EFFICIENT EVALUATION OF GROWTH MEDIA WITH PLANT HORMONES ON MICROPORPAGATION OF A MEDICATIVE HERB: BENGAL BEAN

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Abstract

Bengal bean *or* velvet bean botanically named as *Mucuna prurience* L. belongs to the Fabaceae family is an important ingredient in Ayurveda preparation. In Indian tradition medicinal plants play richest role and in modern economy as well. India is the oldest and most diverse culture in utilizing medicinal plants for curing various diseases. In addition to its medicinal use, it is also an excellent source of cover crop, food and green manure. The plant is pharmacologically investigated for curing various diseases like worms, dysentery, diarrhoea, sexual debility, muscular pain, menstrual disorder, diabetes, cancer and tuberculosis. Seed of the *Mucuna prurience* contains L-Dopa which is used in ayurvedic drugs and medicine. Due to its high pharmacological usage and commercial value, the variety is now in the endangered category. so as to meet the demands of mankind and pharmaceutical company there is a need to develop a reliable protocol for micropropagation of *Mucuna prurience*. Hence, the purpose of the present investigation is to develop an efficient in-vitro regeneration method for this important medicinal plant of India, *Mucuna prurience*. 3% sucrose in Murashige and Skoog 's media with 5.8 -6.0 PH was used for organogenesis. A range of various conc. of different types of auxins (IAA, IBA, and NAA) alone and in different combinations with BAP (6, benzyl amino purine) was studied for shoot regeneration with cotyledon & cotyledonary node as an explant. Shoot apex explant proved to be efficient for multiple shooting. The reading was taken after 20-25 days of incubation. Out of 3 auxins (IAA, IBA, NAA) used in Murashige and Skoog 's media, NAA was found to be most effective. Moderate 5 mg/l conc. of NAA alone showed optimum 95% shoot induction. In present study low to moderate (1-2.5 mg/l) conc. of NAA in combination with high conc. (10 mg/l of BAP) proved to be highly effective for shoot regeneration. The highest shoot proliferation i.e., 92% was found in 2.5 mg/l NAA / 10mg/l BAP. Low conc. of NAA i.e., 1mg/l in combination with high conc. i.e., 5 mg/l BAP also showed good response 90% of multiple shoot induction. Higher and moderate conc. i.e., 5 & 10 mg/l IBA in half strength MS was proved to be the most effective for root regeneration. The plantlets were acclimatized & transferred to small plastic cups filled with sterilized sand & soil mixture (1:1), where 90% of the plantlets showed effective and normal growth. All plantlets were irrigated with Knop 's nutrient solution. After proper hardening all plantlets were shifted to earthen pot for normal growth. Thus, the present investigation provides high and reliable protocol for in vitro micro propagation of *Mucuna prurience*.

Keywords: Knop 's nutrient, leaf primordia, Shoot apex,

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Introduction

Bengal bean or velvet bean botanically named as Mucuna prurience L. belongs to the Fabaceae family is an important ingredient in Ayurveda preparation. In Indian tradition medicinal plants play richest role and in modern economy as well. Additionally, to its medicative use it provides an excellent supply of canopy crop, food and manure. It is a creeping vine named as *Mucuna pruriens* in Latin that grows all over India, particularly in the tropics. There are two varieties of seeds, one black and one white (Pole 2006) Many names given to *Bangal bean* is due to velvet coating of hairs that cover its seedpods which causes severe itching and irritation of the skin, if touched. In Sanskrit language, it is named as, Kapikacchu, which means "one starts itching like a monkey" and Atmagupta, which means "secret self," and shows the healthful value of the seed present within the allergenic seedpod (Vaidya and Gogte 2009). In language English, this creeper is known as Cow hage. There are traditional uses for the root and the trichomes, but it is the seed of *Mucuna pruriens* that is most often employed therapeutically. It is a natural supply of levodopa (L-dopa). It improves stress handle ability of our body. It also helps to maintain healthy blood sugar level (Grover etal.2002). Nutritive value of Mucuna seeds is very identical to other common legumes (Vadivel and Janardhan an 2000).

Mucuna pruriens is useful in The disorders of *tridoshic* origin which involve vata, pitta, and kapha. It is also known as building herb, which promotes muscle mass, body weight. *Mucuna pruriens* is primarily sweet and bitter. It has an affinity for all of the tissues in the body, but is especially suited to balance the nervous, reproductive, and digestive systems. The plant is pharmacologically investigated for curing various diseases like worms, dysentery, diarrhoea, sexual debility, muscular pain, menstrual disorder, diabetes, cancer and tuberculosis. Seed of the *Mucuna prurience* contains L Dopa which is used in Ayurvedic drugs and medicine for providing symptomatic relief in Parkinson ‘s disease. Due to its high pharmacological usage and commercial value, there is a need to establish the glory of this valuable asset. Cultivation of medicinal plants is also difficult due to lack of standardized agronomic practices for the most species and unavailability of source plant materials. Plant tissue culture offers unconventional techniques and important tool for conservation and mass propagation. Plant tissue culture is not only significant to produce large number of plants, but also possible methods for creating genetic variability in in-vitro plants (Lema-Rumińska and Mellem 2017, Huang et al. 2018). Hence, the purpose of the present investigation is to develop an efficient in-vitro tool for Callus formation and subsequent regeneration of this important medicinal plant of India, M*ucuna prurience L*.

