The explants were thoroughly washed with a soap solution containing Tween-20 to remove surface contaminants. Following this, the explants were treated with 1000 ppm Bavistin for fungal control and disinfected with 0.2% HgCl2 for 2–5 minutes. The disinfected explants were then rinsed multiple times with sterile distilled water to remove traces of disinfectant. Using sterile techniques, explants were trimmed and transferred to culture media.

B. Culture Media Preparation

Murashige and Skoog (MS) medium were prepared by supplementing it with 3% (w/v) sucrose and 0.8% (w/v) agar. Plant growth regulators, including BA, NAA, and Indole-3-butyric acid (IBA), were added to the medium at various concentrations. The pH was adjusted to 5.8 before autoclaving the medium at

121°C for 15 minutes. The cultures were maintained at 26±1°C with a 16/8-hour light-dark photoperiod under cool white fluorescent light (30-40 µmol m^{-2} s^{-1}).

C. Shoot Induction

Explants from shoot tips and seeds were placed on MS medium supplemented with varying concentrations of BA (0.5–6.5 mg/L). The response of the explants, including shoot number, shoot length, and overall growth, was recorded after six weeks of culture.

D. Root Induction and Acclimatization

Shoots measuring 2–3 cm were excised and transferred to MS medium containing NAA or IBA (0.5–2.0 mg/L). Data on rooting percentage, root length, and callus formation were collected after five weeks. The rooted plantlets were acclimatized in a greenhouse by transplanting them into plastic pots containing a mixture of sterile garden soil, sand, and cow dung manure (1:1:1). High humidity was maintained by covering the pots with transparent polyethylene sheets. The plantlets were gradually hardened for field transfer over four weeks.

III. RESULTS AND DISCUSSION

A. Shoot Induction with BA

The addition of BA significantly influenced shoot induction in both shoot tip and seed explants. At an optimal concentration of 5.0 mg/L, the shoot tip explants showed the highest response, with 90% of the explants producing up to 25 shoots each, with an average shoot length of 6 cm. Seed explants also exhibited substantial shoot proliferation, albeit at slightly lower efficiency.

Higher concentrations of BA (above 6.0 mg/L) resulted in reduced shoot induction and abnormalities such as browning and callus deformation. These results underline the importance of optimizing BA concentration for effective shoot regeneration.

Table 1: Effect of BA on Shoot Tip and Seed Explants

BA (mg/L)	Explant Type	Response (%)	Average Shoots
0.5	Shoot Tip	80	8
2.5	Shoot Tip	80	12
5.0	Shoot Tip	90	25
6.5	Shoot Tip	60	6

B. Root Induction with NAA

Rooting efficiency was highest when shoots were cultured in MS medium containing 1.0 mg/L NAA. At this concentration, 90% of the shoots developed roots with an average length of 3.5 cm. Lower concentrations resulted in fewer roots, while higher concentrations caused root browning and deformation.

Table 2: Effect of NAA on Root Induction

NAA (mg/L)	Rooting (%)	Average Root Length (cm)	Callus Characteristics
0.5	70	2.1	Minimal callus
1.0	90	3.5	Compact callus
2.0	60	1.8	Browning, deformation

C. Acclimatization

The acclimatization process was critical for transitioning the plantlets to field conditions. Plantlets with robust roots were successfully transplanted to soil, achieving an 85% survival rate. The use of sterile soil mixtures and gradual humidity reduction ensured plant adaptability and growth.

D. Discussion

The study demonstrates the effectiveness of BA and NAA in optimizing shoot and root induction, respectively. BA facilitated the rapid production of multiple shoots, while NAA promoted healthy root development. These findings provide a scalable protocol for the propagation of *M. pruriens*. The tissue culture approach addresses the challenges of conventional propagation and supports the sustainable production of L-DOPA to meet industrial demands.

Further studies could focus on the genetic stability of regenerated plants and the quantification of bioactive compounds to ensure their pharmaceutical efficacy.

IV. CONCLUSION

This research highlights a reliable and efficient method for the large-scale propagation of *Mucuna pruriens*. The optimized protocol ensures rapid multiplication and contributes to the conservation and commercial cultivation of this medicinally important species. The integration of tissue culture methods into agricultural

practices can significantly enhance the availability of M. pruriens for therapeutic applications.

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