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Effect of Growth Regulators on Direct Clonal Propagation *and* Analysis of Total Phenolic Content of Wild and Propagated *Mucuna pruriens*

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ABSTRACT

Mucuna pruriens is an important vegetable cover crop. Almost every part of the plant is thought to contain L-3,4 dihydroxyphenylalanine (L-Dopa). The present study describes a quick and dependable technique for high-constancy miniature spread. Helper nodal explants were refined on Murashige and Skoog's (MS) media boosted with different groupings of cytokinins from 15-day-old refined explants. Throughout the primary culture on 35 to 45 mg/l 6-benzyl amino purine (BAP) greatest no of shoots was delivered. The number of shoots expanded when the flawless shoots were exposed to re-refine on a similar hormonal medium. The stretched shoots created a limit of roots on half-strength MS medium enhanced with 20-40 mg/l naphthalene acidic corrosive (NAA). The Plantlets were adjusted by moving them first to peat greenery: manure (1:1) combination further with sand: soil (1:1) blend, recording 90% endurance. This framework gives high constancy miniature engendering framework for proficient and fast miniature proliferation and evaluation of phenolic content of this significant green compost cover crop with restorative properties.

Keywords: Auxiliary bud, Acclimatization, Cytokinin, Multiple shoots, Rapid micro-propagation.

INTRODUCTION

The class *Mucuna* has a place with the family *Fabaceae* (Leguminosae) and incorporates around 150 types of yearly and enduring vegetables of pantropical circulation. Numerous types of the class offer an amazing source of cover harvest and green fertilizer, notwithstanding their customary practice as feed and food (Janardhanan and Lakshmanan, 1985; Mohan and Janardhanan, 1993; Capo-chichi et al., 2003). Practically every one of the animal varieties is accounted for to contain L-3,4-dihydroxyphenylalanine (L-DOPA), a non-protein corrosive that goes about as antecedent for the synapse dopamine, utilized in the treatment of Parkinson's illness (Manyam, 1995). Notwithstanding this *M. pruriens* is a significant therapeutic plant used to deal with some illnesses like intestinal sickness, epilepsy, Parkinson's infection, the runs, helminthiasis, fruitlessness, snakebite, scorpion stings, and elephantiasis (Lampariello et al., 2011; Okafor et al., 2013; Oyeyemi et al., 2019). It is developed as viable green fertilizer to renew crushed soil because of its capacity to collect supplements in different conditions (Sathiyanarayanan et al., 2007; Lorenzetti et al., 1998) and it displays allelopathy to weed development and is productive in diminishing the nematode worm populace in ranches (Lampariello et al., 2011; Pugalenthi et al., 2005). Several examinations have shown that L-3,4 dihydroxy phenylalanine(L-DOPA), lectin, is flavones, and a few alkaloids confined in *M. pruriens* seeds are answerable for

the colossal bioactivity of its unrefined concentrates. As of late, a few restorative methodologies have been taken on to make a sound living reasonably to all no matter what their status in the general public, since engineered items are costly as well as accompanied by unfriendly impacts (Atanasov et al., 2015; Jimoh et al., 2019a). The difficulties presented by the expense and security of engineered drugs have raised the need to investigate underutilized plant species presumed for high restorative importance (Rautela et al., 2018, Bajpai et al., 2021). From past reports, various pieces of *M. pruriens* have been demonstrated to be great for different helpful purposes (Oyeyemi et al., 2019; Sathiyanarayanan et al., 2007).

