J. bio-sci. 22: 53-58, 2014 **ISSN 1023-8654**
http://www.banglajol.info/index.php/JBS/index

IN VITRO REGENERATION OF *INDIGOFERA VISCOSA* LAM.

E Rajabudeen[1], A Saravana Ganthi[2*] Sivasubramanian S[3], M Padma Sorna Subramanian[4]

[1] Dr. Zahir Husain College, Ilayankudi, Tamil Nadu, India
[2] Rani Anna Govt. College for Women, Tirunelveli, Tamil Nadu, India
[3] JJ College of Arts and Science, Pudukottai, Tamil Nadu, India
[4] Siddha Medicinal Plants Garden, CCRS, Mettur Dam, Tamil Nadu, India

Abstract

Context: *In vitro* propagation or tissue culture of plants offers a rapid means of producing large quality of clonal planting stock and propagation that are difficult to establish conventionally. Biotechnological tools are important for multiplication and genetic enhancement of the plants by adopting techniques such as *in vitro* regeneration and genetic transformations.
Objective: Effect of different plant growth regulators (PGRs) and their concentration on multiple shoot regeneration and callus formation was studied in *Indigofera viscosa*.
Materials and Methods: *In vitro* plant regeneration was achieved in nodal and shoot tip explants. The explants were cultured on MS medium supplemented with BAP and NAA.
Results: The nodal explants exhibited a greater number of healthy multiple shoots. The maximum callus induction was observed on MS medium supplemented with 1.5 mg L^{-1} 2,4-D. NAA and BAP combination proved to be the most effective treatment for promoting shoot multiplication. IBA was found to be the best rooting hormone than IAA or NAA. The plantlets showed high survival rate in the soil.
Conclusion: The present investigation clearly established and demonstrated the method of obtaining the new plantlets in *Indigofera viscosa* supported by different hormone concentrations

Key words: Callus, *In vitro*, *Indigofera viscosa*, nodal and shoot tip explants.

Introduction

Medicinal compounds from plants represent one of the largest and most diverse groups of plant secondary metabolites. *In vitro* propagation of medicinal plants with enriched bioactive principles and cell culture methodologies for selective metabolite production is found to be highly useful for commercial production of medicinally important compounds. Tissue culture protocols have been developed for several plants but there are many other species, which are over exploited in pharmaceutical industries and need conservation. It is now quite apparent that India has made big strides in the realm of biotechnology. The galloping rate, at which this advancement is continuing, holds out great promise for a better future.

The *Fabaceae* family (=Leguminosae) consists of approximately 650 genera and 18,000 species; it is one of the largest Angiosperm families (Polhill *et al.* 1981, Judd *et al.* 1999). Many plants of this family have been used in traditional systems of medicine. Still, several potent plants of *Fabaceae* are unexplored which deserve attention and research. *Indigofera viscosa* Lam. is such plant which has not been explored extensively by the scientific world so far. The genus *Indigofera* comprises around 700 species that are distributed geographically in tropical regions (Bakasso *et al.* 2008). *Indigofera viscosa* Lam. is herb distributed in hill slopes of southern peninsular, India. The macerate of the crushed whole plant is used as rectal application, twice a week for one week, to stop diarrhea (Kusamba 2001). The purpose of this research was to study the effect of various concentrations of growth hormones on multiple shoot induction and callus development.

* Corresponding author *E-mail:* saran_gan@rediffmail.com

Materials and Methods

Indigofera viscosa was collected from Wolf Hills, Sivanthipatti, Tirunelveli and grown in the greenhouse at Department of Botany, St. Xavier's College, Palayamkottai, Tamil Nadu, India. Nodal and shoot tip explants were collected from healthy plants and used for the present study. The explants were washed in running tap water for 30 minutes. Then they were washed in an agitated solution of liquid detergent 2% (w/v) (Teepol) for two minutes and rinsed in distilled water three times. Surface sterilization was performed by immersion of the explants in 70% (w/v) aqueous ethanol for 40 seconds followed by 0.1% (w/v) mercuric chloride for five minutes. Finally, the materials were thoroughly rinsed with sterile distilled water five times to remove the traces of mercuric chloride. All the explants were cut into pieces approximately 10–15 mm long for inoculation.

