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In Vitro Propagation of *Desmodium gangeticum* (L.) DC. from Cotyledonary Nodal Explants

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ABSTRACT

An *in vitro* procedure for rapid multiplication of medicinally important plant *Desmodium gangeticum* (L.) DC. (Fabaceae), has been developed using cotyledonary nodal explant. An average of 9.2 shoots per explant were obtained by culturing cotyledonary nodal explant on Murashige and Skoog's medium containing 8.8 μM BAP and 21.2 μM NAA, in combination, within 28 days. These shoots were rooted on half strength MS medium supplemented with IAA 17.1 μM. Rooted plantlets were hardened using 1:1:1 mixture of soil, river sand and vermiculite under green house conditions.

KEY WORDS: *Desmodium gangeticum* , cotyledonary nodes, regeneration, growth regulators.

ABBREVIATIONS: *BAP - Benzylaminopurine; IAA - Indole-3-acetic acid; IBA - Indole-3-butyric acid; Kn - Kinetin; MS - Murashige and Skoog medium (1962); NAA - α - Naphthaleneacetic acid; S.E.- Standard error.*

INTRODUCTION

Desmodium gangeticum (L.) DC. is an important Ayurvedic medicinal plant, belonging to family fabaceae. It is an erect woody under shrub, 2-4 feet high, grows wild in lower Himalayan regions and through out India. It is also found in Ceylon, Burma, Malay peninsula and Islands, China, Philippines and Tropical Africa (1-3).

Whole plant, mainly roots are used in medicine. The plant is a bitter tonic,digestive, antidysentric, alterative, aphrodisiac, antipyretic, anticatarrhal, febrifuge. It is used to cure typhoid and other fevers, asthma, bronchitis, vomiting, dysentery, piles, biliousness, chorela, scorpion sting and snake bite. The roots of *Desmodium gangeticum* (L.) DC. are used as one of the ingredients of two very important Ayurvedic preparations, '*Dashmoola Kwatha*' and '*Dashamoolarishta*' (4-5).

It is identified as promising plant, which is in great demand and of high commercial potential. Estimated domestic demand for *Desmodium gangeticum* (L.) DC. is about 678.4 tonnes/ year (6). The drug, *Desmodium gangeticum* (L.) DC. is mostly collected from wild sources to meet the requirment of pharmaceutical industries, as such no efforts have yet been made towards its cultivation except Dhan prakash *et al* (7). Department of Indian Systems of Medicine and Homeopathy, Ministry of Health and Family Welfare, Government of India, has formulated a Central Scheme for Cultivation and Development of Medicinal Plants. *Desmodium gangeticum* (L.) DC. is one of the species identified for promoting cultivation in order to reduce pressure on their natural habitat and to meet the shortage against the demand of the industry (8). Efforts on propagation of *Desmodium gangeticum* (L.) DC. by seed and stem cuttings have been successfully made(9-10). There is no report on tissue culture studies of *D. gangeticum* (L.) DC., though such studies on other species of *Desmodium* have been reported such as *D. Heterocarpon* (L.) DC. and *D. ovalifolium* Wall. (11). Therefore, efforts were made to evolve the techniques for rapid multiplication through tissue culture.

MATERIAL AND METHODS

Fresh mature seeds of *Desmodium gangeticum* (L.)DC., were collected from healthy plants growing in garden of Regional Research Institute, Pune, India. The plant

was identified with the help of Flora of British India and confirmed by comparing the authenticated herbarium specimen available in Botanical Survey of India, Western Circle, Pune. Herbarium was prepared, deposited in the herbarium section of the Institute with voucher specimen number 215. Seeds were treated with concentrated H_2SO_4 for 15 minutes to facilitate germination and thoroughly washed under tap water. The seeds were then soaked in 5% Teepol solution for 5 minutes and washed again under tap water. Later, the seeds were treated with 0.1% $HgCl_2$ solution for 1 minute followed by several times washing with sterile distilled water under aseptic condition and inoculated on MS plain medium containing 3 percent sucrose .The pH of medium was adjusted to 5.7 with 1N NaOH/1N HCl, before addition of 0.8 percent agar and autoclaved at 15 lb/Inch^2 pressure and 121^{0}C temperature for 20 minutes. In the initial stage of seed germination, cultures were kept in dark at 25^{0}C and 92% humidity, in Environmental test chamber, for 4-5 days. The cultures were, then transferred to culture room, where they were maintained at 25^0 C$\pm$ 2^0 C and 8/16- h (light/dark) photoperiod provided through white fluorescent tubes with light intensity of 3000 lux.

