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BRIEF COMMUNICATION

In vitro clonal propagation of *Nyctanthes arbor-tristis*

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Abstract

Rapid shoot multiplication of *Nyctanthes arbor-tristis* L. was achieved from axillary meristems on Murashige and Skoog (MS) basal medium supplemented with 1.0 - 1.5 mg dm^{-3} 6-benzylaminopurine (BA), 50 mg dm^{-3} adenine sulfate (Ads) and 3 % (m/v) sucrose. Inclusion of indole-3-acetic acid (IAA) in the culture medium along with BA + Ads promoted a higher rate of shoot multiplication. Maximum mean number of microshoots per explant (6.65) was achieved on the MS medium supplemented with 1.5 mg dm^{-3} BA, 50 mg dm^{-3} Ads and 0.1 mg dm^{-3} IAA after 4 weeks of culture. The elongated shoots rooted within 13 to 14 d on half-strength MS medium supplemented with either indole-3-butyric acid (IBA), IAA or 1-naphthaleneacetic acid (NAA) with 2 % sucrose. Maximum percentage of rooting was obtained on medium having 0.25 mg dm^{-3} IBA and 0.1 mg dm^{-3} IAA. About 70 % of the rooted plantlets survived in the greenhouse. The *in vitro* raised plants were grown normally in the field.

Additional key words: growth regulators, medicinal plant, shoot multiplication.

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Nyctanthes arbor-tristis Linn. is a valuable medicinal plant which belongs to the family *Oleaceae*, and is distributed in Indo-Malayan regions. It is a small tree with scented white flowers. It is a native of India occurring wild in the Sub-Himalayan region. Due to unrestricted large-scale exploitatiobn of the natural resource, coupled with limited cultivation and insufficient attempts for its replenishment, the natural stock of this species has been markedly depleted. Propagation from seed is unreliable due to poor germination and death of many young seedlings under natural conditions (Thapliyal and Naithani 1996). *In vitro* culture is an alternative method for conservation and propagation of this species, but no report has been published so far. Hence, this investigation aimed to develop an efficient protocol for micropropagation of *N. arbor-tristis*, an important medicinal plant.

Elongated shoots (4 - 5 cm long) were collected from field grown plants of *Nyctanthes arbor-tristis* L. and brought to the laboratory with cut ends dipped in distilled water. Stem without leaves were washed in 2 % (m/v) *Teepol* (*Qualigen*, Mumbai, India) and surface disinfected by 0.1 % (m/v) aqueous mercuric chloride solution for 15 min. After rinsing 4 - 5 times with sterile distilled water, stems were cut into smaller segments (*ca*. 0.5 cm long), each with one node was used as explant source.

Nodal explants were placed on semisolid Murashige and Skoog (1962; MS) mineral salts plus 555 μM myo-inositol, 4.06 μM nicotinic acid, 2.43 μM pyridoxine-HCl, 0.296 μM thiamine-HCl supplemented with various concentrations of cytokinins, *i.e*. 6-benzylaminopurine (BA: 0.0, 0.5, 1.0, 1.5 and 2.0 mg dm^{-3}), kinetin (Kn: 0.0, 0.5, 1.0, 1.5 and 2.0 mg dm^{-3}), adenine sulfate (Ads: 25, 50 and 100 mg dm^{-3}) and auxins like indole-3-acetic acid (IAA: 0.0, 0.10, 0.25 and 0.5 mg dm^{-3}) and 1-naphthalene-acetic acid (NAA: 0.0, 0.10, 0.25 and 0.5 mg dm^{-3}) for shoot multiplication. The pH of the medium was adjusted to 5.8 prior to autoclaving. Each treatment was represented by 20 explants and the experiment was repeated three times. The cultures were incubated under a 16-h photoperiod (irradiance of 55 μmol m^{-2} s^{-1}, cool, white fluorescent lamps) at temperature of 25 ± 2 °C.