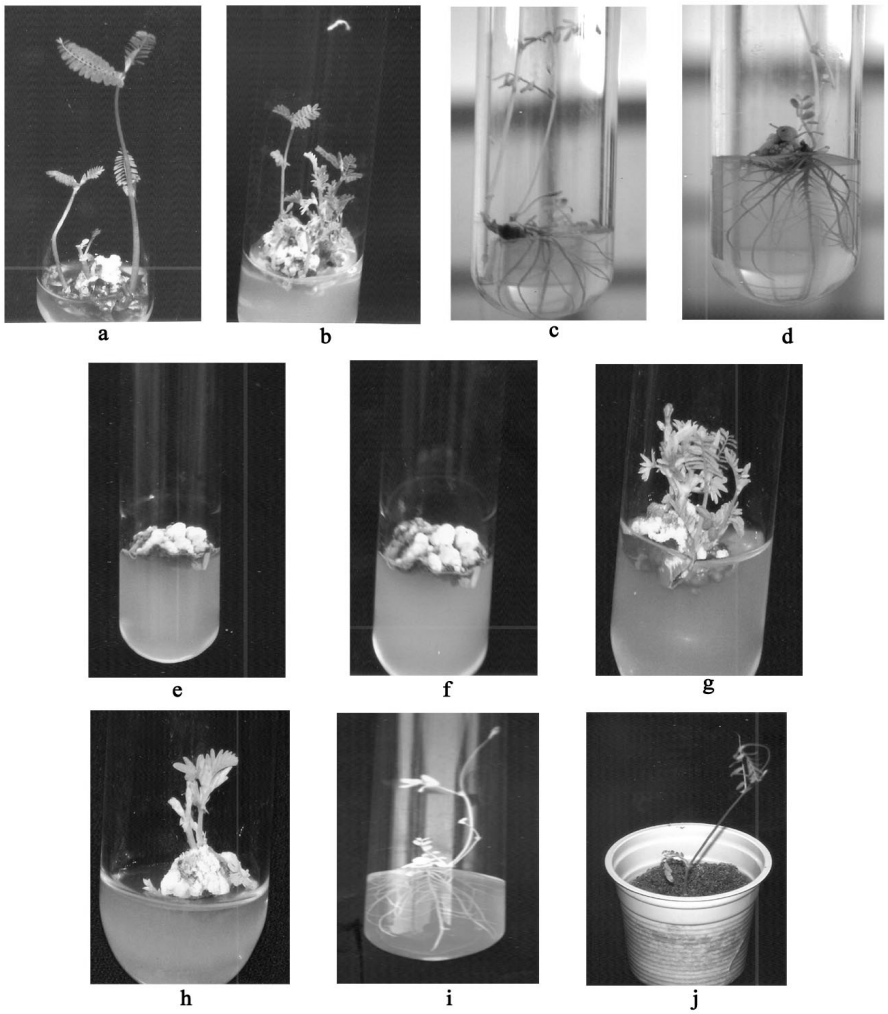
Shoot tip and nodal segments (0.5 cm) of the twigs were cut and cultured on to 8% (w/v) agar gelled MS medium supplemented with various growth regulators (NAA, IAA, IBA, BAP and Kn) at different concentrations and combinations. Sub-culturing was done at 14 - 21-day intervals. Shoot buds were further cultured for elongation in the same medium for two-times at 7-day intervals. Elongated shoot buds were rooted on half strength MS medium fortified with different concentrations of auxins (NAA and IBA) alone. The pH of the medium was adjusted to 5.8 before autoclaving. All cultures were incubated at 25 ± 2^{o}C under 16/8h photoperiods. After 12 weeks, plantlets with roots were successfully planted in pot soil through gradual acclimatization.

