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In vitro propagation of *Indigofera trita* L. F-Highly reputed medicinal plants

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ABSTRACT

Protocol for rapid in vitro propagation of Indigofera trita through nodal explants multiplication was established. Murashige and Skoog (MS) medium with 2 mg/l N-benzyladenine (BA) was best suited for nodal proliferation. Excision and culture of the nodal segments from the In vitro shoots on fresh medium with same concentrations of BA facilitated development of more than 20 shoots / node. Subsequent culturesenhanced the rate of shoot proliferation. Shoots developed were rooted best on half strength MS with 2 mg/l IBA. Starting from a single node explants, 150 rooted shoots were obtained within 125 days. Plantlets established in pots exhibited 75% survival. Micropropagated plants established in garden were uniform and identical to the donor plant with respect to morphological and cytological characteristics.

Key words: *Indigoferatrita,In vitro, plantlet production, nodal explants.*

INTRODUCTION

The conservation and sustainable use of threatened plants is essential to meet the demand for future food security, agricultural productivity and environmental management.According to the reports prepared by the All India Coordinated Research Project of Ethanobiology of Govt. of India, out of 8000 species of medicinal plants, almost 1000 are estimated to be threatened, due to the indiscriminate collection from the natural population. So, there is a need to development of alternative methods of propagation for the mass production of threatened genetic resources, *Indigofera trita* is belonging to the Fabaceae family and livingin the Kolli hill of Tamil nadu[11].It is an under shrub, branches hoary with fine apprised hairs. Leaves trifoliate, leaflets all obovate-oblong with fine gray hairs,flowers small, 6-12 flowers in spicate racemes, salmon coloured. Pods rigid, straight, 4-gonous, spine pointed, not tortulose,silvery with apressed hairs. Seeds 6-10 oblong, they have wide distribution, mostly found in India, Ceylon, South Africa and North Australia[13]. The plant is known as Kattuavuri in Tamil. It is used by the tribes and native medical practioners to treat much kind of diseases such as rheumatism[12,14,15]. The entire plant is traditionally used for various ailments including liver disorders and tumors[9,5,16]. The plants also have strong antioxidant and hepatoprotective activity[10].The antitumor activity of ethanol Extract of *I. trita*was evaluated in Swiss albino mice. The plant also possesses anti-inflammatory and analgesic activity[6]. Therefore in the present study, an attempt has been made to mass propagate this plant through tissue culture. Efficient plant regeneration via nodal explants has been developed. To our knowledge this is the first report on *In vitro* propagation of *I. trita* through nodal explants.

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MATERIALS AND METHODS

Plants were collected from the wild population. The nodal explants were excised and washed with tap water several times. Then it was washed with 5% teepol solution and then rinsed with distilled water for the removal of detergent. Then surface sterilization was done with 0.1% $HgCl_2$ solution for 4-8 minutes and the explants were washed with sterile distilled water. The sterilized explants inoculated in MS medium[7]containing different concentration of cytokinins (BAP, KIN,2,4-D) the nodal explants were inoculated and maintained at 25±2°C 16 hrs light period. After the regeneration of the shoots, they were transfer into rooting medium supplemented with IBA .the rooted plantlets were transferred to the plastic cups containing vermiculate supplemented with ½ strength MS liquid medium. The seedlings were covered with perforated polythene bags and maintained for 7 days, and then they were maintained without polythene bag for another week. The acclimatized seedlings in the plastic cup were transferred to garden soil with vermiculate, FYM and sand (1:1:1) under controlled environmental conditions for 7 days. The hardened plantlets were transferred to the garden.

RESULT AND DISCUSSION

The surface sterilized nodal explants were inoculated in MS medium supplemented with different concentration of cytokines (BAP, KIN, 2,4-D (0.5 mg/l -3 mg/l)). Among the three, BAP shows the best result of proliferation. Shoots were regenerated after 15 days of inoculation. Maximum number of shoots was proliferated at BAP (2 mg/l) (Table.1) similar results were observed in *S.Lycopersicoidos*[3],*Curculigo orchioides*[2].Shoots of above 2cm length was harvested from the explants. The original explants were sub-cultured on the same fresh medium for further shoot production.

