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Study on the influence of selected plant growth regulators on the growth and induction of callus in *Mucuna pruriens* - The Marvelous Natural drug.

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Abstract- **Mucuna pruriens(Fabaceae)., a medicinal plant exhibits various medicinal properties. All parts of Mucuna possess medicinal properties. The high demand for plant material from Mucuna pruriens in the production of ayurvedic medicine, switches the technique of plant-tissue culture into one of the alternatives for the improvement of crops. The purpose of this study was to develop an efficient micropropagtion system for Mucuna pruriens, an important medicinal plant in India. A range of plant growth hormones were investigated for inducing callus from 17 days in vitro raised seedlings. The protocol could thus be helpful for invitro mass propagation of Mucuna pruriens. Callus induction of Mucuna pruriens was investigated by using different combinations Auxins and cytokinins such as NAA, 2, 4-D, BAP. Efficient and simple, direct organogenesis(rooting) system was developed when invitro derived nodal explants cultured on Murashige skoog (MS) medium supplemented with various plant growth regulators. The induced shoots produced adventitious roots on MS medium supplemented with Auxins and cytokinins. Highest frequency of root multiplication was observed with 2.5mg/l of NAA and 2.5mg/l of BAP. The maximum number of rootlets (12-16) was induced on full strength MS medium. The highest frequency of callus induction was observed in medium containing 2 mg/l , 3 mg/l 2,4-D with full strength MS medium at pH 5.8. Callus derived from nodal explants was found to be big and friable and yellow in colour. This work aims to develop an efficient protocol for the in vitro propagation of Mucuna pruriens.**

Key Words: Naphthalene acetic acid, 2,4-Dichlorophenoxy acetic acid, Benzylaminopurine(BAP), Plant tissue culture, Mucuna pruriens

INTRODUCTION

Mucuna pruriens is the marvelous herbal drug in Ayurvedic system of medicine known as Cowitch or Kaunch and velvet bean. It belongs to the Fabaceae family. This is a twining herb which has been used as a herbal drug for the management of male sterility(Anon, 1961: Farooqi et al., 1999)., nervous disorders, and also as an aphrodisiac. It also has been used as antidiabetic, antineoplastic, antiepileptic drug (Sathyanarayanan et al., 2007) M. pruriens is recognized as an aphrodisiac in Ayurvedic medicine, and it has been shown to increase the testosterone levels leading to deposition of protein in the muscles, as well as increased muscle mass and strength (Anonymous, 2003).

It has been used in ayurvedic medicine to treat Parkinsons disease a neurodegenerative disease. Mucuna pruriens leaf extract has antivenom activity against cobra venom poisoning. The importance of M. pruriens as a medicinal plant is mainly due to the presence of L-Dopa (3, 4-dihydroxy-L-phenylalanine), a neurotransmitter precursor being used for symptomatic relief of Parkinson's disease (Sathiyanarayanan et al., 2007). It is produced via the oxidation of tyrosine by the enzyme tyrosinase. Once formed L-Dopa can be converted into several neurologically important catecholamines such as the neurotransmitter dopamine and the important hormones adrenaline and noradrenaline. It is present in all parts of the plant, but in varying concentrations(Riley 1997). Parkinson's disease is a degenerative disorder that causes rigidity, tremors, slowness of speech and eventually dementia. This also causes the changes in enzymes of energy metabolism of the myocardium, followed by the neurogenic injury (Lee et al., 1996 and Lee et al., 1999). The bean, if applied as a paste on scorpion stings, is thought to absorb the poison (Jeyaweera, 1981). Human contact results in an intensely itchy dermatitis, caused by mucunain (Infante et al.,1990). All parts of M. pruriens possess valuable medicinal properties and it has been investigated in various contexts, including for its anti-diabetic, aphrodisiac, anti-neoplastic, anti-epileptic, and anti-microbial activities (Sathiyanarayanan et al., 2007). Since it has antioxidant activity it has antitumour and anticancer activity. The biochemical potential of plant cell cultures to produce specific secondary metabolites such as drugs, flavors, pigments, and agrochemicals is of considerable interest due to

their uses in biotechnology (Chakraborty and Chattopadhyay 2008). So invitro cultivation of this Marvelous drug will be useful as alternative method for producing L- DOPA. The principle aim of the present study was to study the effect of various plant growth regulators on the invitro callus induction of Mucuna pruriens.

