

In vitro propagation of *Nyctanthes arbor-tristis* L.

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Mass propagation of tree species has always been a challenging task. An efficient protocol for rapid *in vitro* mass propagation of *Nyctanthes arbor-tristis* L. has been developed from shoot tip and internodal explants. Explants were cultured on Murashige and Skoog (MS) medium supplemented with 6-benzylamino purine (BAP) and thidiazuron (TDZ) in various concentrations. 2.0 mg/l BAP was proved to be best for high frequency multiple shoot induction from shoot-tip explants and 2.5 mg/l BAP was produced maximum shoots proliferation from internodal explants. The well elongated healthy shoots were transferred for rooting on MS medium supplemented with indole-3-butyric acid (IBA) in different concentration. Maximum numbers of roots were achieved in 2.0 mg/l of IBA medium. The rooted plantlets were hardened and transferred to field conditions. All the regenerated plants with 80-85% survival rate were normal with respect to morphology and growth characteristics.

Keywords: *In vitro* propagation, *Nyctanthes arbor-tristis*, plant regeneration, culture condition.

Introduction

Night jasmine or coral jasmine *Nyctanthes arbor-tristis* L., belonging to the family Oleaceae, is a woody small tree, with greenish white rough bark. It is native of India, occurring wild in the Sub-Himalayan region, from Chenab to Nepal up to 1500 m, Chota Nagpur, Rajasthan, Madhya Pradesh and South wards to Godavari. Leaves, flowers and seeds are extensively used in the treatment of various diseases such as asthma, chronic fever, constipation, grayness of hair, baldness, bronchitis and skin diseases¹. Besides, this plant has antiamoebic, antiviral², analgesic and antipyretic³, antimicrobial⁴, immunomodulatory, antimalarial⁵, anti-inflammatory⁶, hypnotic and tranquillizing⁷, antileishmanial and antifungal activities. The orange colour bright corolla tubes of the flowers contain a colouring substance nyctanthin, which is matching with α -crocetin (C₂₀H₂₄O₄) from saffron. The corolla tubes were used in dyeing industries. Nyctantic acid, beta-sitosterol, friedelin and oleanolic acid isolated from leaves were used for antiviral activity⁸. Ayurvedic medicines like Nashtapushpantaka rasa and part of Shankhapushpi taila are formulated using *Nyctanthes arbor-tristis*⁹.

Propagation of *N. arbor-tristis* through seeds is very difficult because of low seed-setting and poor

germination due to the insect attack during the seed ripening stage. This type of limitation leads to the death of many young seedlings under natural conditions in several medicinal plant species¹⁰. Hence, studies need to be conducted to produce this plant on large scale for commercial, ornamental and conservation purposes. *In vitro* propagation is one of the scintillating tools to mass propagate this species and to improve the quality of the plant. The objective of this study was to establish a trustworthy protocol for *in vitro* propagation of *N. arbor-tristis* from shoot tip and internodal explant and optimize conditions for acclimatization and transfer into *ex vitro* conditions.

Materials and Methods

Plant Material and Surface Sterilization

N. arbor-tristis seeds were obtained from Pachamalai hills, Thuraiyur, Tamil Nadu. In the present study both water-soaked (24 hours) and non-water soaked seeds were selected for *in vitro* seed germination. Healthy seeds were kept under running tap water for 15 minutes, then washed with 5% Teepol (W/V) for surface sterilization and 0.1% (W/V) Bavistin (fungicide) for 5 minutes and again washed thoroughly with running tap water. Thereafter the seeds were again surface sterilized with 0.1% HgCl₂ for 4 minutes, followed by washings with sterile double distilled water inside the laminar airflow chamber.

