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IN VITRO MICROPROPAGATION OF *DESMODIUM GANGETICUM* (L.) DC. THROUGH NODAL EXPLANTS

ASMITA BEHERA AND M. THIRUNAVOUKKARASU*

Natural Products Department, Regional Research Laboratory, Bhubaneswar - 751013

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SUMMARY

Nodal stem segments from one year old plants of *Desmodium gangeticum* were cultured on Murashige and Skoog (MS) medium supplemented with different concentrations and combinations of BA and IAA. Explants cultured on MS basal medium supplemented with 1.0 mg/l BA + 0.25 mg/l IAA showed highest rate of shoot multiplication. *In vitro* shootlets were rooted on to the half-strength MS basal media supplemented with 1.0 mg/l IBA or IAA. Rooting was better in IBA supplemented medium than IAA. Rooted shoots were transplanted in the greenhouse for hardening and their survival was 80 % in the field condition.

Key words: *Desmodium gangeticum*, medicinal plant, micropropagation, nodal segment

INTRODUCTION

The genus *Desmodium* comprises of perennial or annual herbs or shrubs found throughout the tropical and sub-tropical regions of the world. About 38 species have been reported in India of which *Desmodium gangeticum* (L.) DC (Syn. *Hedeyserum gangeticum*) commonly known as Salaparni is used in the Indian system of medicine, particularly in the Ayurveda. It belongs to the family Fabaceae (Faboidea). The plant has high medicinal value and is used as bitter tonic, febrifuge, digestive, anti-catarrhal, anti-emetic, in inflammatory conditions of the chest and in various other inflammatory conditions, which are due to vata-disorders (Chopra *et al*. 1956, Rathi *et al*. 2004). The plant shows anthelmintic, aphrodisiac, astringent and diuretic properties. It is used in general anasarca, cough, diarrhea, dysentery, chronic fever including enteric fever, piles, respiratory disorder, vomiting, worms, asthma, snake bite and scorpion sting (Anonymous 1952, Ghosal and Bhatacharya 1972). Three ptero-carpenoids namely gangetin, gangetinin and desmodin were isolated from roots of *D. gangeticum* (Purushotaman *et al*. 1971). Alkaloid isolated from aerial part comprises indol-3-alkyl-amines and β-carbolines and has anticholinesterase, smooth muscle stimulant, CNS stimulant and depressor response (Ghosal and Bhatacharya 1972, Rathi *et al*. 2004).

Due to increasing demand of plant based raw materials hitherto collected through wild, the supply line is adversely affected. So, the urgency now is to conserve wild population for future uses and at the same time, produce enough planting materials by adopting improved and efficient propagation approaches including mass cultivation of these species. *D. gangeticum* is one of the important medicinal plants which is being exploited from the wild by the pharmaceutical industries. Development of an appropriate mass propagation technique is warranted to meet the growing demand. There is not much information on *in vitro* micropropagation of this species and hence an attempt was made for mass scale propagation of this important species using *in vitro* techniques.

* Corresponding author

MATERIALS AND METHODS

Healthy stems with active buds were collected from one year old plants of *D. gangeticum* maintained in the experimental garden of the Aromatic and Medicinal Plants Department, Regional Research Laboratory, Bhubaneswar. They were cut into 0.5 cm length with single node intact. Then, they were washed in a mild, non-phytotoxic liquid detergent (2% Labolene; Qualigens, India), stirred for about 10 min and then washed in tap water. It was followed by a dip in a 0.1% (w/v) mercuric chloride ($HgCl_2$) solution for another 5 minutes. Finally, the axillary bud explants were washed thoroughly with sterile water before the inoculation onto sterilized nutrient agar media prepacked in culture tubes. All the above operations were performed under aseptic conditions in a laminar airflow cabinet.

