IN VITRO PLANT REGENERATION FROM SHOOT TIP AND NODAL EXPLANTS OF RHINACANTHUS NASUTUS (L.) KURZ.

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ABSTRACT

An efficient, rapid and large-scale in vitro clonal propagation of the valuable medicinal shrub Rhinacanthus nasutus (Acanthaceae) by enhanced shoot proliferation in nodal and shoottip segments was designed. Both the explants gave rise to multiple shoots when cultured on MS medium supplemented with different concentrations of BAP and KIN. The highest rate of shoot multiplication (26.33 shoots/ explants) as well as beneficial shoot length (10 cm) was obtained in nodal explants on MS containing 2.0 mg/L BAP. Subculturing of regenerated shoots on similar medium enabled continuous production of healthy shoots with similar frequency. Rooting was highest (16 roots/explant) in nodal explants on full strength MS medium containing 2.0 mg/L IBA. Micropropagated plants established in a mixture garden soil, farmyard (manure) and sand (2:1:1) were uniform and identical to the donor plant with respect to growth characteristics as well as floral features. These plants grew normally without showing any morphological variation.

KEY WORDS Rhinacanths nasutus, medicinal plant, in vitro, plant regeneration, multiple shoots

Abbreviations

BAP Benzyl aminopurine
KIN Kinetin
IBA Indole-3-butyric acid
MS Murashige and Skoog

INTRODUCTION

Rapid and progressive deforestation is endangering several plant species. Micropropagation systems have the potential for rapidly multiplying economically important genotypes for reforestation, which help to increase forest productivity. In vitro regeneration of whole plants is a necessary step in the implementation of genetic transformation systems, therefore the assessment of different regeneration and micropropagation alternatives is required prior to any genetic transformation attempt. This study was aimed to find an appropriate system to regenerate plants through shoot tip and nodal explants**.**

Rhinacanthus nasutus (L.) Kurz. belonging to the family Acanthaceae a shrub with a height of 1 - 2 meters and widely distributed in South China and India. It is commonly called as snake jasmine, nagamalli (Kirtikar and Basu, 1984). The plant is acrid, bitter, thermogenic, blister producer, revulsive, depurative, antihelmentic, anti-septic, aphrodisiac, antiparasitic, and anti-cancerous. This plant in traditional system of medicine is used to treat condition like vata, kapha, eczema, septic-ulcer, cancer, herpes, dhobi's itch, leprosy, helminthiasis, prickly heat, ring worm, scurvy and inflammation (Orient Longman). The plant has been recently cultivated for use in Thai Folk remedy for

the treatment of various diseases including cancer, hepatitis, diabetes, hyper tension and skin diseases in Taiwan (Farnsworth and Bunyapraphatsara, 1992). The decoction of its roots or whole plant is drunk for treatment of some cancer forms (Rojanapo et al., 1990).The part of the plant that is used for medication purpose is the leaf. It is used to relieve colds, fever, early stage of tuberculosis, headache from hypertension, constipation and sore throat. It can also refresh the lungs, and reduce blood pressure (Cheeptham and Towers, 2002).

Rhinacanthus nasutus plant is well known as the sources of flavonoids, steroids, triterpenoids, anthraquinones, lignans and especially naphthoquinone analoges (Kodama et al., 1993). Naphthoquinone compounds have been reported to possess in vitro antiproliferative activity towards various cancer cells (Wu et al., 1988). Rhinacanthone also showed antitumor activity against Dalton's lymphoma ascites cells bearing mice (Siripong et al., 2008). In addition, rhinacanthin-C, a main naphthoquinone ester and the active chloroform and aqueous extracts from the roots and stems, significantly suppressed the growth of Meth-A-sarcoma-bearing mice (Siripong et al., 2006b).

There are only a few reports on this plant for rapid multiplication, prompting the authors for attempting to propagate plants from shoot tips and nodal explants under in vitro conditions. This study describes the effects of BAP and KIN on shoot bud multiplication, elongation, rooting from shoot tip and nodal explants of R. nasutus.