After germination of seeds, cotyledonary nodes of 1-1.5 cm length with both cotyledons were excised from 14 days old seedlings and cultured on MS medium (12) supplemented with BAP and Kn, singly or in combination with IAA or NAA, in different concentrations, by embedding 2-3 mm of the epicotyle end of the embryonic axis in the medium.

Well developed shoots regenerated from cotyledonary nodal explants were excised and rooted on half and full strength MS plain medium supplemented with or without IAA and IBA, singly, as well as in different concentrations.

Observations were made every day. Final observation was taken on 28th day, with respect to number of cultures responded for callusing, shooting and rooting, average number of shoots per explant, average height of the shoot, average number and length of roots per shoot. All the experiments were repeated thrice with 24 replicates. Data obtained from the experiments were analyzed for mean and standard error using software SYSTAT 11 (Stat Soft Inc.,).

Plantlets with well developed roots were removed carefully from culture tubes and washed under running tap water to remove medium sticking to the surface. Then the plantlets were dipped in 1% aqueous solution of Bavistin for 10 minutes and washed with water. The treated plantlets were transferred to small plastic pots containing sterile soil: river sand: vermiculite (1:1:1). The pots were covered with plastic bags to maintain high humidity, for 14-15 days. The plants were sprayed with half strength MS salt solution twice a week for a period of 2 weeks. Then the hardened plants were transferred to green house. Well grown plants were shifted to bigger earthen pots containing garden soil and farm yard manure, after 2-3 months and watered on alternate days.

RESULTS AND DISCUSSION

Effect of phytohormones on shoot regeneration from cotyledonary nodes

Cotyledonary nodal explants with both cotyledons were excised from *in vitro* grown seedlings were cultured on MS medium supplemented with BAP and Kn, singly or in combination with IAA or NAA, in different concentrations, to find out their influence on shoot regeneration(Fig.A). Cotyledonary nodal explants inoculated on MS plain medium showed callus formation, root initiation at cut end dipped in the medium and regeneration of 1-2 shoots, directly from axillary buds, at the end of 28 days. Cotyledonary nodes, which were inoculated on MS medium supplemented with different concentrations (4.6-18.4 μM) of kn also showed response towards shooting and callusing but failed to improve the number of proliferating shoots and on an average 2.7 shoots per explant were regenerated from cotyledonary nodal explants directly on MS+ Kn (9.2 μM) (Table 1).

However incorporation of BAP in the medium improved shoots proliferation. The number of shoots per explant was highest on MS medium containing BAP (8.8μM), singly, where over 3 shoots of 0.5 cm were regenerated from cotyledonary explants after 28 days. The number of shoots per explant increased with increase in concentration of BAP up to optimal level (8.8 μM). However increase in concentration of BAP resulted in decrease of shoot length. The number of shoots per explant declined when the concentration of BAP was increased beyond 8.8 μM (Table 2). This supports the findings of Gulati and Jaiwal (13) and Cheng *et al* (14). Effectiveness of BAP in shoot regeneration from cotyledonary nodes has been reported in several other species of leguminaceae e.g. *Dalbergia latifolia* Roxb. (15), *Arachis hypogea* L. (16), *Vigna mungo* (L.) Hepper (17), *Acacia nilotica* subsp. *indica* Brenan (18) and *Sterculia urens* Roxb. (19).

BAP was found to be more effective than Kn when added in MS medium, either in combination with IAA or NAA, in different concentrations. Addition of IAA or

Table 1: Effect of Kn on cotyledonary nodal explant of Desmodium gangeticum (L.) DC.

Medium	Shooting %	Callusng %	Av. No. of shoots ± S.E.	Av. height of shoots in cm ± S.E.
MS plain	100	100	1.2 ± 0.100	1.5 ± 0.470
MS + Kn (4.6µM)	100	100	2.6 ± 0.306	0.6 ± 0.153
MS + Kn (9.2µM)	100	100	2.7 ± 0.300	0.5 ± 0.116
MS + Kn (13.8µM)	100	100	2.1 ± 0.493	0.8 ± 0.100
MS + Kn (18.4µM)	100	100	2.1 ± 0.322	0.6 ± 0.100

Values are mean of 3 experiments, each with 24 replicates.

Table 2: Effect of BAP on cotyledonary nodal explant of Desmodium gangeticum (L.) DC.

