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Induction of Callus From Stem Explant By Using Auxins In *Desmodium gangeticum* (L.) DC

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ABSTRACT
Desmodium gangeticum (L.) DC is an important medicinal plant belongs to family Fabaceae (Leguminoceae). It is known as Salparni in Sanskrit. It is a sub-erect, under-shrub 0.6–1.2m high with irregular angled, branched woody stem. Leaves are unifoliate or trifoliate. Flowers are small, pink to purple in color. Whole plant or mainly the roots are used in medicines. It is used in Ayurvedic preparations like Dashmoola-Kwatha and Dashamoola-rishta. In the present investigation, an efficient protocol for callus induction of *D. gangeticum* is developed. The stem explant was inoculated with basal cut surface down on Murashige and Skoog medium supplemented with 0.25, 0.5, 0.75, 1 mg/l IAA, NAA and 2,4- D for callus induction. The better and profuse callus induction was found in MS medium supplemented with 0.5mg/l 2, 4 D from the stem explants.

Keywords: *Desmodium gangeticum*, Callus induction, Explants.

INTRODUCTION

Desmodium gangeticum (L.) DC belongs to family Fabaceae (Leguminoceae). It is known as Salparni in Sanskrit. It is a sub-erect, under-shrub 0.6–1.2m high with irregular angled, branched woody stem. Leaves are unifoliate or trifoliate. Flowers are small, pink to purple in color (Chopra et al., 1956). It is found in India, China, Africa, Australia, Ceylon, Burma, Malay Peninsula, Islands, Philippines and Tropical Africa (Anonymous, 1952; Cook, 1967; Hooker, 1973). Whole plant or mainly the roots are used in medicines. In Ayurveda, it is used to treat the various conditions such as snakebite, ulcer and diabetes (Dharmani et al., 2001), in asthma, bronchitis, dysentery, fever (Dharmani and Palit, 2006), in heart diseases (Kirtikar and Basu, 1935). It is used in Ayurvedic preparations like Dashmoola-Kwatha and Dashamoola-rishta (Kirtikar and Basu, 1935; Chopra et al., 1956). In the Ayurvedic system of medicines, it is used as an analgesic, antiarthritis, against cough, rheumatism, astringent, in diarrhea, tonic, diuretic, fever, biliousness, cough, vomiting, asthma, snake-bite, scorpion- sting (Anonymous, 1992).

The drug *D. gangeticum* is mostly collected from wild sources to meet the requirement of pharmaceutical industries. 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. *D. gangeticum* is one of the species identified for promoting the cultivation in order to reduce the pressure on natural habitat and to meet the shortage against the demand of the industry (Rawat and Sharma, 1998). It is identified as a promising plant which is in great demand and of a high commercial potential. An estimated domestic demand for *D. gangeticum* is about 678.4 tones/year (Anonymous, 2001). *In vitro* plant regeneration from various explants has been reported in *D. gangeticum* (Patil and Deokule, 2012, 2014, 2016) but there is no report on callus induction from stem explant. Due to its highly chemical properties, it is essential to study the plant for future benefits to meet basic need of chemical constituents. In the present study, the callus inductions have been studied from the stem explant supplemented with the various concentrations of auxins in MS medium.

MATERIALS AND METHODS

Collection and Identification of Plant Material

The plant material was collected from Western Ghats of Maharashtra, India. Efforts were made to collect the plant material in flowering and fruiting condition for the correct botanical identification and authentication. It was identified with help of Flora of Presidency of Bombay (Cook, 1967). Herbarium specimens were prepared and it was authenticated from Botanical survey of India, Pune. The voucher specimen number is BSI/WRC/Tech/2011/PAVNDGI.

Inoculation of Nodal Explants

The *in vitro* plant material were used after attaining the height about 15 -20 cm for shoot regeneration. The stem nodal explants were cut by scalpel and inoculated on Murashige and Skoog's (MS) medium (Murashige and Skoog, 1962) supplemented with 0.25, 0.5, 0.75, 1mg/l IAA, NAA and 2,4- D for callus induction. The pH of the medium was adjusted to 5.8 with 0.1N

NaOH / HCl before addition of 0.8% agar. Medium was autoclaved at 121°C at 15lbs for 20 min. The cultures were incubated at 25±2°C under photoperiod 16/8 hr (light/dark). The light source used was cool white florescent tubes providing an illumination of 2000/lux /m^2 /s. The explants were cultured under laminar air flow chamber. After four weeks, the data were recorded and were sub-cultured on the new medium of same concentrations.

RESULTS

In order to induce the callus in *D. gangeticum*, the stem explant was cultured on MS medium containing different concentrations of NAA, IAA, 2,4-D (0.25, 0.5, 0.75 and 1mg/lit). After 28 days the calli were harvested and calculated the moisture content and dry weight of callus. In the present study, we have evaluated the effect of auxins (IAA, NAA and 2,4- D) on stem explants. The results are presented in (Table 1, 2 and Figure 1). All the hormonal concentrations were found to be responsible for the development of callus from the stem explant, the 0.5mg/l 2, 4-D and 0.5mg/l IAA in MS medium were found to be the best hormone concentrations used for profuse callus induction among all the concentration. Moreover, the size and physical appearance of the calli formed on various concentrations of the same auxins did not show any difference except the color. As the concentrations of the auxins were increased, the size and appearance of the calli were found to be decreased. This is the first report of such induction of callus in various concentrations of auxins.