MS basal medium (Murashige and Skoog 1962) was used throughout. For shoot induction, the medium was supplemented with 0-1.5 mg/l N^6-benzyladenine (BA) and 0.25 or 0.5 mg/l Indole-3-aceticacid (IAA) either individually or in combinations. For root induction, shoots were cultured on half-strength MS medium supplemented with either IAA (indole-3-acetic acid) or IBA (indole-3-butyriacid) in the concentrations of 0.5, 1.0 or 1.5 mg/l. The pH of the medium was adjusted to 5.8 before gelling with agar (8 g/l, Bacteriological grade, Qualigens, India). One explant per culture tube was used. All the cultures were incubated in a growth room with a 16 h photoperiod (cool, white fluorescent light - 30 µmol m^{-2} s^{-1}) and the temperature was maintained at 25 ± 2°C, with 50-80% relative humidity. Each treatment consisted of 10 replicates and repeated thrice.

Rooted plantlets were removed from the culture medium and the roots were washed under running tap water to remove agar. Then the plantlets were transferred to poly pots containing pre-soaked vermiculite (TAMIN, India) and maintained inside a growth chamber set at 28°C and 70-80% relative humidity. After three weeks they were transplanted to poly bags containing mixture of soil + sand + manure (FYM) in 1:1:1 ratio and kept under shade house for a period of three weeks .The potted plantlets were irrigated with Hogland's solution every 3 days for a period of 3 weeks.

RESULTS AND DISCUSSION

Bud break and shoot proliferation

The response of *D. gangeticum* nodal explants cultured on different shoot proliferation media over a period of six weeks is presented in table 1. Culture medium devoid of growth regulators (control) failed to stimulate the bud break response in the cultured explants even when the cultures were maintained beyond the normal observation period of four weeks. MS medium with growth regulator supplements produced better results in terms of percentage explant response, shoots/explant, average shoot length and average number of nodes produced per shoot. In such media combinations bud break was noticed within 6-8 days of culture (Figure 1 A & B). Of the combination tested, MS + BA (1.0 mg/l) + IAA (0.25 mg/l) elicited optimal response in which an average of 5.5 ± 0.3 shootlets (Figure 1C) with a mean shoot length of 3.5 ± 0.3 cm per explant was recorded. The second best shoot multiplication (4.5 ± 0.2) was obtained in the medium MS + BA (1.0 mg/l) with a mean shoot length of 2.5 ± 0.3 cm. Higher concentration of BA (1.5 mg/l) in combination with IAA (0.5 mg/l) showed callusing explants with fewer shoots. In such cultures shoots were stunted with a mean shoot length of 1.4 ± 0.2 cm. The dependence of cultured explants on bud break response and shoot multiplication has already been established and extensively discussed (George and Sherrington 1984). This has also been recently reported in the case of micropropagation of many other medicinal plants like *Hemidesmus indicus* (Patnaik and Debata 1996), *Gmelina arborea* (Thirunavoukkarasu and Debata 1998) and *Plumbago zeylanica* (Sahoo and Debata 1998).

In the present study, nodal explants of *D. gangeticum* showed significantly higher response in the medium with the combination of BA (1.0 mg/l) + IAA (0.25 mg/l). The quality of shoots and the overall growth response in terms of average shoot length was better in this growth regulator combination. A comparatively lower response was recorded when BA was used alone in the medium. Review of literature indicates that the addition of either of IAA or NAA in the culture medium improved the response in a number of species in terms of shoot

Table 1. Shoot formation in nodal explants of *Desmodium gangeticum* cultured on semisolid MS medium supplemented with various concentrations of BA and IAA (10 explants per treatment, data scored after 6 weeks)