Medium	Shooting %	Callusing %	Av. No. of shoots ± S.E.	Av. height of shoots in cm ± S.E.
MS plain	100	100	1.2 ± 0.100	1.5 ± 0.470
MS + BAP (4.4 µM)	100	100	2.8 ± 0.200	0.6 ± 0.058
MS + BAP (8.8µM)	100	100	3.0 ± 0.577	0.4 ± 0.116
MS + BAP (13.2µM)	100	100	2.1 ± 0.493	0.3 ± 0.058
MS+BAP (17.6µM)	100	100	2.0 ± 0.577	0.4 ± 0.153

Values are mean of 3 experiments, each with 24 replicates.

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Table 3: Effect of Kn+ IAA on cotyledonary nodal explant of Desmodium gangeticum (L.)DC.

Medium	Shooting %	Callusing %	Av. No. of shoots ± S.E.	Av. height of shoots in cm ± S.E.
MS + Kn (4.6µM) + IAA (5.7µM)	100	100	1.5 ± 0.289	3.7 ± 0.436
MS + Kn (4.6µM) + IAA (11.4µM)	100	100	1.6 ± 0.306	5.0 ± 0.322
MS + Kn (4.6µM) + IAA (17.1µM)	100	100	1.7 ± 0.351	5.3 ± 0.153
MS + Kn (4.6µM) + IAA (22.8µM)	100	100	1.2 ± 0.291	1.9 ± 0.404
MS + Kn (9.2µM) + IAA (5.7µM)	100	100	1.4 ± 0.306	2.3 ± 0.458
MS+ Kn (9.2µM) + IAA (11.4µM)	100	100	1.6 ± 0.306	3.2 ± 0.513
MS+ Kn (9.2µM) + IAA (17.1µM)	100	100	1.7 ± 0.153	3.5 ± 0.361
MS + Kn (9.2µM) + IAA (22.8µM)	100	100	2.0 ± 0.577	3.5 ± 0.504
MS + Kn (13.8µM) + IAA (5.7µM)	100	100	2.3 ± 0.300	1.7 ± 0.404
MS+Kn (13.8µM) + IAA (11.4µM)	100	100	1.8 ± 0.200	1.6 ± 0.208
MS+Kn (13.8µM) + IAA (17.1µM)	100	100	1.6 ± 0.521	1.5 ± 0.351
MS+Kn (13.8µM) + IAA (22.8µM)	100	100	1.5 ± 0.289	1.2 ± 0.200

Values are mean of 3 experiments, each with 24 replicates.

Table 4: Effect of Kn+ NAA on cotyledonary nodal explant of Desmodium gangeticum (L.) DC.

Medium	Shooting %	Callusing %	Av. No. of shoots ± S.E.	Av. height of shoots in cm ± S.E.
MS + Kn (4.6µM) + NAA (5.3µM)	100	100	1.1 ± 0.100	1.7 ± 0.300
MS+ Kn (4.6µM) + NAA (10.6µM)	100	100	1.1 ± 0.100	2.9 ± 0.351
MS+ Kn (4.6µM) + NAA (15.9µM)	100	100	1.2 ± 0.200	3.6 ± 0.328
MS+ Kn (4.6µM) + NAA (21.2µM)	100	100	0.9 ± 0.100	1.8 ± 0.153
MS + Kn (9.2µM) + NAA (5.3µM)	100	100	1.8 ± 0.200	1.7 ± 0.153
MS+ Kn (9.2µM) + NAA (10.6µM)	100	100	1.7 ± 0.153	3.1 ± 0.404
MS+ Kn (9.2µM) + NAA (15.9µM)	100	100	1.7 ± 0.379	3.7 ± 0.473
MS+ Kn (9.2µM) + NAA (21.2µM)	100	100	1.0 ± 0.000	2.8 ± 0.436

MS+ Kn (13.8µM) + NAA (5.3µM)	100	100	1.1 ± 0.100	4.4 ± 0.458
MS+Kn(13.8µM) + NAA (10.6µM)	100	100	1.2 ± 0.200	2.7 ± 0.351
MS+Kn(13.8µM)+ NAA (15.9µM)	100	100	1.5 ± 0.500	2.4 ± 0.493
MS+Kn(13.8µM) + NAA (21.2µM)	100	100	1.4 ± 0.116	1.8 ± 0.351

Values are mean of 3 experiments, each with 24 replicates.

Table 5: Effect of BAP+ IAA on cotyledonary nodal explant of Desmodium gangeticum (L.) DC.