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Culture Conditions

Surface sterilized seeds were inoculated on Murashige and Skoog (1962)¹¹ solidified agar medium devoid of any growth regulators for germination. The shoot tip and internodal segments were collected from 24 days old *in vitro* seedlings and inoculated on MS medium containing various growth regulators, 3% (w/v) sucrose, and solidified with 0.8 % (w/v) agar (Hi-Media). The pH of the medium was adjusted to 5.7 before gelling with agar. Culture tubes with media were steam sterilized under 1.1 kg/cm² pressure at 121°C. All cultures were maintained at 25 ± 2°C in a culture room under cool white fluorescent lamps (Phillips, India) at an intensity of 50 μ mol m⁻² s⁻¹ with 16 hrs photoperiod.

Effect of PGR on Shoot and Root induction

Shoot tip and internodal explants were cultured on MS medium containing 3% (w/v) sucrose and 0.8% (w/v) agar and supplemented with various concentrations of 6 benzyl amino purine (BAP) (1.0 to 3.0 mg/l) and thidiazuron (TDZ) (0.5 to 2.5 mg/l) with 5% coconut water (CW) for multiple shoot induction. The well elongated shoots were transferred to the rooting medium containing indole butyric acid (IBA) (1.0 to 2.5 mg/l) in different concentrations. Well rooted plantlets were transferred to the environmental condition for hardening process.

Statistical Analysis

Each treatment consisted of 10 replicates and the experiment was repeated thrice. Observations were recorded on the percentage of response (number of cultures responding for shoot proliferation and root induction), the number of shoots per explant, mean shoot length, roots per shoot and mean root length respectively. The treatment means were compared using Duncan's Multiple Range Test (DMRT) at a 5% probability level¹².

Results and discussion

In Vitro Seed Germination

In vitro seed germination studies were conducted with normal seeds (non-water-soaked) and water-soaked seeds for 24 hours. The percentage of seed germination was recorded after 14 days of initial inoculation. The highest percentage (50%) of *in vitro* seed germination was observed in 24 hours water-soaked seeds. Normal seed germination was very low (15%) when compared to water-soaked seeds. Hence, the result of seed germination percentage

clearly shows the poor germination viability of the *N. arbor-tristis* seeds.

Induction of Multiple Shoots

The practice of tissue culture starts with excising explants from mother plant. Explants can be collected either from field plants or from plants grown in aseptic condition. When we depend on field plant, the problems such as contamination and poor response are likely to occur. On the other hand, explants from aseptic seedlings could overcome the above handicaps. In the present study, shoot tip and internodal segments excised (1-2.5 cm) from 24 days old *in vitro* seedlings were used as explants for induction of microshoots. Various concentrations of BAP (1.0 to 3.0 mg/l) and TDZ (0.5 to 2.5 mg/l) were used with 5% CW for multiple shoots induction. No multiple shoots were formed, when explants were cultured on hormone free MS medium. Microshoots were first observed 12 days after inoculation of explants. The efficiency of shoot production from shoot tip explants was significantly higher than that of internodal explants when evaluated five to six weeks after incubation. As a supplement, 2.0 mg/l BAP with 5% cw resulted in maximum multiple shoots proliferation and the highest percentage of response (90%) was noted in shoot tip explants. The shoot tip explants produced the highest number of 23.1 ± 0.94 shoots per culture with a mean length of 11.8 ± 1.06 cm (Table 1 and Fig-1 b & c). With reference to internodal explants, the highest percentage of response (73%) and maximum number (14.4 ± 1.12) of shoots per culture with mean shoot length of 11.4 ± 1.12 cm was observed at 2.5 mg/l BAP (Table 1, Fig 1 d & e). When the explants were cultured on TDZ-based medium only 23 - 65% of them responded. Among different concentrations of TDZ tested, best results in terms of percentage of response, number of shoots per culture and mean shoot length were observed on MS medium fortified with 1.5 mg/l TDZ in shoot tip explants and 2.0 mg/l in internodal explants. In these treatments the highest number of shoots per explant and mean shoot length were 17.0 ± 0.85 and 7.5 ± 0.54 cm for shoot tip explants and 9.3 ± 1.09 and 8.3 ± 0.10 for internodal explants. When the concentration of BAP and TDZ increased to above optimal concentration, shoot induction frequency decreased and basal callus formation occurred. Similar results were observed in *Prosalia corylifolia*¹³ and *Terminalia arjuna*¹⁴. Further shoot