MATERIALS AND METHODS

Plant material and Explants

Healthy young shoot tip and nodal explants with dormant axillary buds were collected from the mature plants of Rhinacanthus nasutus (L.) Kurz. grown in the Botanical and Experimental Garden of A.V.V.M. Sri Pushpam College, Poondi, Thanjavur district. After removing leaves, the explants (1.0 - 1.5 cm) were excised and then washed thoroughly under running tap water for 15 min, followed by a treatment with aqueous solution of detergent 10% v/v Teepol (Reckitt Benckiser, India Ltd.) for 10 min., and 70% (v/v) ethanol for 15 seconds and washing with autoclaved sterile distilled water three to five times. The explants were then surface disinfected with 0.1% (w/v) aqueous mercuric chloride (Merck, India) solution for 5 - 6 minutes and finally rinsed with autoclaved distilled water (five to seven changes). The shoot tip and nodal segments were then trimmed at both ends prior to inoculation on culture media.

Medium and culture conditions

Murashige and Skoog's (1962) (MS) medium containing 3% sucrose (w/v) was used in all the experiments. The pH of the medium was adjusted to 5.8 prior to the addition of 0.8% (w/v) agar (SLR, India). Molten medium was dispensed in 15 ml aliquots into culture tubes (25× 150 mm) and closed with non-absorbent cotton plugs. The medium was autoclaved at 1.1 kg/cm^2 pressure and 121ºC temperature for 15 min. All the cultures were incubated in a culture room maintained at 25 ± 2ºC and 55 to 65% RH under 12 h photoperiod of 50-60 µmolm^{-2} s^{-1} light intensity provided by cool white fluorescent tubes.

Shoot proliferation

MS basal medium supplemented with various concentrations (0.0 to 3.0 mg/L) of plant growth regulators, benzylaminopurine (BAP) or KIN individually, were used for culture initiation and shoot regeneration and multiplication of shoots. All the cultures were transferred to fresh medium after 2-3 weeks duration. The mean number of shoots and their lengths were evaluated after 6 weeks of inoculation.

Root formation

For rooting, the in vitro raised shoots (2-5 cm) were excised and transferred to full strength MS basal medium containing 3% (w/v) sucrose and 0.8% (w/v) agar. The medium was further supplemented with individual concentration of 0.0 - 3.0 mg/L indole – 3 butric acid (IBA). Data on the number of roots were recorded after 4 weeks.

Hardening and Acclimatization

Rooted plants were removed carefully from culture vessels, washed under running tap water to remove the remains of agar and transferred to pots containing sterilized vermiculite. The potted plantlets were kept under transparent polythene membranes for 2 weeks to ensure high humidity, and then kept in open diffused light for hardening. After one month, the surviving plants were transferred to pots containing mixture garden soil, farmyard (manure) and sand (2:1:1) and maintained in green house for acclimatization. At least 20 - 24 explants were cultured in each treatment and all the experiments were repeated three times.

Result and Discussion

Proliferation of multiple shoots was obtained with high frequency from shoot tips and nodes. These explants were capable of directly developing multiple shoots on MS containing different concentrations of cytokinins. Multiple shoot initiation from both explants was observed within 10 - 15 days after inoculation. The highest number of shoots (26/explant) was observed in the medium containing BAP (2.0 mg/L) followed by KIN (2.0 mg/L) with 19 shoots. Of the two cytokinins (BAP and KIN) tested, BAP was found to be more suitable than KIN for initiation and proliferation of multiple shoot buds (Table - 1).

The frequency of multiple shoot bud induction was higher in nodes (26.33 ± 1.52) than shoot tips (20.00 ± 1.00) (Table -1; Figs.1.b). The elongation of shoots and proliferation of nodes were achieved on the same parental medium. In the present study, the relative effectiveness of BAP and KIN varied for in vitro multiple shoot regeneration from shoot tips and nodes. BAP (2.0 mg/L) was found to be the best concentration for generation of maximum number of shoot buds (25 - 26). Shoots were harvested every 35 - 40 days and new shootlets were harvested periodically. Eighty five per cent of the plantlets produced roots on the rooting medium containing IBA (2.0 mg/L) after a week (Figs. 1.e ; Table 2). The in vitro regenerated plantlets were successfully transferred to plastic cups and then to the field (Fig. 1.f).