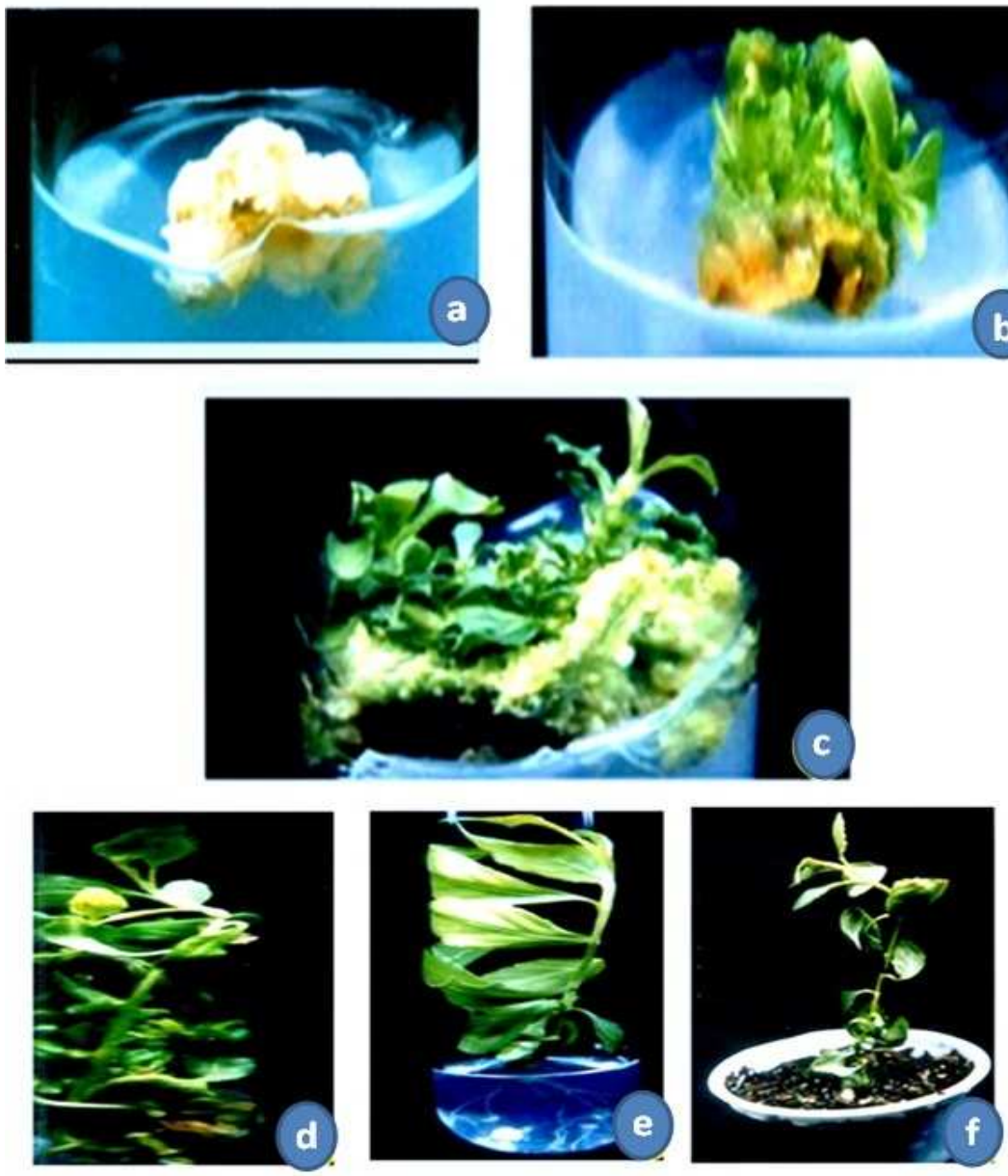
Rooting

The elongated shoots were harvested and transferred to the rooting medium. This medium is supplemented with different concentrations of auxins for root induction. Root ignition started after 7th day of inoculation. High frequency of root induction was observed in the concentration of 2 mg/l of IBA (Table.2 andFigure 1).Similar results was obtained in *Cayratia pedata*[1],*Datura metel*[8],*L.esculentum*[4]. The rooted plant lets were transplanted to the sterilized vermiculate in plastic pots and were moisturized ½ strength medium, then successfully transferred to the soil after hardening(Figure 1) the survival rate of the hardened plantlets were 75%.

Table 1: Effect of different concentrations of BAP, KIN and 2,4-D on shoot regeneration from nodal explants of *I. trita*

Growth regulators(mg/l)	Percentage of shoot proliferation	Shoot length(cm)	No. of shoot/plant Mean ± SD
BAP			
0.5	76.6	2.8	33.0 ± 1.28
1.0	83.3	3.0	39.3 ± 0.66
1.5	90.0	3.2	48.8 ± 1.26
2.0	**96.2**	**3.9**	**56 .5 ± 0.30**
2.5	94.3	3.0	48.8 ± 1.25
3.0	90.0	2.9	40.6 ± 1.56
KIN			
0.5	34.8	2.0	15.0 ± 1.05
1.0	38.3	2.1	18.0 ± 1.37
1.5	43.2	2.2	22.8 ± 1.05
2.0	**51.3**	**2.6**	**25.0 ± 1.94**
2.5	48.7	1.9	20.0 ± 1.98
3.0	40.9	1.6	19.6 ± 0.90
2,4-D			
0.5	22.8	1.0	3.0 ± 0.75
1.0	25.0	1.2	4.0 ± 0.52
1.5	26.0	1.5	5.5 ± 0.91
2.0	27.8	1.3	8.0 ± 0.73
2.5	**38.6**	**2.3**	**9.8 ± 0.12**
3.0	29.6	1.2	5.4 ± 0.40

Fig. 1: Plant regeneration from nodal explants of *Indigofera trita*



(a) Initiation of shoots (b) Shoot bud formation (c) Multiple shoots (d) Shoot elongation (e) Rooting (f) Hardening

CONCLUSION

The findings of this study would help in the conservation and propagation of *Indigoferatrita* and provide a possible lead to the synthesis and extraction of active compounds form the plant.

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