Material and Method

Seeds of Mucuna L. obtained from native medicative plant agency, were surface sterilized with 0.1% HgCl2 (W/V) for five min then rinsed in sterile Double distilled water for 4-5 times. The seeds were germinated on sterile wet paper in Petri plates/sterile wet cotton in flasks at 22-25 □ in dark. Shoot apex and leaf primordia explants were removed from four to five days previous seedlings and placed aseptically on semisolid MS medium containing 3% saccharose and 0.8% agar. 5.6-5.8 was the pH of the media, adjusted with the assistance of 0.1N sodium hydroxide and/or 0.1 N Hydrochloric acid before adding the agar. The media were supplemented with filter sterilized auxins, 1-Naphthaleneacetic acid (NAA), Indole butyric acid (IBA),2, 4 Dichlorophen oxyacetic acid (2, 4-D) and Cytokinin 6-Benzylaminopurine (BAP) both individually and together at an amount of 0.1 – 10mg/litre. Cultures were maintained under white fluorescent light with light dark cycles of 16 hr/8 hr at 25 ± 2^0C.

After shoot induction they were subculture onto MS medium fortified with various concentrations of IBA for root induction. The rooted shoots were transferred to plastic cups containing a mixture of sterilized garden soil, and sand (1: 2). Rooted plantlets were grown under laboratory conditions of regulated humidity and temperature for two weeks, irrigated with knop’s nutrient broth once in every three days. Then the plants were placed under shade for 4 weeks and then transferred to full sunlight. Readings was taken after 3540 days of culture for shoot and root response. Only readings which showed some favorable effects are included within the tables. All the experiments were repeated 3 times with 19 explants per treatment.

Result and Discussion

Evaluation of the effect of various conc. of Auxin alone and together with Cytokinin on shoot multiplication and complete plantlet regeneration was done. The best shoot regeneration frequency (95 %) was seen within the medium supplemented with high conc. of BAP (10 mg / l) alone in shoot

apex explants. 5.0 mg / l of NAA also showed 94 % of shoot morphogenesis in shoot apex part as shown in Table-I

Different concentrations of 2, 4-D alone was tested for callus induction and shoot growth. Low conc. of 2,4-D i.e., 0.1mg/l 2,4-D showed effectiveness for callus and shooting (70%) as well. but increase in 2,4-D conc. (2.5 mg/L) showed decreased suitability for shoot regeneration (14%) when used alone. Suitability of lower conc. (0.1mg/l) of 2, 4-D (70%) and its adverse effect (14%) on shooting at high conc is in agreement with results obtained previously in Sugarcane, French beans etc. (Kona et al., 2018). When the same low conc. i.e., 0.1mg/l 2, 4-D used in combination with 5mg/l BAP showed excellent **(**84%) shooting response. These results indicates that lower conc. of 2,4-D when interacted with cytokinin i.e., BAP gave fairly good response of shooting. Such sort of results was observed in the studies on chrysanthemum varieties (Xu et al. 2012, Kazeroonian et al. 2018).

When various conc.of only BAP (5&10 mg/L) was used, it showed good number of shoot bud formation with both shoot apex **(**87% & 95%) and leaf primordia (64% & 84%). (Table I). Combined treatment of Auxin NAA & Cytokinin BAP i.e. (1mg/l NAA /5 mg / l conc. BAP) and (2.5 mg/l NAA/10mg/l BAP) exhibited high rate of shoot regeneration i.e., 70% & 93% respectively with shoot apex explant. The effect of interaction of auxin and cytokinin and explant type on in-vitro micropropagation has been demonstrated in several medicinal plants viz. *Rawvolfia tatraphyla* (Faisal and Anis 2002), *Rotulla aquatica* (Martin 2003), *Chrysanthemum*(Lim et al. 2012, Teixeira et al. 2015), *Santolina canescense* (Cascado et.al.2002), sugarcane (Dibax et.al.2011) and *C. morifolium* (Chowdhury et.al.2022).The present study also supported the shoot induction capacity of combined treatment of high conc. of BAP with low conc. of NAA as shown in Table I. In present study low to moderate (1-2.5 mg/l) conc. of NAA together with high conc. (10 mg/l of BAP) proved to be highly effective for shoot regeneration. The highest shoot proliferation i.e., 93% was found in 2.5 mg/l NAA / 10mg/l BAP with Shoot apex explant. 1 mg/l NAA together with 10 mg/l BAP also showed good response i.e., 90% of multiple shoot induction with shoot apex.

