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Efficient *in vitro* Regeneration of a Medicinal Plant Harsinghar (*Nyctanthes arbor-tristis* L.)

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Abstract

In vitro propagation of *Nyctanthes arbor-tristis* L. has been successfully established from axillary bud explants on MS. Maximum number of multiple shoots was obtained on MS containing BAP (22.2 μM). Half strength of MS (2% sucrose) supplemented with NAA (10.74 μM) provided the maximum frequency of root initiation. The plantlets were successfully hardened.

Introduction

Medicinal plants are the natural and safer source of phytochemicals to fight against new strains of a microorganism. Currently, a great deal of public interest is witnessed in the use of herbal remedies; furthermore many western drugs had their origin in plant extract (Arulmozhi and Sathiya 2007). Due to rapid destruction of habitat the natural populations of a number of medicinal plants have been reduced considerably and several have become extinct. Among the different approaches, their *in vitro* culture provides new means of conserving and rapid propagation of valuable, rare and endangered medicinal plants (Karuppusamy and Pullaiah 2007).

Nyctanthes arbor-tristis L. (Oleaceae), commonly known as 'Harsinghar' is a valuable medicinal plant, and grows in Indo-Malayan region. It is cultivated in gardens almost throughout India (Nair and Mahanan 2001). Different parts of this plant species are known to cure various ailments (Vats et al. 2009). Flowers are used as stomachic, carminative, astringent for bowel, antibilious, expectorant, hair tonic and in the treatment of piles, skin diseases, etc. (Khatune et al. 2003).

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Powdered stem bark is used to treat rheumatic joint pain, malaria (Suresh et al. 2010), and bronchitis (Kirtikar and Basu 1993). Leaves are used as a laxative, diaphoretic, diuretic (Tuntiwachwuttiku et al. 2003), and against high blood pressure and diabetes (Nawaz et al. 2009). Seeds are used as anthelmintic and for treating alopecia and bilious fevers (Nair et al. 2005), affections of scalp, piles and skin disease (Sasmal et al. 2007). Leaves contain β-amyrin, β-sitosterol, benzoic acid, nyctanthic acid (Talakal et al. 2000). Seeds contain fixed oil having glucosides of linoleic, oleic, stearic, palmitic acid and β-sitosterol.

Due to poor seed germination on account of the presence of phenolic compounds and alkaloids in the pericarp and seed coat (Bhattacharya et al. 1999), many young seedlings die under natural conditions (Anon. 1988). The present study therefore aims at developing an efficient protocol for *in vitro* propagation of this medicinally important plant *Nyctanthes arbor-tristis*.

Materials and Methods

Healthy plants of *Nyctanthes arbor-tristis* L. were collected from the nursery of Jawaharlal Nehru Krishi Vishwavidyalaya, Jabalpur. Axillary buds were excised into 0.5 - 0.7 cm and washed under running tap water to remove soil and other superficial contamination.

To minimize contamination caused by fungus, endogenous and exogenous bacteria, explants were surface sterilized for 2 min in 70% (v/v) ethanol and surface disinfected with an aqueous solution of 0.1% $HgCl_2$ for 3 min. The disinfectant was removed by rinsing the material with sterilized distilled water five to six times. The explants were then dried on sterile filter paper, sectioned and aseptically inoculated to the medium.

The culture media consisted of MS basal constituents supplemented with different concentration BAP and Kn. The media were supplemented with 3% sucrose and 0.8% agar was used as the gelling agent. The pH of the media was adjusted between 5.6 - 5.9 by 0.1 N NaOH and autoclaved at 121ºC, 15 lb pressure for 45 min. The cultures were maintained at 25 ± 2ºC, 16/8 hr (light/dark) photoperiod with a light intensity of 1500 lux at relative humidity (RH) of 60 - 70%. For root induction *in vitro* grown 8 weeks old micro-shoots were transferred to half strength of MS with 2% sucrose devoid of agar supplemented with different concentrations of IBA, NAA and IAA, respectively. Plantlets with well grown roots were removed from the culture tubes; dipped in 1% bavistin solution, washed in tap water and then transferred to poly cups containing sterilized soil: sand: farmyard manure (1 : 1 : 1). The regenerants were hardened for four weeks, by covering the cups with a thin perforated transparent

polythene bag to maintain humidity and were watered with distilled water for first week then with tap water.