Because of the enormous scope, and unhindered double-dealing of the entire plant to fulfil its always-expanding need by the drug business, combined with restricted development and deficient endeavors for its recharging, the wild supply of this important restorative plant has uniquely drained. In nature, this species spreads just through seed, Engendering through seed presents issues because of the great hypersensitive properties of cases that cause uncontrolled tingling for those dealing with the seeds, and low germinability and unfortunate suitability (Arora and Bhojwani, 1989; Sudha and Seeni, 1994; Rautela et al., 2018). Subsequently, customary spread through seed is certainly not a satisfactory answer to satisfy the need for this plant. Thus, concocting a strategy for huge scope duplication for business production is helpful. Tissue culture innovation is effectively used in the proliferation of plants with poor and questionable reactions to regular engendering. As of late, there has been an expanded interest *in vitro* culture procedure, which offer a suitable device for mass spread and germplasm protection of therapeutic and sweet-smelling plants (Pattnaik and Chand, 1996; Joshi and Dhar, 2003; Anis and Faisal, 2005; Rautela et al., 2020). The goal of the review revealed here was to foster a productive and quick technique for *in vitro* spread of *M. pruriens* utilizing helper nodal explants through the improvement of basal media, pH, cytokinins, auxins and cementing specialists, trailed by the effective outside foundation of recovered plants. Also, examination of its complete phenolic content, and the conventions introduced in this paper could likewise go far for the future hereditary improvement programs in this significant restorative plant.

MATERIAL AND METHODS

Plant material

The explants that were taken for investigation were nodal explants from the sound mother plant of *Mucuna pruriens*. The primary plant was gathered from various areas of Dehradun (valley zone of Uttarakhand in North India). Mother plant significant or material congregated from the local district of Vikas Nagar and Herbertpur Dehradun, Uttarakhand.

Surface sterilization of explants

For micropropagation, surface sanitization assumes an exceptionally essential part. Surface disinfection of *Mucuna pruriens* starts with cutting off the nodal portions and washing under the tap of running water for 4 minutes to 10 min alongside a drop of Tween-20 cleanser. Then wash it with 0.1% (w/v) bavistin after that 0.1% (w/v) polyvinyl pyrrolidone (PVP) for 10-20 min. These explants were then washed with over two-thirds of C_2H_5OH briefly, and Anti-microbial $HgCl_2$ 0.5% (w/v) for 20-30 sec (Yadav and Singh, 2011; Rautela et al., 2018). Then, washed with autoclaved refined water 3 to 4 times. Helper buds estimating 1cm lengths were aseptically immunized onto original capacity MS medium enhanced with different shoot enlistment chemicals.

Medium and culture conditions

Logical grade synthetic substances got from HI media research facilities and chemicals and nutrients from Sigma-Aldrich synthetic compounds were utilized for setting up the stock arrangements and ensuing media planning. Murashige and Skoog's (1962) salt with 3% (w/v) sucrose was utilized as a basal medium. Subsequent to adding the development controllers, the pH of the medium was acclimated to 5.8 followed by gelling with 0.8 % of agar in the event of a strong medium. The media was autoclaved at 121 ^{0}C and 1.06 kg/cm^2 tension for 20 min. Every one of the ways of life was brooded in a development chamber kept up at a temperature of 25±2 ^{O}C, relative stickiness, 70-80%, and photoperiod of 16:8 h term under photon motion thickness given by light fluorescent cylinders (Sharma et al., 2016: Rautela et al., 2018).

Multiple shoot initiation

For initial numerous shoot induction, the explants were refined on Murashige and Skoog's medium enhanced with BAP (20-80 mg/l), and mixes of BAP (30-40 mg/l) + kinetin (Kn) (30 - 50mg/l), BAP (30-40 mg/l) + NAA (40-50 mg/l). The initiated shoots were permitted to develop for 20 days. Toward the finish of 20 days period, the explants creating the most extreme number of numerous shoots were refined on MS+BAP (30-40mg/l) for 30 days additional for the creation of a more prominent number of shoots (Saini et al., 2021; Gaurav et al., 2018; Rautela et al., 2018; Pant et al., 2021).

Rooting

The extended shoots of 3-4 cm in length were hence moved to a half-strength fluid MS medium enhanced with NAA

(20-40 mg/l) for establishment. Boundaries were recorded in the span of about fourteen days of culture. The way of life without plant development chemicals filled in as reins for both shooting and establishing. NAA as a vital chemical in prompting rhizogenesis is additionally revealed in a few laid-out miniature engendering conventions like *M. pruriens* (Chattopadhyay et al., 1995).