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Abbreviations: Ads - adenine sulfate; BA - 6-benzylaminopurine; IAA - indole-3-acetic acid; IBA - indole-3-butyric acid; MS medium - Murashige and Skoog medium, NAA - 1-naphthaleneacetic acid.

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Elongated shoots (2 - 3 cm long) were excised from the culture and transferred to half-strength semisolid MS medium supplemented with different concentrations of IBA, IAA and/or NAA (0, 0.1, 0.25, 0.5 and 1.0 mg dm^{-3}) and 2 % (m/v) sucrose for root induction. One excised shoot was cultured in each tube (25 × 150 mm) with 15 cm^3 of the culture medium. All the cultures were incubated under conditions mentioned above. The percentage of shoots forming roots and the number of roots per shoot was examined periodically up to 4 weeks of culture. Rooted micropropagules were thoroughly washed to remove the adhering gel and planted in 2.5 cm^3 earthen pots containing a mixture of soil:sand:dry cow-dung at the ratio of 1:1:1 (v/v) and kept in the greenhouse for acclimatization. The plants were watered at 2-d intervals and were supplied with ¼ strength MS inorganic solution twice a week before transfer to the open field. The survival rate was recorded one month after transfer to pots. The data were analyzed statistically by the Duncan's multiple range test (Harter 1960).

Meristem growth and multiplication were initiated on MS medium supplemented with different concentrations of BA, Kn and Ads alone or in combinations. The growth of the axillary meristems and subsequent multiplication could not be achieved in medium without growth regulators. The media having Kn alone or in combination with Ads showed a low rate of shoot multiplication and slower shoot elongation as compared to BA alone or BA + Ads (Table 1). The combination of Kn plus IAA or NAA did not improve shoot multiplication. The medium having BA in combination with Ads enhanced the rate of shoot multiplication within 2 weeks of culture (Fig. 1*A*). The medium containing BA + Ads + IAA or NAA produced higher number of multiple shoots as compared with BA + Ads (Table 1). Medium having IAA or NAA alone did not induce shoot multiplication or growth.

Fig. 1. *In vitro* propagation of *Nyctanthes arbor-tristis*. *A* - Development of single axillary shoot from nodal explants on MS medium supplemented with 1.5 mg dm^{-3} BA, 50 mg dm^{-3} Ads after 2 weeks of culture. *B* - Development of multiple shoots from nodal explants of on MS medium supplemented with 1.5 mg dm^{-3} BA, 50 mg dm^{-3} Ads, 0.1 mg dm^{-3} IAA and 3 % sucrose after 4 weeks of subculture. *C* - Induction of roots from microshoots on half strength MS medium supplemented with 0.25 mg dm^{-3} IBA, 0.1 mg dm^{-3} IAA and 2 % sucrose after two weeks of culture. *D* - *In vitro* raised plantlets grown in the soil.

Table 1. Effect of different concentrations of BA, Kn and Ads [mg dm^{-3}] added to the MS medium on shoot proliferation from axillary meristems of *Nyctanthes arbor-tristis* after 4 weeks of culture. Values are mean ± SE, 20 cultures per treatment, repeated three times. Within a column, means having the same letters are not significantly different at the 5 % level according to Duncan's multiple range test.

BA	Kn	Ads	Regeneration frequency [%]	Number of shoots [$explant^{-1}$]
1.0	0	0	56.6 ± 1.2f	2.32 ± 0.6
1.5	0	0	68.2 ± 1.0h	3.81 ± 0.7
2.0	0	0	74.4 ± 0.8i	3.88 ± 0.8
0	1.0	0	24.4 ± 1.1a	1.82 ± 0.5
0	1.5	0	36.2 ± 1.0c	2.12 ± 0.7
0	2.0	0	42.6 ± 1.1d	2.76 ± 0.8
0	0	25	28.2 ± 0.8b	2.11 ± 0.7
0	0	50	45.6 ± 1.0e	3.13 ± 0.8
0	0	100	62.2 ± 0.8g	3.43 ± 0.7
1.5	0	25	76.8 ± 1.4j	4.52 ± 0.8
1.5	0	50	80.2 ± 1.1k	4.22 ± 0.5
2.0	0	50	78.4 ± 0.8k	3.88 ± 0.6
0	1.5	50	46.4 ± 0.8e	1.85 ± 0.7
0	2.0	50	41.8 ± 0.7d	1.68 ± 0.6