Table 1: Influence of Auxins alone for the production of callus in stem derived explant

MS + PGR/s (mg/l)			Moisture %	D. W. mg
IAA	NAA	2,4 – D		
Control			-	-
0.25			92.9%	157±1.6
0.5			93.6%	151±1.2
0.75			**93.7%**	**66±1.5**
1			91.8%	169±1.2
	0.25		92.6%	158±1.7
	0.5		92.9%	151±1.5
	0.75		**93.2%**	**164±1.9**
	1		91.8%	96±1.3
		0.25	94.4%	166±1.2
		0.5	**95.1%**	**182±1.6**
		0.75	92.2%	118±1.8
		1	91.3%	145±1.6

Table 2: Proliferation of callus on MS media fortified with BA in combination with Auxins.

MS + PGRs (mg/l)			Stem derived callus	
IAA	NAA	2,4-D	Callus response	Remark
Control			-	-
0.25			++	Callus fail to survive
0.5			++++	Callus proliferation
0.75			+++	Callus proliferation
1			++	Callus proliferation
	0.25		++	Callus proliferation
	0.5		++++	Callus proliferation
	0.75		++	Callus proliferation
	1		+	Callus fail to survive
		0.25	++	Callus proliferation
		0.5	++++	Callus proliferation
		0.75	++	Callus proliferation
		1	+	Callus fail to survive

Data scored after 4 weeks: Degree of callusing: - absent, + poor callus, ++ medium callus, +++ good callus, ++++ profuse callus, Nature of callus - friable yellowish white and greenish white in color.

0.5mg/l NAA 0.5mg/l IAA 0.5mg/l 2,4-D

Figure: Callus induction in various concentrations of auxins from stem explant

DISCUSSION AND CONCLUSIONS

Yellowish white and greenish white friable calli were developed from the stem explant in various concentrations of auxins. But the greenish white is found to be better calli for the fast growing, friable callus. Callus initiation was observed within 8 days and induction within 28 days. Stem explant was showing early and profuse callus induction in MS medium supplemented with 0.5 mg/l 2, 4-D and 0.5mg/l IAA. As we go to higher and lower hormone concentration to get optimum response of callus. Finally, the protocol of efficient callus induction from stem explant is selected in MS medium supplemented with 0.5 mg/l 2, 4-D and 0.5mg/l IAA for biotechnological appliances.

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REFERENCES

Anonymous, (1992) Preparation of kwath and Dashmulakwath in Bangladesh National Formulary of Ayurvedic Medicine, Approved by the Govt. of Bangladesh vide Ministry of Health and Family Welfare, Memo No. Health-1/Unani-2/89 I: 20-32, 116

Anonymous, (1952) The Wealth of India (Raw materials); CSIR New Delhi D-E : 41-43

Anonymous, (2001) Agenda for the meeting of medicinal plants board; (Annexure), Dept. of ISM & H, Ministry of Health and Family Welfare, Govt. of India, New Delhi, 2 : 910, 38, 44, 48-50, 55, 65

Chopra, R.N., Nayar, S.L. and Chopra I.C. (1956) Glossary of Indian Medicinal Plants. Council of Scientific and Industrial Research, New Delhi : 94

Cook, T. (1967) The Flora of Presidency of Bombay, Botanical Survey of India, Calcutta 13: 379-380

Dharmani, P. and Palit G. (2006) Exploring Indian medicinal plants for antiulcer activity. Indian J Pharmacol, 38 (2): 95-99

Dharmani, P., Mishra, P.K., Maurya, R., Chauhan, V.S. and Palit, G. (2001) *Desmodium gangeticum*: a potent anti-ulcer agent. Indian J Exp Biol, 43(6): 517-521

Hooker, J.D. (1973) Flora of British India; Reprinted edition, Periodical Experts, Delhi, 2 : 168

Kirtikar, K.R. and Basu, B.D. (1935) Indian Medicinal Plants; Vol.1 (Lalit Mohan Basu and Co., India : 204-212,758-760

Murashige, Y. and Skoog, F.A. (1962) Revised medium for rapid growth and bioassay with Tobacco tissue cultures, Physiol Plant, 15: 473-497

Patil, V.N. and Deokule, S.S. (2012) *In-vitro* seeds germination and plantlets development of *D. gangeticum* (L.) DC, International Journal of Current Research, 4(8): 21-24

Patil, V.N. and Deokule, S.S. (2014) Rapid *In vitro* regeneration of *Desmodium gangeticum* (L) DC, Asiatic journal of biotechnology resources, 4(3):48-53

Patil, V.N. and Deokule, S.S. (2016) High frequency of multiple shoot induction in *Desmodium gangeticum* (L.) DC. by using different concentrations of BAP, International Journal of Life sciences, A6: 101-104

Rawat G.S. and Sharma, A.K. (1998) Stratrergies for the conservation medicinal plants in the Himalayas; In Prosects of Medicinal Plants, (Indian Society of Plant Genetic Resources, New Delhi, India : 29-36