Hardening and acclimatization of plantlets

After 24 days, plantlets of 3-4 cm level with advanced roots were painstakingly eliminated and washed completely under running regular water for 2-3 minutes eliminating the hints of the medium. Plantlets were then moved to plastic pots containing autoclaved peat greenery and fertilizer blends (1:1) and covered with polythene packs to keep up with stickiness. After beginning solidifying in the development chamber for about fourteen days, the plantlets were moved to pots containing sand: soil (1:1) blend for 30 days before at last moving to the field.

Total Phenolic Content

Crude extraction

Ethanol and Methanol of normal logical class were utilized for extraction. The Soxhlet technique for the extraction was utilized. 50 gm of powder-dried plant material was utilized for the extraction and 250 ml every one of the methanol and ethanol as dissolvable were utilized and Soxhlet run for 8 to 10 cycles and the remaining parts after filtration were aggregated together and thorough to 2% of its special volume through vanishing at dense pressure in a rotatory dissipating device. Then the gathered concentrate was saved in the fridge at 4°C for additional purposes (Gaurav, 2016; Sundriyal et al., 2021).

$$\text{Rate Yield or produce (\%)} = \frac{\text{Weight of plant separate}}{\text{Weight of dry Sample}} \times 100$$

Evaluation of total phenolic content in wild and *in vitro* propagated plant extract

The complete phenolic content of the concentrate was assessed utilizing the modified Folin-Ciocalteu method (Jimoh et al., 2019b). Gallic corrosive (standard) was ready in methanol at an underlying grouping of 200 micrograms/ml. This was subsequently weakened with methanol as per all out-response blends and evaluated in a progression of 20-200 microgram/mL, ready in discrete test tubes. The rough concentrate was likewise ready in methanol at a grouping of 1 mg/ ml. To every 0.5 ml of the evaluated grouping of standard arrangements, 50 microliters of Folin-Ciocalteu arrangement was added and, thusly, 300 microliters of anhydrous NA_2CO_3 (20% w/v). The combination was vortexed and hatched for 20 min at room temperature, after brooding, the absorbance of the arrangement was estimated at 755 nm utilizing a UV-300 PC spectrophotometer. The absolute phenolic part was assessed as milligrams of Gallic corrosive same (GAE) per gram of unrefined concentrate extrapolated from the standard condition y= 0.1197 x-0.0008(R2 = 0.9991) by the equation Y=mx+c given above.

RESULT AND DISCUSSION

Induction of multiple shoots

Various shoots were seen toward the finish of the second week, subsequent to vaccinating the helper buds. Assistant buds refined on chemical-free MS medium brought about the development of a single shoot. More than one shoot was recovered on MS medium enhanced with development controllers separately as well as in blends. The best number of shoots delivered per explant on various mixes of chemicals is displayed in Table 1. Rate reaction for shoot arrangement from the explant contrasted in various media enhanced with different groupings of plant development controllers. A limit of 85.5% (information not shown) of explant refined on MS medium containing 35 mg/l BAP framed shoots following 24 days of culture. A similar medium additionally brought about the largest number of shoots per assistant bud with a typical shoot length of 1cm. MS + Kn (30 mg/l) actuated 5 to 7 shoots for every assistant bud. Consistent expansion in the number of shoots was seen up to 35 mg/l if there should be an occurrence of BAP and 30 mg/l in the event of Kn, separately (Figure 1 A, B, C). Notwithstanding, sub-refined shoots acquired on BAP (35 mg/l) on to similar hormonal mode for 20 days of advanced shoot augmentation by almost 2.5 times (Table 2). The stimulatory impact of an expanded section of subculturing on shoot bud acceptance is additionally detailed in *E. alba*. Among the blends of chemicals attempted, even the best hormonal mixes like MS + BAP (40 mg/l) + Kn (45 mg/l), MS + BAP (40 mg /l) + NAA (50 mg/l), just 4-5 shoots for every axillary bud. This shows that, in *M. pruriens*, a blend of chemicals doesn't create synchronized results towards enormous scope bud-break prompting various shoot arrangements as seen in other *M. pruriens* assortments. Chattopadhyay et al. (1995) have prior revealed the greatest various shoot acceptance on NAA+2-isopentyladenine (2ip) and NAA+BAP in *M. pruriens* and one more assortment of *Mucuna* (not determined), separately. In any case, the last hormonal blend created just 4-5 shoots.