Medium	Shoot-ing %	Callus-ing %	Av. No. of shoots ± S.E.	Av. height of shoots in cm ± S.E.
MS+ BAP (4.4µM) + IAA (5.7µM)	100	100	3.4 ± 0.167	1.9 ± 0.208
MS+BAP(4.4µM) + IAA (11.4µM)	100	100	3.5 ± 0.289	1.8 ± 0.116
MS+BAP(4.4µM) + IAA (17.1µM)	100	100	4.6 ± 0.306	1.7 ± 0.116
MS+BAP(4.4µM) + IAA (22.8µM)	100	100	3.7 ± 0.351	1.6 ± 0.306
MS+ BAP (8.8µM) + IAA (5.7µM)	100	100	1.9 ± 0.208	0.8 ± 0.058
MS+BAP(8.8µM) + IAA (11.4µM)	100	100	2.2 ± 0.416	0.9 ± 0.200
MS+BAP(8.8µM) + IAA (17.1µM)	100	100	2.9 ± 0.208	1.3 ± 0.306
MS+BAP(8.8µM) + IAA (22.8µM)	100	100	2.2 ± 0.153	1.0 ± 0.333
MS+BAP(13.2µM) + IAA (5.7µM)	100	100	3.0 ± 0.351	0.8 ± 0.067
MS+BAP(13.2µM)+IAA (11.4µM)	100	100	5.2 ± 0.436	1.0 ± 0.167
MS+BAP(13.2µM)+IAA (17.1µM)	100	100	6.6 ± 0.379	1.0 ± 0.133
MS+BAP(13.2µM)+IAA (22.8µM)	100	100	4.7 ± 0.351	0.8 ± 0.100

Values are mean of 3 experiments, each with 24 replicates.

Table 6: Effect of BAP+ NAA on cotyledonary nodal explant of Desmodium gangeticum (L.) DC.

Medium	Shoot-ing %	Callus-ing %	Av. No. of shoots ± S.E.	Av. height of shoots in cm ± S.E.
MS+BAP (4.4µM) + NAA (5.3µM)	100	100	4.0 ± 0.577	2.5 ± 0.406
MS+BAP(4.4µM)+ NAA (10.6µM)	100	100	3.2 ± 0.200	2.4 ± 0.346
MS+BAP(4.4µM)+ NAA (15.9µM)	100	100	2.7 ± 0.153	2.2 ± 0.351
MS+BAP(4.4µM)+ NAA (21.2µM)	100	100	2.6 ± 0.379	1.3 ± 0.173
MS+BAP (8.8µM) + NAA (5.3µM)	100	100	3.3 ± 0.300	1.3 ± 0.153
MS+BAP(8.8µM)+ NAA (10.6µM)	100	100	4.6 ± 0.306	1.2 ± 0.116
MS+BAP(8.8µM)+ NAA (15.9µM)	100	100	8.4 ± 0.306	1.0 ± 0.333
MS+BAP(8.8µM)+ NAA (21.2µM)	100	100	9.2 ± 0.757	0.8 ± 0.100
MS+BAP(13.2µM)+ NAA (5.3µM)	100	100	3.5 ± 0.500	0.6 ± 0.208
MS+BAP(13.2µM)+NAA(10.6µM)	100	100	3.4 ± 0.306	0.6 ± 0.208
MS+BAP(13.2µM)+NAA(15.9µM)	100	100	3.3 ± 0.351	0.5 ± 0.058
MS+BAP(13.2µM)+NAA(21.2µM)	100	100	2.5 ± 0.500	0.4 ± 0.058

Values are mean of 3 experiments, each with 24 replicates.

Table 7: Effect of IBA on in vitro rooting in Desmodium gangeticum (L.) DC.

Medium + Phytohormone	Rooting %	Av.number of roots ± S.E.	Av.length of roots ± S.E.	No. of days required for rooting
1/2 MS plain	100	3.3 ± 0.057	3.6 ± 0.145	7
MS plain	100	1.8 ± 0.115	1.1 ± 0.057	14
1/2 MS + IBA (4.9µM)	100	4.2 ± 0.153	7.6 ± 0.231	7
1/2 MS + IBA (9.8µM)	100	5.0 ± 0.500	8.1 ± 0.265	7
1/2 MS + IBA (14.7µM)	100	6.2 ± 0.416	10.0 ± 0.577	7
1/2 MS + IBA (19.6µM)	100	6.5 ± 0.500	10.8 ± 0.436	7
MS + IBA (4.9µM)	100	2.1 ± 0.100	2.3 ± 0.379	14
MS + IBA (9.8µM)	100	3.0 ± 0.577	2.8 ± 0.153	14
MS + IBA (14.7µM)	100	3.2 ± 0.200	2.9 ± 0.100	14
MS + IBA (19.6µM)	100	3.4 ± 0.400	3.7 ± 0.351	14

Values are mean of 3 experiments, each with 24 replicates.