MATERIALS AND METHODS

Mature seeds of Mucuna prureins were collected from NBPGR, New Delhi were washed with running tap water and rinsed in teepol (5 times dilute) for 2 minutes. Seeds were surface sterilized in 70% ethanol for 1 minute and immersed in 0.1% HgCl2 for 2 minutes, then rinsed with autoclaved double distilled water (5 washes, each for 5 minutes). Seeds were inoculated in test tube (10 X 1.2 cm) containing MS basal media (Murashige and Skoog , 1962) . Explants obtained from in-vitro plantlets were used as culture materials. (Shalini et al., 2007).Solid MS medium containing 3% sucrose with varying concentration of 2, 4-D, NAA, , (0.5,1.0, 1.5, 2.0, 2.5, 3.0 mg/l) were used for callus formation. Combination of auxins and cytokinins were also used. The pH of media was adjusted to 5.8 before gelling with agar (0.8%w/v) and was autoclaved for 15-20 minutes at 15 psi at 120 ˚C. The seeds were inoculated on to the culture medium(15ml) in culture bottles and incubated in culture room (Shalini et al., 2007). Cultures were incubated at 26±1˚C under cool florescent light (1500-200 lux) with a 16hours and 18 hours light and dark cycle (Shalini et al., 2007).Explants (shoot and leaf) were excised from 10-15 days old invitro grown seedlings and transferred to MS medium supplemented with 2, 4-D(1.5, 2.0, 2.5, 3.0 mg/l) and NAA (0.5, 1.5, 2.0, 2.5 mg/l), BAP(1.5, 2.0, 2.5 mg/l) for inducing callus production. Combination of BAP (cytokinin) and NAA (auxin) was supplemented for regeneration system at various concentrations(1.5, 2.0, 2.5 mg/l) (Shalini et al., 2007).

RESULTS AND DISCUSSION

Table:1 Invitro seedlings produced from the seeds of Mucuna pruriens

Seed Accession Number	Percentage of response(%)	Root length (cm)	Number of lateral roots produced	Shoot length (cm)
IC385926	53	2.06±0.37^{a}	5.8±0.44^{c}	2.9±0.22^{d}
IC326953	30	3.2±0.79^{b}	4.5±1.7^{b}	2.3±0.47^{a}
IC385842	55	3.2±0.64^{b}	5.6±0.54^{c}	2.6±0.54^{c}
IC392241	52	3.0±0.79^{b}	5.4±1.6^{c}	2.5±0.61^{b}
IC471870	50	2.8±0.75^{a}	3.2±0.83^{a}	2.3±0.44^{a}

Data represent treatment means ± SE followed by different letter (s) within a column indicate significant differences according to DMRT test (P< 0.05).

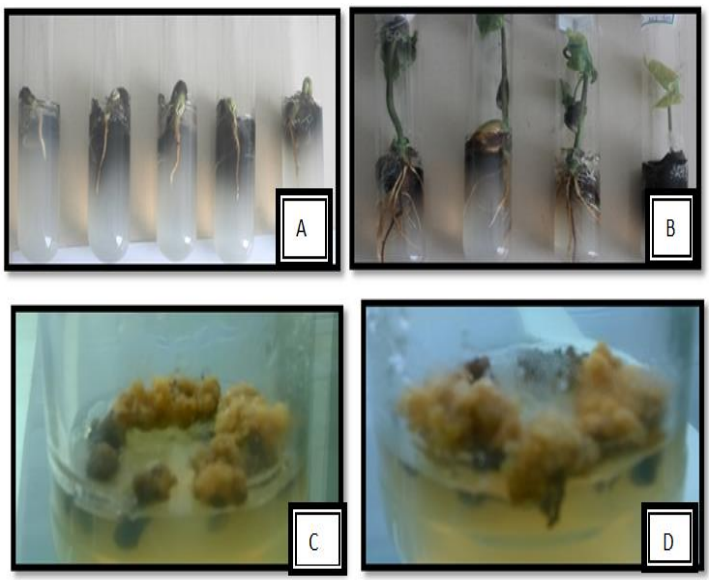
Table: 2:Effect of auxin and cytokinin on root organogenesis from invitro raised nodal explants of Mucuna pruriens on MS media