Results and Discussion

Multiple shoot formation

The present investigation was carried out to explore the morphogenic potential of *Indigofera viscosa* by using different combinations of growth regulators. The nodal explants resumed new bud growth by proliferating the axillary shoot within 1–2 weeks of culture. Nodal segments inoculated on MS medium fortified with Kn and NAA for the proliferation of axillary bud. Proliferation of axillary bud was observed on MS medium having Kn (1 mg L^{-1}) and NAA (0.5 mg L^{-1}) but these proliferated axillary buds remained undifferentiated till one month. However, axillary buds proliferated on NAA (1.5 mg L^{-1}) and BAP (1.0 mg L^{-1}) underwent multiplication of shoots (Fig.a). A notable frequency of shoot formation from node explants was observed (69.42%) on regeneration medium with NAA (2.5 mg L^{-1}) and BAP (2.0 mg L^{-1}) (Fig. b). Some of these shoots were rooted on the same medium (Fig.c, d). Within 4 weeks of inoculation, axillary meristem elongated up to 3.5 - 4 cm in height. NAA and BAP combination proved to be the most effective treatment for promoting shoot multiplication (3–5 shoots/explant, each shoot having 3 to 4 nodes) within four weeks of subculture (Table 1). Similar results were reported in legume trees *Acacia mearnsii* and *Albizia odoratissima* (Beck *et al.* 2001, Rajeshwari and Paliwal 2008). Contrary to the present results, modified MS medium along with BAP (1.5 mg/l) and BAP (1mg/l) in MS medium also stimulated the multiplication of shoots in *Acacia nilotica* and *A. senegal* respectively (Dewan *et al*. 1992, Khalafalla and Daffalla 2008).

In vitro studies on *Indigofera viscosa Lam.*



a. Induction of multiple shoots on MS medium containing NAA (1.5 mg L^{-1}) and BAP (1.0 mg L^{-1}) from nodal explant after 25 days of culture.
b. Induction of multiple shoots on MS medium containing NAA (2.5 mg L^{-1}) and BAP (2.0 mg L^{-1}) from nodal explant after 20 days of culture.
c.& d. Elongation and rooting of adventitious shoots in culture medium with BAP and NAA without intervening callus phase.
e. Callus formation on MS medium supplemented with 1.5 mg L^{-1} 2, 4 – D after 10 days of culture.
f. Callus formation on MS medium supplemented with 0.5 mg L^{-1} 2, 4 – D after 12 days of culture.
g. & h. Multiple shoot tip along with the callus at the base on MS medium supplemented with BAP and IAA
i. Microshoot rooted on MS medium supplemented with 0.2 mg L^{-1} IBA showing well developed root system from callus.
j. Hardened plant in polycup containing sterile soil and vermiculate

Fig. a - j.

Table 1. Effect of different concentrations and combinations of growth regulators in MS medium on direct proliferation of shoot from nodal and shoot tip explants of *I. viscosa.*

NAA mg L^{-1}	KIN mg L^{-1}	BAP mg L^{-1}	No. of explants used	Nodal explants			Shoot tip explants		
				% of regenerated shoots	Mean number of shoot explants	Mean length of shoot (in cm)	% of regenerated shoots	Mean number of shoot explants	Mean length of shoot (in cm)
0	0.5	0	20	-	-	-	-	-	-
0.5	1.0	0	20	45	5.0 ± 0.2	11.1 ± 0.4	60	3.2 ± 0.3	4.4 ± 0.2
1.0	1.5	0	20	50	5.3 ± 0.2	14.2 ± 0.2	45	3.8 ± 0.3	3.6 ± 0.5
1.5	2.0	0	20	65	6.2 ± 0.1	15.4 ± 0.1	50	3.3 ± 0.5	3.4 ± 0.3
2.0	2.5	0	20	55	5.1 ± 0.6	7.6 ± 0.5	35	1.8 ± 0.2	3.1 ± 0.5
2.5	3.0	0	20	60	2.5 ± 0.2	3.8 ± 0.4	15	1.6 ± 0.3	2.8 ± 0.4
0.5	0	0	20	-	-	-	-	-	-
1.0	0	0.5	20	60	4.3 ± 0.1	8.1 ± 0.2	35	4.74± 0.3	3.7 ± 0.7
1.5	0	1.0	20	70	5.2 ± 0.6	11.2 ± 0.3	55	2.58 ± 0.4	4.4 ± 0.4
2.0	0	1.5	20	40	4.7 ± 0.1	9.6 ± 0.2	15	3.42 ± 0.2	2.7 ± 0.2
2.5	0	2.0	20	70	4.2 ± 0.8	8.8 ± 0.1	25	3.32± 0.3-	3.5 ± 0.2
3.0	0	2.5	20	55	3.9 ± 1.1	7.3 ± 0.3	-		-