Table 1 — Effect of BAP and TDZ in combination with 5% CW on multiple shoots induction from shoot tip and internodal explants of *Nyctanthes arbor-tristis* L after six weeks of culture

PGR (mg/l)	Shoot tip explant			Internodal explant		
	% of responded cultures	Number of shoots/ explant M ± SE	Mean shoot length (cm) M ± SE	% of responded cultures	Number of shoots/ explant M ± SE	Mean shoot length (cm) M ± SE
BAP						
Control	-	-	-	-	-	-
1.0	56	12.2 ± 0.33e	2.8 ± 0.30de	36	5.4 ± 0.34ef	4.4 ± 0.34ef
1.5	76	19.9 ± 0.78b	8.8 ± 0.95 b	43	7.2 ± 0.30de	6.2 ± 0.30de
2.0	90	23.1 ± 0.94a	11.8 ± 1.06a	63	11.2 ± 0.90b	10.2 ± 0.90b
2.5	66	15.2 ± .39cd	4.5 ± 0.24cd	73	14.4 ± 1.12a	11.4 ± 1.12a
3.0	53	10.5 ± 0.43e	2.6 ± 0.24de	50	7.6 ± 0.23cd	6.7 ± 0.23cd
TDZ						
Control	-	-	-	-	-	-
0.5	-	-	-	-	-	-
1.0	46	8.3 ± 0.27f	4.7 ± 0.31cd	23	1.3 ± 0.13 _{gh}	2.1 ± 0.20 _h
1.5	68	17.0 ± 0.85c	7.5 ± 0.54b	30	2.4 ± 0.24g	3.0 ± 0.24g
2.0	60	14.5 ± 0.54d	5.7 ± 0.30c	53	9.3 ± 1.09bc	8.3 ± 0.10bc
2.5	40	4.0 ± 0.22 _g	1.6 ± 0.29e	33	4.1 ± 0.32fg	3.9 ± 0.32fg

Control – MS basal medium

30 explants were used for each treatment and data (M ± SE) recorded after six weeks in culture

Mean values within a column with same alphabets are not significantly different (P=0.05) according to Duncan's Multiple Range Test.

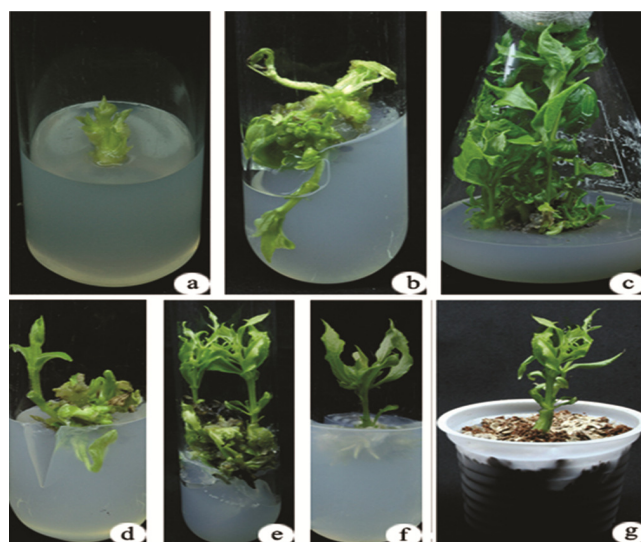


Fig. 1 (a-g) *In vitro* propagation of *Nyctanthes arbor-tristis* L: (a & b) Microshoots initiation from shoot tip explant, (c) High frequency of multiple shoot induction from shoot tip explant, (d) Microshoots initiation from internodal explant, (e) Maximum number of shoot formation from internodal explant, (f) Rooting of elongated shoots, (g) *In vitro* regenerated plants in plastic cups.