Table 8: Effect of IAA on in vitro rooting in Desmodium gangeticum (L.) DC.

Medium + Phytohormone	Rooting %	Average number of roots ± S.E.	Average length of roots ± S.E.	No. of days required for rooting
1/2 MS+IAA (5.7µM)	100	6.1 ± 0.100	2.8 ± 0.152	7
1/2 MS+IAA (11.4µM)	100	6.8 ± 0.416	3.2 ± 0.252	7
1/2 MS+IAA (17.1µM)	100	10.0 ± 0.577	4.1 ± 0.153	7
1/2 MS+IAA (22.8µM)	100	6.9 ± 0.493	3.5 ± 0.321	7
MS+IAA (5.7µM)	100	7.6 ± 0.306	3.9 ± 0.208	14
MS+IAA (11.4µM)	100	8.5 ± 0.252	4.3 ± 0.300	14
MS+IAA (17.1µM)	100	10.3 ± 0.351	5.1 ± 0.100	14
MS+IAA (22.8µM)	100	5.8 ± 0.416	5.0 ± 0.577	14

Values are mean of 3 experiments, each with 24 replicates.

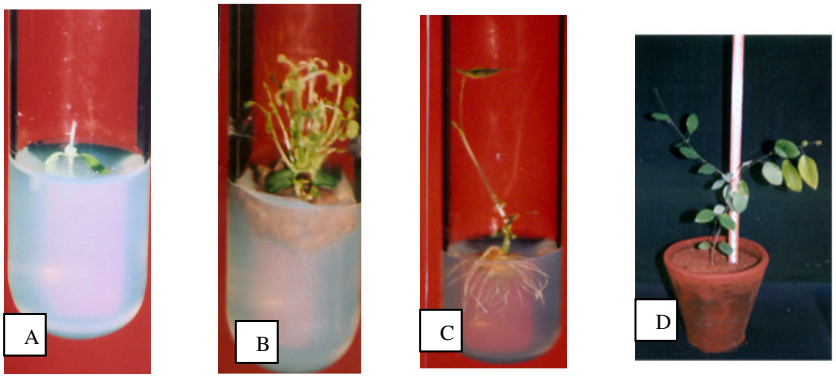

Fig. 1 (A - D)

A- *Cotyledonary nodal explant*

B- *Multiple shoot regeneration from cotyledonary node on MS+BAP(8.8µM)+ NAA(21.2µM)*

C- *Rooting of in vitro shoots on 1/2MS+IAA (17.1µM)*

D- *Hardened plant of Desmodium gangeticum (L.) DC.*

NAA in combination with Kn did not improve the number of shoots per explant except that the length of shoot was increased (Table 3 and 4). Highest number of shoots *i.e.*, 2.3 was observed in explants inoculated on MS+ Kn (9.2µM) + IAA (5.7µM), whereas when BAP in combination with IAA or NAA was incorporated in MS medium the number of shoots per explant was considerably increased. However, the number of shoots per explant was relatively lower on the medium supplemented with BAP + IAA as compared to BAP + NAA (Table 5 and 6). Highest number of shoots (9.2) per explant was obtained on MS+BAP (8.8 µM) + NAA (21.2µM), but these shoots failed to elongate, average shoot length being 0.8 cm only (Fig. B). Duhoux and Davies (20) reported regeneration of multiple shoots in *Acacia albida* Delille. from cotyledonary nodes inoculated on MS medium containing BAP and NAA in combination.

Effect of phytohormones on in vitro rooting

Root initiation was observed in shoots of *Desmodium gangeticum* (L.) DC. inoculated on both full strength and half strength MS medium supplemented with or without IAA or IBA (Table 7 and 8). IAA was found to be the best for rooting.100 percent rooting with maximum average number (10.3) of roots were obtained in shoots inoculated either on half strength or full strength MS medium supplemented with IAA (17.1µM) (Fig. C). Root initiation was found earlier in shoots inoculated on half strength MS medium either supplemented with IAA or IBA.

Hardening

The plantlets were transferred to small plastic pots containing sterile soil: river sand: vermiculite (1:1:1) for hardening (Fig. D). Fully developed rooted plantlets were transferred to field after acclimatization (hardening), where they showed 80% survival rate. This efficient and simple protocol reported herein could be useful for rapid multiplication of this medicinally important plant.

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