- No response

Callus induction

Callus induction was recorded on all regeneration media (Table 2). However, the intensity of callus formation increased with the increase of concentration of 2,4-D. Lower concentration of 2, 4 5 – T did not favour the callus induction. Callus was initiated from the node in 10 – 15 days. The maximum of 72.4% callus induction was observed on MS medium supplemented with 1.5 mg L^{-1} 2,4-D (Fig. e). 2,4-D supplemented media with 0.5 mg L^{-1} also showed the best response after 12 day of culture and 61.7% explants showed callus proliferation in this combination (Fig. f). Combination of 2,4-D and 2, 4, 5 –T is moderately support the growth of callus. All the concentrations of 2,4-D were proved better than 2, 4, 5 – T and the results were statistically significant. Generally the callus exhibits white colour and compact.

Shoot regeneration

Node and shoot tip explants derived calli were cultured on MS medium supplemented with various concentrations and combinations of BAP and IAA (shoot regeneration medium). Calli turned green compact and shoot buds were induced after four weeks of culture (Fig. g, h). All of the explants derived calli were not assorted in shoot bud regeneration. Higher frequency of shoot regeneration was obtained in MS medium supplemented with BAP (0.5 mg L^{-1}) and IAA (0.2 mg L^{-1}) (Table 3). The observation on the synergistic effect of BAP in combination with IAA on promotion of shoot regeneration of this medicinal plant species is in agreement with that of several plant species (Vidya *et al.* 2005). However, maximum number of shoots was obtained in MS medium fortified with IAA (0.2 mg L^{-1}). The beneficial (2 – 4 cm long) shoot length was observed in all the concentrations and combinations of shoot regeneration media after 8 weeks of culture.

Table 2. Response of different treatments of 2,4-D and 2, 4, 5 – T on callus induction on nodal and shoot tip explants in *I. viscosa.*

Plant growth regulators mg L^{-1}		Number of explants	Days to initiate callus	Nodal explants		Shoot tip explants	
2,4 - D	2, 4, 5 - T			% of success	Nature of callus	% of success	Nature of callus
0.5	-	25	10 – 15 days	60	White & compact	12	White & compact
1.0	-	25	10 – 15 days	56	White & compact	20	White & compact
1.5	-	25	10 – 15 days	72	White & compact	24	White & compact
2.5	-	25	10 – 15 days	60	Pale compact	-	-
3.0	-	25	-	-	-	-	-
-	0.5	25	-	-	-	-	-
-	1.0	25	10 – 15 days	24	White & compact	32	White & compact
-	1.5	25	10 – 15 days	20	White & compact	44	White & compact
-	2.5	25	10 – 15 days	48	White & compact	48	Pale Green
-	3.0	25	10 – 15 days	40	White & compact	-	-
0.5	0.5	25	10 – 15 days	44	Pale green	28	Pale compact
1.0	0.5	25	10 – 15 days	56	Pale greenish yellow	36	White & compact
1.5	0.5	25	10 – 15 days	32	Pale compact	40	Pale green
2.5	0.5	25	-	-	-		

Table 3. Shoot organogenesis from initiated callus on nodal and shoot tip explants in *I. viscosa.*

Treatment mg L^{-1}	Days to initiate shoots	Number of explants	Node		Shoot tip	
			% of success	Mean No. of multiple shoots	% of success	Mean No. of multiple shoots
MS + BAP 0.2	15 – 20	25	12	2.2	12	1.4
MS + BAP 0.5	15 – 20	25	12	2.3	20	2.2
MS + IAA 0.2	15 – 20	25	32	3.9	20	2.5
MS + IAA 0.5	15 – 20	25	44	2.1	32	3.4
MS + BAP 0.5 + IAA 0.2	15 – 20	25	72	3.1	36	3.0

Rooting of regenerated shoots

Elongated shoots were excised from regeneration medium and then transferred to MS medium supplemented with 0.2 – 1.0 mg L^{-1} IBA or NAA (Table 4). However, IBA was found to be the best rooting hormone than IAA or NAA. Similar results are well documented (Rehman *et al.* 2003). Higher frequency (81.2%) of root induction was obtained in MS medium supplemented with 0.2 mg L^{-1}IBA (Plate: i).The maximum number of roots was observed in 0.6 mg

L^{-1}IBA. However, the root length was varied in each treatment. MS medium supplemented with IBA (0.8 mg L^{-1}) was produced thick and hair-like secondary roots.