About 78 % of cultures showed multiple shoots in a medium having 1.5 mg dm^{-3} BA, 50 mg dm^{-3} Ads and 0.10 mg dm^{-3} IAA (Fig. 1B). The increase of IAA concentration higher than 0.25 mg dm^{-3} suppressed the rate of shoot multiplication and stunted growth. The maximum number of multiple shoots (6.65) was obtained in the medium containing 1.5 mg dm^{-3} BA, 50 mg dm^{-3} Ads and 0.1 mg dm^{-3} IAA in 4 weeks after culture initiation (Table 2). Similar observations indicating a cytokinin and auxin effect on shoot multiplication had been reported earlier in Clerodendrum colebrookianum (Mao et al. 1995), Plumbago zeylanica (Rout et al. 1999), Lawsonia inermis (Rout et al. 2001), Ocimum gratissimum (Gopi et al. 2006) and Aristolochia indica (Soniya and Sujitha 2006). There were differences between treatments in both the percentage of cultures with multiple shoots and the mean number of shoots per culture. Many authors reported that cytokinin is required in optimal quantity for shoot proliferation in many species but inclusion of a low concentration of auxin along with cytokinin increased the rate of shoot multiplication (Sharma et al. 1993, Sharma and Singh 1997, Shasany et al. 1998, Rout et al. 2000, Rout 2005, Rani et al. 2006). A higher concentration of BA (> 2.0 mg dm^{-3}) in the culture medium inhibited the growth of the shoots and stimulated small callusing at the basal end. The number of multiple shoots per explant varied from 3.74 to 6.65 between different treatments. The rate of multiplication increased as the number of subcultures increased. The number of multiple shoots was remaining constant after 16^{th} subcultures (data not shown). This was probably due to adaptation of the explants to *in vitro* conditions. Similar observations have been reported for *Gentiana kurroo* (Sharma *et al*. 1993) and *Plumbago* species (Rout *et al*. 1999).

Table 2. Effect of different concentrations of BA, Ads, IAA and NAA [mg dm^{-3}] on shoot multiplication from axillary meristems of *Nyctanthes arbor-tristis* after 4 weeks of culture (+ - callusing at the basal end). Means ± SE, 20 cultures per treatment, repeated three times. Within a column, means having the same letters are not significantly different at the 5 % level according to Duncan's multiple range test.

BA	Ads	IAA	NAA	Explants with multiple shoots [%]	Number of shoots [$explant^{-1}$]
0	0	0	0	0	0
1.0	25	0.10	0	65.8 ± 1.2c	5.12 ± 0.5c
1.5	50	0.10	0	78.4 ± 1.1g	6.65 ± 0.6d
1.5	50	0	0.10	72.6 ± 1.0f	4.78 ± 0.7b
1.5	50	0	0.25	62.2 ± 1.1b	4.86± 1.0b
1.5	50	0.25	0	66.4 ± 0.8c	5.34 ± 0.8c+
2.0	50	0.25	0	70.2 ± 1.0e+	5.28 ± 0.6c+
2.0	50	0	0.25	68.3 ± 0.9d+	5.12 ± 0.7c
2.0	100	0.10	0	58.8 ± 1.2a+	3.74 ± 0.6a+
2.0	100	0	0.10	62.1 ± 0.8b+	3.80 ± 0.8a+

Elongated shoots were excised and placed in half-strength MS medium supplemented with various concentrations of IBA or IAA or NAA for induction of root. Full-strength MS medium without growth regulators did not promote root induction; roots were observed in media containing half-strength MS medium supplemented with NAA, IAA or IBA with 2 % sucrose. However, optimal rooting and growth of microshoots were observed in medium containing 0.25 mg dm^{-3} IBA, 0.10 mg dm^{-3} IAA with 2 % sucrose 13 - 14 d after culture without intervening callus. The percentage of shoots forming roots and number of roots per shoot significantly varied depending on concentrations of IBA. The maximum percentage of rooting (72.6 %) was obtained in medium containing 0.25 mg dm^{-3} IBA and 0.1 mg dm^{-3} IAA (Fig. 1*C*). Root development was; however, slow at higher concentrations of IBA or IAA. Faisal and Anis (2006) reported that low concentration of IBA favour root induction from microshoots of *Psoralea corylifolia*.