Treatments (mg/l)		% Explant response	Days to bud break	Mean number of shoots/ explant ± S.E.	Mean shoot length (cm) ± S.E.	Mean number of nodes/ shoot ± S.E.
BA	**IAA**					
0	0	-	-	-	-	-
0.25	0	30	10-12	2.0 ± 0.2	1.5 ± 0.2	1.0 ± 0.2
0.5	0	50	10-12	2.5 ± 0.3	2.0 ± 0.1	2.5 ± 0.3
1.0	0	70	8-10	4.5 ± 0.3	2.5 ± 0.3	2.5 ± 0.3
1.5	0	70	8-10	3.5 ± 0.1	2.0 ± 0.3	2.0 ± 0.3
0.25	0.25	40	6-8	3.0 ± 0.2	2.5 ± 0.2	2.0 ± 0.2
0.5	0.25	60	6-8	4.0 ± 0.3	3.2 ± 0.1	2.2 ± 0.2
1.0	0.25	80	6-8	5.5 ± 0.3	3.5 ± 0.3	2.8 ± 0.2
1.5	0.25	60	6-8	3.0 ± 0.0	2.8 ± 0.2	2.5± 0.2
0.25	0.5	40	6-8	2.8 ± 0.2	2.2 ± 0.2	2.2 ± 0.2
0.5	0.5	50	8-10	2.2 ± 0.3	1.6 ± 0.5	2.0 ± 0.3
1.0	0.5	40	8-10	2.0 ± 0.3	1.4 ± 0.2	1.0 ± 0.3
1.5	0.5	50	8-10	1.8 ± 0.3	1.4 ± 0.2	1.0 ± 0.3

S.E. – Standard error of mean

Table 2. Influence of different levels of IAA and IBA on rooting response of *in vitro* generated shootlets of *Desmodium gangeticum* (10 replicates/treatment, data scored after 4 weeks of culture)

Growth regulators augmented with ½ strength MS basal medium (mg/l)		% Response	Days to root initiation ± SE	Mean root numbers	Mean root length (cm) ± SE
IAA	IBA				
0	0	-	-	-	-
0.5	0	-	-	-	-
1.0	0	40	10-12	2.8 ± 0.2	2.0 ± 0.3
1.5	0	40	10-12	2.5 ± 0.4	2.1 ± 0.1
0	0.5	30	8-9	2.5 ± 0.2	2.4 ± 0.1
0	1.0	70	6-8	3.5 ± 0.2	2.8 ± 0.1
0	1.5	50	6-8	2.6 ± 0.2	2.2 ± 0.1