Table 4. Effect of growth hormones on average rooting of *in-vitro* developing shoots of *I. viscosa.*

Plant growth regulators mg L^{-1}		% of rooting	No. of roots per shoots	Average root length (cm)
IBA	0.2	81.2 ± 2.6	3.6 ± 0.1	3.4 ± 0.6
	0.4	47.2 ± 2.2	4.4 ± 0.1	6.4 ± 0.71
	0.6	35.8 ± 2.6	5.2 ± 0.6	8.2 ± 0.6
	0.8	46.5 ± 1.1	2.1 ± 0.4	9.1 ± 0.2
	1.0	32. 3 ± 1.4	1.5 ± 1.5	5.5 ± 0.3
NAA	0.2	18.62 ± 1.5	3.0 ± 0.3	2.4 ± 0.3
	0.4	22.2 ± 2.6	4.9 ± 0.1	4.9 ± 0.1
	0.6	43.2 ± 1.7	3.2 ± 0.2	5.2 ± 0.2
	0.8	25.8 ± 0.6	3.6 ± 0.1	3.6 ± 0.1
	1.0	-	-	-

- Values are the mean of three replicates with 15 explants.

Acclimatization and establishment in soil

The rooted plantlets were carefully taken from the culture tubes and then washed with tap water and followed by sterile water. After washing, the rooted plantlets were successfully transferred to poly cups containing sterile soil and vermiculate (1:1). The acclimatized plantlets were successfully established in the field with 70% survivability (Fig. j).

References

Bakasso S A, Lamien-Meda C E, Lamien M, Kiendrebeogo J, Millogo A G, Ouedraogo Nacoulma O G. 2008. Polyphenol contents and antioxidant activities of five *Indigofera* species (*Fabaceae*) from Burkina Faso, *Pak. J Biol Sci* 11, 1429-1435. http://dx.doi.org/10.3923/pjbs.2008.1429.1435, PMid: 18817242

Beck S L, Dunlop R, Staden J V .2001. Meristem culture of *Acacia mearnsii*, *Plant Growth Regulation* 3, 49-58.

Dewan A, Nanda K, Gupta SC. 1992. *In-vitro* micropropagation of *Acacia nilotica* subsp, i*ndica Brenan* via cotyledonary nodes, *Plant Cell Report* 12, 18–21. http://dx.doi.org/10.1007/BF00232415,PMid:24201723

Polhill R M, Raven P H Stirton C H. 1981. Evolution and systematics of the Leguminosae, In: *Advances in legume systematics* edited by RM Polhill & PH Raven, Part 1, (Royal Botanic Gardens, Kew), 425.

Judd W S, Campbell C S, Kellogg E A, Stevens P F.1999. Plant Systematics: a phylogenetic approach. (Sinauer Associates, Sunderland
p. 464.

Kusamba Chifundera. 2001. Contribution to the inventory of medicinal plants from the Bushi area South Kivu Province Democratic Republic of Congo, *Fitoterapia* 72, 351-368. http://dx.doi.org/10.1016/S0367-326X(00)00294-X

Rajeshwari V, Paliwal K. 2008. *In-vitro* adventitious shoot organogenesis and plant regeneration from seedling explants of *Albizia odoratissima* L.f. (Benth.) *In-vitro cellular and Developmental Biology* 44 (2), 78-83. http://dx.doi.org/10.1007/s11627-008-9120-7

Khalafalla M M, Daffalla H M. 2008. *In-vitro* Micropropagation and Micrografting of Gum Arabic Tree, *Acacia senegal* (L.) Wild. *Internationa J Sustainable Crop Production* 3(1), 19 -27.

Vidya S M, Krishna V, Manjunatha B K, Shankar Murthy K. 2005**.** Micropropagation of *Entala pursaetha* DC. – An endangered medicinal plant of Western Ghats, *Indian J Biotech* 4, 561 – 564.

Rehman M M, Amin M N, Jahan H S, Ahmed R, 2003. *In-vitro* regeneration of plantlets of *Curcuma longa* L. a valuable spice, *Asian J Plant Science* 3, 306-3.