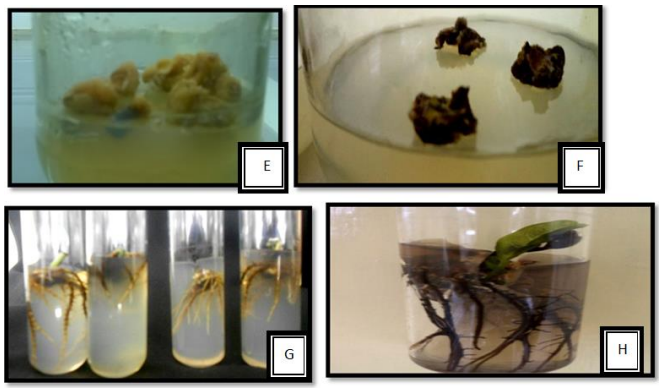
Combinations of hormone (Auxin ansd Cytokinin NAA and BAP	No of roots/explants	Average root length(cm)
NAA(2.0)+BAP(0.5)	5.0±0.3^{a}	4.6±0.05^{a}
NAA(2.0)+BAP(1.0)	5.0±0.3^{a}	5.8±0.1^{b}
NAA(2.0)+BAP(1.5)	7.0±0.2^{c}	5.8±0.1^{b}
NAA(2.0)+BAP(2.0)	11.0 ±0.2^{f}	12±0.2^{f}
NAA(0.5)+BAP(2.0)	6.0±0.4^{b}	8.7±0.2^{e}
NAA(1.0)+BAP(2.0)	6.0±0.4^{b}	7.5±0.15^{d}
NAA(1.5)+BAP(2.0)	8.0±0.3^{d}	8.5±0.2^{e}
NAA(2.5)+BAP(2.5)	12.0 ±0.5^{g}	16±0.15^{g}

When combination of auxins and cytokinins were used in the culture, root formation was observed, and measured about 4-16cm in length, 5-12 roots per explants. In the present study, BAP and NAA induced organogenesis it seems that BAP is essential to induce organogenesis. The auxin and cytokinin used were NAA & BAP. When nodal segments were transferred in media containing NAA & BAP (2.5mg/l) root induction was observed to be maximum with a maximum root length. The results showed that the concentration of various growth regulators affect callus induction and organogenesis in invitro grown plantlets of Mucuna pruriens.

In Conclusion, a present work demonstrates a simple protocol for callus induction, shoot and root formation of Mucuna pruriens using leaf and nodal explants.

Figure 1





In vitro germinated seedling developed and callus induction from Mucuna pruriens.

A. Invitro raised seedlings of Mucuna pruriens shows rooting on MS basal media(7th day)

B. Invitro raised seedlings of Mucuna pruriens on shows root and shoot formation MS basal media(17th day)

C. Optimum callus biomass developed from nodal explants of Mucuna pruriens on MS medium supplemented with2,4-D (3mg/l)

D. Optimum callus biomass developed from nodal explants of Mucuna pruriens on MS medium supplemented with2,4-D (4mg/l)

E. Optimum callus biomass developed from nodal explants of Mucuna pruriens on MS medium supplemented with NAA (3mg/l)

F. Optimum callus biomass developed from leaf explants of Mucuna pruriens on MS medium supplemented with 2,4-D (3 mg/l)

G. Invitro root formation from nodal explants of Mucuna pruriens on MS basal medium supplemented with NAA and BAP 2.5mg/l.

H. Regeneration of root and shoot formation from the nodal explants of Mucuna pruriens on MS basal medium supplemented with BAP 2.5mg/l.

Table: 3 Morphological characteristics of calli produced on the medium supplemented With different combinations of growth regulators in Mucuna pruriens

Growth regulators	Nature of Explant	Morphology of Calli (callus texture)	Colour of the callus
2,4-D	Stem	Friable	Pale Yellowish cream-white
NAA	Stem	Compact callus	Pale Yellowish
BAP	stem	Compact callus, Hard	Yellowish green
2,4-D	Leaf	Friable	Brown-White
NAA	Leaf	Friable	Brown-White
BAP	Leaf	Compact callus, Hard	Brown-White

Type of callus was greatly affected by the type and age of explants and growth regulators used. Initially there was formation of pale yellowish callus on the surface of the explant as well as on the cut region and leaf margin. Combination of IAA (2.5 mgl-1) and BAP (2.5 mgl-1), IAA produced pale yellowish callus; whereas BAP produced compact, hard green callus. When invitro raised stem and leaf were cultured on 2,4-D 3.0mg/l and 4mg/l yellow friable calli and brown compact calli were noticed respectively within 2 weeks Thus the callus showed differential response according to the growth regulators used. Our results are in accordance with the study of Janarthanam and Sumathi (2015) who obtained callus induction from invitro cotyledonary leaf explants of Mucuna pruriens on MS supplemented with , 2,4-D, NAA, BAP alone.

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