The present findings revealed that during subsequent subculture in hormone free MS medium, it enhanced the number of multiple shoots (82%). Therefore, MS medium with no exogenous hormone i.e., hormone free medium was used for further shoot proliferation and shoot elongation. Several reports also supported efficiency of hormone free MS medium for multiple shoot development and shoot elongation (Xu et al. 2012, and Huang et al. 2018). It was also prominently seen that in shoot formation and multiplication, spontaneous rooting occurred when continuous subcultures were done in hormone free MS medium i.e., without application of any exogenous plant hormone but at the same time it had been also seen that after a while there was sudden reduce in further growth. This might be due to presence and activeness of endogenous plant growth regulators for a short period after inoculation and after that when endogenous hormones are exhausted there was sudden decline in response. Similar results were also observed by Praveen kumar et. al. 2017 in his experiments.

All the regenerated shoots were transferred to varied conc. of IBA, IAA, and NAA alone and together with BAP and also in hormone free medium for rhizogenesis. Regenerated shoots (90-95%) produced healthy roots within 6 to 7 weeks of culture, which was just like the chrysanthemum varieties (Kazeroonian et. al. 2018).

IBA alone (2.5 mg / l &5mg / l) showed 85% & 95% root formation respectively. Thus, IBA was found to be superior out of the three auxins (IAA, NAA, IBA) tested for rhizogenesis, MS medium without PGR was also equally effective (84%) for rhizogenesis, as shown in Table II. Effectivity of IBA on Rhizogenesis has been reported in past in *Sesbania aculeata* (Bansal and Pandey, 1993), Vigna *sinensis* (Pandey and Bansal,1989) *Glycine whitii* (Pandey and Bansal,1992) and a few medicinal plants like *Aloe polyphylla* (Abrie and van Staden 2001), chrysanthemum (Miler 2014) and *Tylophora Indica* (Faisal and Anis 2003) etc.

Plantlets with roots were transplanted to the pots containing 2:1 garden soil and sand irrigated with knops nutrient broth covered with transparent polythene bags, gradually they were transferred to larger pots and at last acclimated to outdoor conditions.

Conclusion

The research was performed to assess the regenerative capability of distinct hormones,

auxins and cytokinin combinations and single one on the explants. The methodology performed in the research is reproducible and it can be used for micropropagation of significant medicinal plants in large scale. Production of regenerated plants through direct or indirect organogenesis are not only applicable to produce large number of plants, but these are also possible methods for creating genetic variability and also for germplasm conservation in coming research days.

Table I: Leaf primordia and Shoot apex explants showing effect of growth regulators on shoot regeneration

GR (mg/l)	explants	% Shoot bud producing explant	Explants Producing Shoot (mean ±SE)
No PGR	SE	82%	14.3 ± 0.16
1 NAA	SE	70%	13.3± 0.25
2.5 NAA	LP	38%	6.6± 0.35
2.5 NAA	SE	68%	12.6± 0.30
5 NAA	SE	94%	18± 1.12
0.1 2,4-D	LP	22%	3.6± 0.35
	SE	70%	14± 0.16
2.52,4-D	SE	14%	2.6± 0.36
5 BAP	LP	64%	11.6± 0.30
	SE	87%	15.78± 0.31
10 BAP	LP	84%	16.3± 0.22
	SE	95%	12.66± 0.22
5 BAP+0.5 NAA	LP	38%	6.66± 0.16
5 BAP+1 NAA	LP	74%	14± 0.17
	SE	70%	17± 0.38
10 BAP+1NAA	LP	64%	12± 0.32
	SE	90%	13.33± 0.22
10BAP+2.5NAA	SE	93%	17.66± 0.30
0.1 2,4-D+5BAP	SE	84%	15.66± 0.30

Values are mean± Standard Error (SE). Mean with standard error of three repeated experiment. GR- Growth regulators, LP- leaf primordial, SE- Shoot apex

Table II- Roots on Micro shoots (mean± SE)

PGR	% Roots produced	Roots produced (mean ±SE)
Without PGR	84%	16.3± 0.22
0.5 IBA	20%	3.6±0.350
1.5 IBA	70%	13.3±0.252
2.5 IBA	85%	16.3±2.5
5 IBA	95%	18±0.519
0.5 BAP+10 IBA	60%	11.3±0.246
5 BAP+10 IBA	65%	12.3±0.309

Values are mean ± Standard Error (SE). Mean with standard error of three repeated experiment. P GR- Plant Growth regulators, LP- leaf primordia, SE- Shoot apex

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