multiplication experiments were carried out with optimal concentration of BAP (shoot tip 2.0 mg/l and internode 2.5 mg/l) with 5% coconut water¹⁵. The cultures were maintained in the same hormone (six weeks) concentration for sufficient shoot elongation. Shoot tip explants were found more suitable in terms of percentage of response, number of shoots per culture and mean shoot length than internodal explants. BAP was most effective than TDZ

for inducing shoot formation rate. Earlier reports also indicate that BAP could produce maximum number of shoots in *Momordica charantia*¹⁶ and *Coleus blumei*¹⁷.

The number of shoots produced was relatively low in the internodal explants, besides the hormonal requirement is more for internodal explants compared to shoot tip explants. The results clearly point out the three general hypotheses, (a) apical meristem in shoot apex is responsible for higher shoot frequency (b) endogenous level of cytokinin are naturally high in shoot tip explants and (c) shoot apex tissues are more sensitive to cytokinins. Hence, the hormonal requirement is relatively lower in shoot tip explants and higher in internodal explants.

Rhizogenesis and Acclimatization

Well elongated excised shoots (from shoot tip and internodal explants) were transferred to MS medium containing 1.0 to 2.5 mg/l IBA for rhizogenesis. The MS medium supplemented with IBA (2.0 mg/l) was most effective on rooting, resulting in highest rooting percentage (85%). A well established root system was formed in 4 to 5 weeks (Fig 1f). Maximum number of roots 7.5 ± 0.28 was achieved with 2.0 mg/l IBA and mean root length was 4.3 ± 0.40 cm (Table 2). Similar results were also obtained from *Abrus precatorius*¹⁸ and *Piper methysticum*¹⁹.

Rooted plantlets were carefully removed from the medium and treated with 10% bavistin for protecting the root system from fungal infection. After this treatment the root system was washed with distilled

Table 2 — Effect of different concentrations IBA on root induction from *in vitro* grown microshoots of *Nyctanthes arbor-tristis* L after five weeks of culture

IBA concentration (mg/l)	% of Response	Number of roots/explant M ± SE	Mean length of roots (cm) M ± SE	Days to emergence of roots
1.0	40	3.6 ± 0.24d	2.1 ± 0.11c	18-22
1.5	70	6.4 ± 0.32b	3.2 ± 0.23b	16-20
2.0	85	7.5 ± 0.28a	4.3 ± 0.40a	14-20
2.5	55	5.3 ± 0.22c	2.6 ± 0.30bc	18-22

20 microshoots were used for each treatment and data (M ± SE) were recorded after 5 weeks in culture.

Mean values within a column with same alphabets are not significantly different (P=0.05) according to Duncan's Multiple Range Test.

water 6 to 8 times and was transferred in plastic cups containing 1:2:1 red soil, vermicompost and vermiculite. These plantlets were kept at room temperature for 28 to 35 days, latter they were transferred to the field condition. The transplantation survival rate was 80 – 85% of the rooted plantlets. The acclimatized plantlets showed rapid growth, without showing any morphological variation compared to the mother plant.

Conclusion

In conclusion, an efficient protocol for plant regeneration via *in vitro* propagation from shoot tip and internodal explants of *Nyctanthes arbor-tristis* by optimizing the plant growth regulators concentration and culture conditions were developed. The obtained results have shown that, rapid and high multiplication rate, rooting and successful transfer of plantlets to the *ex vitro* condition makes this protocol reliable for large-scale multiplication as well as *ex situ* conservation of this important medicinal plant. Continuous production of plants through *in vitro* propagation would reduce the exploitation of species on natural population and thus support the *ex situ* conservation. The plants propagated through *in vitro* did not show any detectable variation in respect to growth or morphological characteristics when compared with the mother plant. Hence, it can efficiently be utilized for mass propagation of *Nyctanthes arbor-tristis*.

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