Results and Discussion

The effect of cytokinins and auxins on morphogenesis of axillary bud explants are presented in Tables 1 and 2. Emergence of multiple shoot buds from axillary explants on MS supplemented with BAP was observed. Nodal explants were found to be the best source of multiple shoot induction. A number of authors have also suggested the same type of explants for propagation of other medicinal plants, such as *Rouwolfia serpentina* (Roy et al. 1995), *Emblica officinalis* (Rahaman et al. 1999), *Holarrhena antidysenterica* (Ahmed et al. 2001) and *Enicostemma hyssopifolium* (Seetharam et al. 2002).

Table 1. The effect of PGRs on regeneration from axillary buds of *N. arbor-tristis* (Values are Mean ± SE).

Sl.No	PGR	Conc. (μM)	FSI (%)	MSN	MSL (cm)	MNN
1.	Control	0	52.77	1.66±0.130	0.81±0.080	-
2.	BAP	0.44	84.37	2.86±0.294	2.41±0.169	1.86±0.191
3.		2.22	81.25	4.26±0.407	4.18±0.321	2.6±0.235
4.		4.44	75.00	7.13±0.653	3.26±0.306	2.2±0.200
5.		6.66	**93.75**	8.53±1.004	2.82±0.174	2.13±0.273
6.		17.76	84.35	13.46±0.950	0.91±0.086	1.33±0.125
7.		22.2	90.62	**14.13±0.999**	0.813±0.072	1.20±0.118
8.	Kn	0.46	71.87	1.8±0.144	2.90±0.219	2.53±0.153
9.		2.32	43.7	2.33±0.270	3.05±0.210	2.6±0.190
10.		4.64	43.9	4.26±0.383	3.63±0.220	2.73±0.228
11.		6.96	46.87	3.4±0.305	**4.87±0.276**	**4.13±0.169**
12.		9.28	71.86	2.06±0.236	4.04±0.225	3.13±0.191
13.		23.2	53.12	1.73±0.210	2.20±0.322	1.93±0.266

FSI = Frequency of shoot initiation, MSN = Mean shoot number, MSL = Mean shoot length, MNN = Mean node number.

Different degrees of morphogenetic response were observed in the presence of BAP and Kn. Explants inoculated on BAP showed better response as compared to Kn. The maximum FSI (93.75%) was observed on BAP (6.66 μM) supplemented medium (Table 1) contrary to the previous reports of Jahan et al. (2011) who reported low regeneration frequency on this concentration. Multiple shoots were also observed on different concentrations of BAP (0.44, 2.22, 4.44, 6.66, 17.76, 22.2 μM). Similar results were obtained by Siddique et al. (2006) while

the contrary was reported by Rout et al. (2007). Maximum number of shoots (14.13 ± 0.999) was obtained on MS supplemented with BAP 22.2 µM (Table 1, Fig. B). The maximum shoot length (4.87 ± 0.276) was obtained on Kn supplemented medium (6.96 µM) (Fig. D) contrary to the previous reports by Rout et al. (2007).