S.E. – Standard error of mean

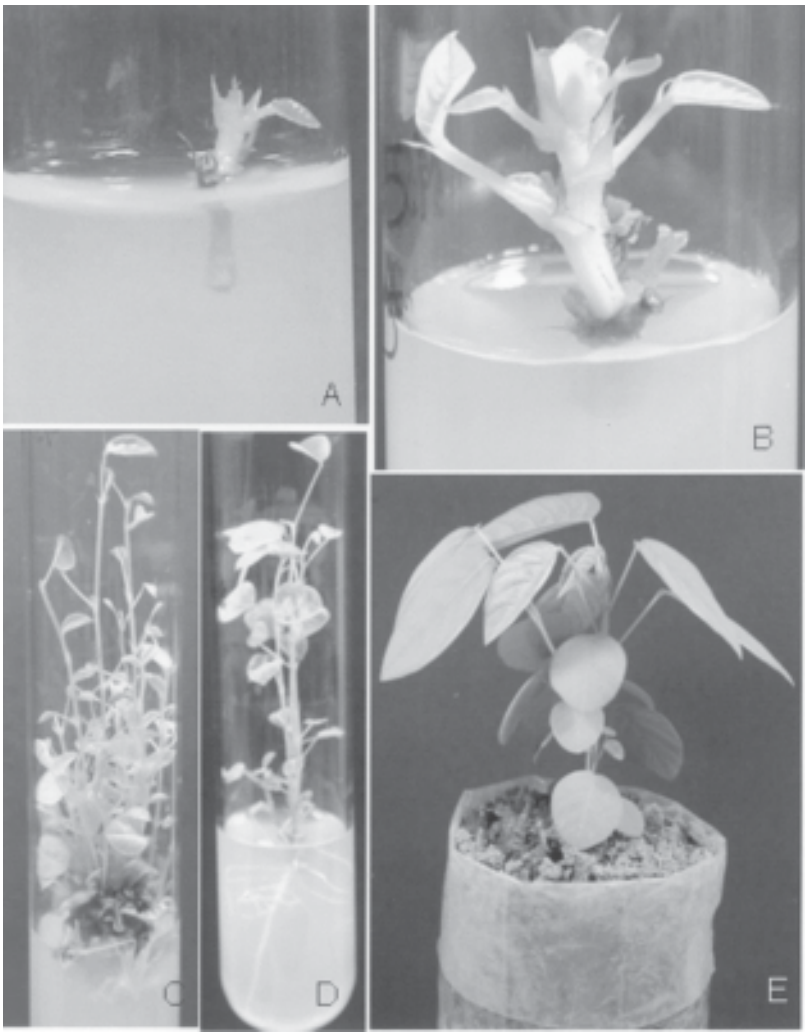

Fig. 1. Micropropagation of *Desmodium gangeticum* (L.) DC

A & B = Bud break in cultured nodal explants on MS + 1.0 mg/l BA + 0.25 mg/l IAA
C = Multiple shoot induction on MS + 1.0 mg/l BA + 0.25 mg/l IAA
D + *In vitro* shoot rooted on 1/2 MS + 1 mg/l IBA
E + Plant after 4 weeks of growth in a growth chamber

growth. It has been reported that shoots of *Spathiphyllum floribundam* when cultured on media with BA supplement alone, a limited proliferation of explants with a maximum of average of 1.8 shoots per cultured explant was observed, while addition of IAA produced an average number of 11.6 shoots per explant (Ramirez-Magon *et al*. 2001). Similar observation was reported in *Hovenia dulcis* nodal culture (Echeverrigaray *et al*. 1998). Hypocotyl explants of *Eucalyptus nitens* and *E. globulus* showed high regenerative capacity when they were cultured on to MS basal medium supplemented with BAP and NAA (Bandyopadhyay *et al*. 1999). Further observation revealed that addition of 2,4-D did not show regenerative ability of the explants. In the present work also it was observed that addition of IAA in low concentration (0.25 mg/l) showed improved response over BA alone.

Rooting and establishment of plants in soil

The rooting responses of shoots on different media, which included rooting percentage, days required for root

initiation, mean number of roots/shoot and mean root growth over a period of three weeks were different (Table 2). There was no rooting in case of shoots planted on auxin free basal media (control). Similarly, at lower level of IAA (0.5 mg/l) treatments, there was hardly any rooting in the cultured shoots during the 4 weeks observation period. However, higher concentration of IAA (1.0 & 1.5 mg/l) and IBA at all concentration tested responded well. Rooting was better in the culture which had combination of ½ MS + 1 mg/l IBA where about 70 % cultures responded for rooting with an average number of 3.5 ± 0.2 roots per plantlet and an average root length of 2.8 ± 0.2 cm was recorded (Figure 1D). The second highest response (50 %) was recorded at 1.5 mg/l of IAA and IBA. It was observed that root primordia emerged from the shoot base starting from day 6 to12 days after shoot inoculation and soon after that the root growth was rapid. IBA was more effective than IAA in induction of rooting as days required to rooting was only 6-8 days as against the 10 to 12 days required for similar response in case of the latter.

Production of plantlets with profuse rooting *in vitro* is important for successful establishment of regenerated plants in soil (Ohyama 1970). The auxins IAA and IBA were used singly to induce rooting from *in vitro* raised shootlets. A range of concentration of IAA and IBA was tested (0.5 – 1.5 mg/l) for rooting in the present studies. Both these auxins were effective in the concentration of 1.0 mg/l. In comparison to IAA, IBA even at 0.5 mg/l concentration produced better rooting response. Higher concentration of these auxins also resulted in basal callusing, reduction in root number/shoot as well as poor root growth. IBA has been widely used as a root-inducing hormone in difficult-to-root plants both under *in vitro* and *in vivo* conditions (Minocha 1987).

Upon transfer to vermiculite medium, plants started producing fresh shoots after one week of transplanting (Figure 1E). Later they were transferred to the field and the survival rate was 80 %. The efficient micropropagation technique described here may be highly useful for raising desirable genotypes of *D. gangeticum* for commercial cultivation.

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