The rooted plantlets were transferred to pots for acclimatization. About 70 % of the rooted plantlets survived in the pot after one month of transfer. The plants grew normally (Fig. 1*D*).

In conclusion, successful production of multiple shoots and *in vitro* root formation depended on the nutrient medium and the culture environment. This study might provide new opportunities for clonal propagation and germplasm conservation of *Nyctanthes arbor-tristis*.

References

Faisal, M., Anis, M.: Thidiazuron induced high frequency axillary shoot multiplication in *Psoralea corylifolia*. - Biol. Plant. **50**:437-440, 2006.

Gopi, C., Nataraja Sekhar, Y., Ponmurugan, P.: *In vitro* multiplication of *Ocimum gratissimum* L. – through direct regeneration. - Afr. J. Biotechnol. **5**: 723-726, 2006.

Harter, H.L.: Critical values for Duncan's multiple range test. - Biometric **16**: 671-685, 1960.

Mao, A.H., Wetten, A., Fay, M., Caligari, P.D.S.: *In vitro* propagation of *Clerodendrum colebrookianum* Walp.: a potential natural anti-hypertension medicinal plant. - Plant Cell Rep. **14**: 493-496, 1995.

Murashige, T., Skoog, F.: A revised medium for rapid growth and bioassays with tobacco tissue culture. - Physiol. Plant. **15**: 473-497, 1962.

Rani, G., Talwar, D., Nagpal, A., Virk, G.S.: Micropropagation of *Coleus blumei* from nodal segments and shoot tips. - Biol. Plant. **50**: 496-500, 2006.

Rout, G.R.: Micropropagation of *Clitoria ternatea* Linn. (Fabaceae) – an important medicinal plant. - *In vitro* cell. dev. Biol. Plant **41**: 516-519, 2005.

Rout, G.R., Das, G., Samantaray, S., Das, P.: *In vitro* micropropagation of *Lawsonia inermis* (Lythraceae). - Rev. Biol. Trop. **49**: 957-963, 2001.

Rout, G.R., Samantaray, S., Das, P.: *In vitro* manipulation and propagation of medicinal plants. - Biotechnol. Adv. **18**: 91-120, 2000.

Rout, G.R., Saxena, C., Samantaray, S., Das, P.: Rapid clonal propagation of *Plumbago zeylanica* Linn. - Plant Growth Regul. **28**: 1-4, 1999.

Sharma, N., Chandel, K.P.S., Paul, A.: *In vitro* propagation of *Gentiana kurroo*: an indigenous threatened plant of medicinal importance. - Plant Cell Tissue Organ Cult. **34**: 307-309, 1993.

Sharma, T.R., Singh, B.M.: High frequency *in vitro* multiplication of disease-free *Zingiber officinalis* Rosc. - Plant Cell Rep. **17**: 68-72, 1997.

Shasany, A.K., Khanuja, S.P.S., Dhawan, S., Yadav, V., Sharma, S., Kumar, S.: High regenerative nature of *Mentha arvensis* internodes. - J. Biosci. **23**: 641-646, 1998.

Soniya, E.V., Sujitha, M. : An efficient *in vitro* propagation of *Aristolochia indica*. - Biol. Plant. **50**: 272-274, 2006.

Thapliyal, R.C., Naithani, K.C.: Inhibition of germination in *Nyctanthes arbor-tristis* (Oleaceae) by pericarp. - Seed Sci. Technol. **24**: 67, 1996.