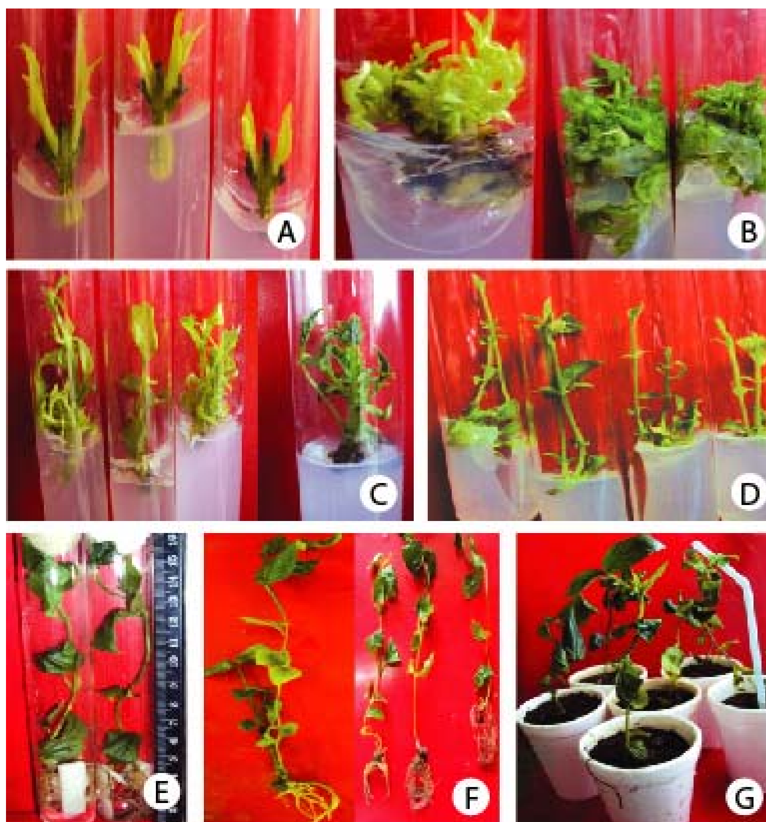

Fig. 1A-G: A. Shoot initiation on different concs. of PGRs, B. Multiple shooting on MS + BAP (22.2 µM), C. Shoot elongation in BAP, D. Shoot elongation in Kn, E. Rooting of *in vitro* shoots, F and G. Hardening and acclimation of *in vitro* regenerated plants.

Rooting failed to occur on the full strength of MS (3% sucrose) but half strength of MS with 2% sucrose was found suitable for root induction. Similar reports were observed by Rout et al. (2007). Individual shoots when inoculated in half strength of MS (2% sucrose) supplemented with auxins, enhanced the rate of rooting. The maximum frequency of root initiation (83.33%) was obtained on half strength of MS (2% sucrose) in combination with NAA (10.74 µM). Maximum mean root number (13.2 ± 1.8) was obtained on IBA (9.86 µM) in combination with half strength of MS and 2% sucrose (Table 2, Fig. E).

Table 2. Rooting from *in vitro* regenerated shoots of *N. arbor-tristis* (Values are Mean ± SE).

PGR	Conc. (µM)	MSL (cm)	MNN	FRI (%)	MRN	MRL
½ MS 2%	-	9.32 ± 0.335	7.26 ± 0.266	-	-	-
½ MS 2%	0.49	8.91 ± 0.746	6.4 ± 0.400	-	-	-
+ IBA	9.86	11.91 ± 0.400	7.4 ± 0.272	33.33	13.2 ± 1.826	2.54 ± 0.512
	26.85	11.23 ± 0.640	7.8 ± 0.261	16.66	8.06 ± 0.441	4.92 ± 1.097
½ MS 2%	0.57	9.94 ± 0.601	7.33 ± 0.287	-	-	-
+ IAA	11.4	12.89 ± 0.733	7.4 ± 0.235	33.33	6.26 ± 0.602	2.226 ± 0.218
	28.55	7.93 ± 0.277	6.06 ± 0.206	-	-	-
½ MS 2%	0.53	7.85±0.408	5.8 ± 0.380	-	-	-
+ NAA	10.74	10.36 ± 0.532	7.46 ± 0.363	83.33	6.93 ± 0.402	1.82 ± 0.245
	26.85	11.69 ± 0.291	6.06 ± 0.206	-	-	-

FRI = Frequency of root initiation, MSL = Mean shoot length, MNN = Mean node number, MRN = Mean root number, MRL= Mean root length.

When shoots of the *in vitro* regenerated plantlets attained a height of 9-13 cm bearing healthy shoots and a good root system, the plants were subjected to hardening (Fig. F, G). They were subsequently transferred to poly cups containing presterile soil : sand : farmyard manure (1 : 1 : 1) covered with perforated polythene. Finally the plantlets were transferred to field. Approximately 90% plantlets survived.

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