Table 1: Showing the effect of various concentrations and combinations of growth regulators on shoot induction from nodal explant of *M. pruriens*.

S.N.	Growth regulators (mg/l)	No. of explants	No. of shoots per culture (Mean±SE)
1	BAP20	10	4.67±0.33
2	BAP25	12	5.67±0.33
3	BAP 30	12	5.33±0.33
4	BAP35	15	6.67±0.33
5	BAP40	15	5.67±0.33
6	BAP45	10	4.67±0.33
7	Kn 20	10	1.67±0.33
8	Kn 25	10	3.67±0.33
9	Kn 30	12	3.67±0.33
10	Kn35	12	4.67±0.33
11	BAP40 +Kn 30	15	3.67±0.33
12	BAP40+Kn45	15	5.00±0.58
13	BAP40 +NAA40	15	4.67±0.33
14	BAP 40 +NAA50	15	5.00±0.58

Table 2: Showing the effect of different concentrations of BAP on subculturing of multiple shoots.

S.N.	BAP(mg/l)	No. of shoots per culture (Mean±SE)
1	BAP 20	12.67±0.33
2	BAP 25	14.67±0.33
3	BAP 30	13.67±0.33
4	BAP 35	15.67±0.33
5	BAP 40	13.67±0.33
6	BAP 45	14.67±0.33

Rooting

The prolonged shoots were established on half-strength fluid MS medium enhanced with NAA (20-40 mg/l). The consequences of establishing tests utilizing various convergences of NAA are displayed in Table 3. Hundred percent of societies showed establishing on NAA (30 mg/l), delivering the greatest number of roots per shoot with normal root length (Figure 1D) in the multi-day of culture. NAA as a critical chemical in prompting rhizogenesis is additionally revealed in a few laid-out miniature proliferation conventions like *M. pruriens*, *Pisonia alba*, *Hyptis suaveolens*, *Jatropha curcas* (Chattopadhyay et al., 1995).

Table 3: Effect of different concentrations of NAA on root induction.

S.N.	NAA(mg/l)	No. of roots per culture (Mean±SE)
1.	NAA 20	3.0±0.58
2.	NAA 25	5.0±0.58
3.	NAA 30	6.0±0.58
4.	NAA 40	15±0.58

Hardening and acclimatization of plantlets

Plantlets straightforwardly moved from attaching medium to sand: soil blend (1:1) displayed a low pace of endurance (60-70%). To undermine this, plantlets were first moved to an autoclaved combination of peat greenery and manure blend (1:1) and kept up in the development chamber for 14 days. The plantlets were then moved to the sand: soil (1:1) combination and permitted to solidify for 30 days before at last moving to the field (Figure 1 F). This brought about 95% endurance of the plants. Every one of the endure plants was effectively relocated to the dirt.

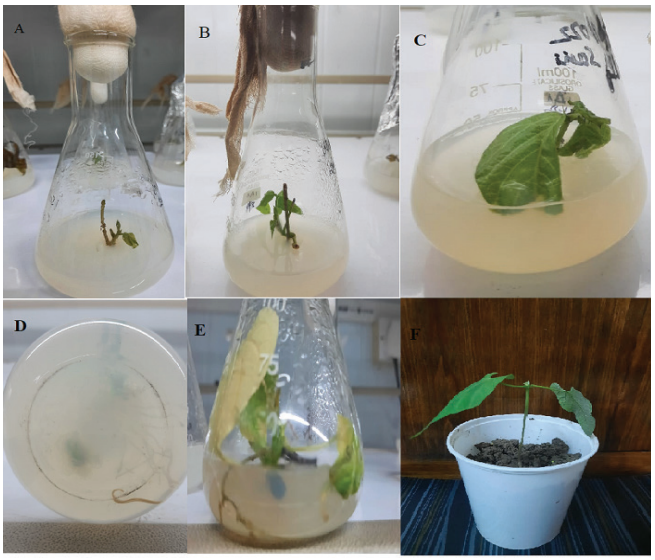

Figure 1: Rapid micropropagation of *M. pruriens* using nodal explant

A. Bud formation in 15days old nodal explant of *Mucuna purines*.
B. *In vitro* multiplication of shoots from auxiliary buds on MS+BAP (35 mg/l) after 30 days of culture.
C. Multiplication of shoots, after subculturing the shoot on the same medium.
D. Complete rooting on half-strength MS medium supplemented with NAA (40mg/l).
E. Regenerated complete plantlets
F. Acclimatization of *in vitro* regenerated plantlet in the pot.

Extraction of wild and propagated plant material of *M. pruriens*.

Table 4 delineates the delivered level of the methanol concentrate of wild and spread plants contrasted with the heaviness of the dried plant test previously extricated. The parched weight (50 grams) of the squashed example gave 7.9 % methanol concentrates of wild and 5.96% of methanol concentrate of the proliferated plant.

Table 4: Produce percentage of extract of *M. pruriens* plant material.

S. N.	Type of plant material	Weight of desiccated plant sample used (gram)	Weight of plant extract (gram)	Percentage (%) produced	Colour of extract
1	Wild plant material	50	3.95	7.9	Dark green
2	Propagated Plant material	50	2.95	5.92	Dark green

Total phenolic content

The total phenolic content of the methanolic extract of the wild and propagated plant was presented in Table 5, Figure 2. The total phenolic content of methanoic extract of both wild and propagated plants is very high and it is a strong sign that *M. pruriens* is rich in phenols.

Table 5: Showing the Total phenolic content in the wild and propagated plant extract.

S.N.	Phytochemical	Wild Plant Extract	Propagated Plant Extract
1	Total Phenol content	234 µg/ml	175.5 µg/ml

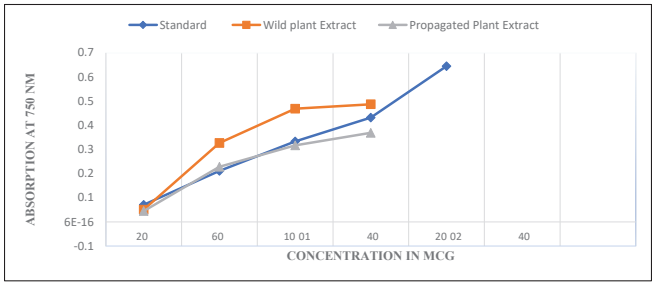

Figure 2: Showing the different absorption at 750 nm of wild and propagated *M. pruriens* and Gallic acid.

Declaration: *We also declare that all ethical guidelines have been followed during this work and there is no conflict of interest among authors.*

CONCLUSION

M. pruriens, the captivating spice is lived with complex purposes. All pieces of the plant are being utilized in pharmacological planning. All in all, a basic, productive, and high-loyalty convention for mass proliferation of *M. pruriens* from assistant bud explant has been laid out. Utilizing this convention, it is feasible to deliver reasonable, uniform, and sound plants with the greatest endurance rate for the proposed *in vitro* germplasm. The convention ought to likewise give an effective means to enormous scope development and *in vitro* control of *M. pruriens*, a significant green excrement cover crop with therapeutic properties. High phenolic content is a significant driver of the cell reinforcement and restorative exercises of *M. pruriens*. Though further work is supposed to exploit the full-scale probability of the plant in the space of pharmacology.

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