The capacity of shoot bud differentiation and shoot proliferation from shoot tip and nodal explants of R. nasutus depended on hormonal variation. There was a good shoot bud initiation and proliferation response only in the presence of cytokinin and no response in the basal medium. Similar observation was seen in several medicinal plant Centella asiatica (Karthikeyan et al., 2009), Asteracantha longifolia (Panigrahi et al., 2006). Sharon and Marie (2000) reported that the shoot tip and nodal explants were preferred over meristem to produce large number of genetically identical clones in Bixa ovellana L. BAP and KIN alone induced a higher frequency of multiple shoots. Similar results were obtained by Karthikeyan et al., (2007), in Phyllanthus niruri and Chandran et al. 2007 in Withania somnifera. From our study it was clear that 2.0 mg/L BAP and 2.0 mg/l KIN were significantly more effective for inducing shoot organogenesis. Kulkarni and Rao (1999) reported that KIN did not support the proliferation of multiple shoots in Acorous calamus. This result was in contrast to the present study where KIN was found to increase the frequency and the number of shoots.

Pawar et al. 2002 reported that BAP and KIN individually and in combination induced a higher frequency of adventitious shoots from a single explant of Solanum xanthocarpum and in Solanum trilobatum (Sathish Kumar et al., 2011). This result was similar to that recorded in the present study.

Well developed shootlets when transferred to MS containing IBA induced roots. In the present study, we observed the highest percentage and maximum number of rootlets per shootlets on medium supplemented with 2.0 mg/l IBA. Here the result showed consistency with other studies where the addition of IBA promoted the induction of roots in several systems including Woodfordia fruticosa (Islam et al., 2009) and Ophiorrhiza eriantha (Jaimsha et al., 2010). From our experimental data, it is evident that BAP and KIN are best suited for inducing multiple shoots and IBA for rooting. The nodal explants showed better response compared to shoot tips. In conclusion, this communication describes an efficient rapid propagation system and gene modifications programmes of R.nasutus.

	Shoot tip explants			Nodal explants		
Growth hormones Shoot (mg/L)	**% of culture Response**	**No. of multiple shoots/ shoot tip**	**Shoot length (cm)**	**% of culture response**	**No. of multiple shoots/nodal explants**	**length (cm)**
BAP						
0.5	35	02.15 ± 0.52	1.23 ± 0.53	30	03.33 ± 0.57	02.24 ± 0.55
1.0	50	06.00 ± 1.00	4.33 ± 0.57	55	08.66 ± 0.57	05.68 ± 0.53
1.5	70	12.42 ± 1.52	6.00 ± 1.00	75	19.00 ± 1.00	08.66 ± 0.57
2.0	88	20.00 ± 1.00	8.66 ± 0.53	95	26.33 ± 1.52	10.00 ± 1.00
2.5	75	13.66 ± 0.54	7.33 ± 0.59	85	15.66 ± 1.52	06.62 ± 0.53
3.0	60	08.00 ± 1.00	4.00 ± 1.00	65	10.66 ± 1.52	04.64 ± 0.57
KIN						
0.5	30	02.00 ± 1.00	1.66 ± 0.55	35	03.00 ± 0.00	02.66 ± 0.59
1.0	45	06.23 ± 0.47	3.33 ± 0.59	50	08.00 ± 1.00	04.66 ± 0.57
1.5	65	10.64 ± 0.56	6.33 ± 1.17	70	16.00 ± 1.00	07.00 ± 1.00
2.0	85	15.00 ± 0.59	7.00 ± 1.00	90	19.00 ± 1.00	09.00 ± 1.00
2.5	70	12.33 ± 0.55	5.33 ± 0.57	75	15.33 ± 0.57	06.00 ± 1.00
3.0	60	06.00 ± 1.00	4.00 ± 1.00	50	08.00 ± 1.00	05.00 ± 1.00

Table - 1. Effect of different concentrations of BAP and KIN on multiple shoot induction from shoot tip and nodal explants of R. nasutus.

Each value represents 20 replicates and each experiment was repeated at least thrice.

	Shoot tip		Nodal explants	
IBA (mg/L)	**% of root**	**Average No. of**	**% of root**	**Average No. of**

	Induction from shoots	roots/shoot	Induction from shoots	roots/shoot
0.5	30.0	06.00 ± 1.00	36.06	06.66 ± 1.24
1.0	40.0	07.33 ± 0.57	52.0	10.00 ± 1.00
1.5	63.6	12.66 ± 0.57	72.0	13.66 ± 1.24
2.0	76.6	15.00 ± 1.00	81.3	16.00 ± 0.81
2.5	66.00	10.00 ± 1.00	78.6	11.66 ± 1.24
3.0	50.00	08.03 ± 1.15	60.0	08.33 ± 0.47

Table 2. Effect of IBA on root induction in micropropagated plantlets of R. nasutus

Each value represents an average of 20 replicates and each experiment was repeated at least thrice.

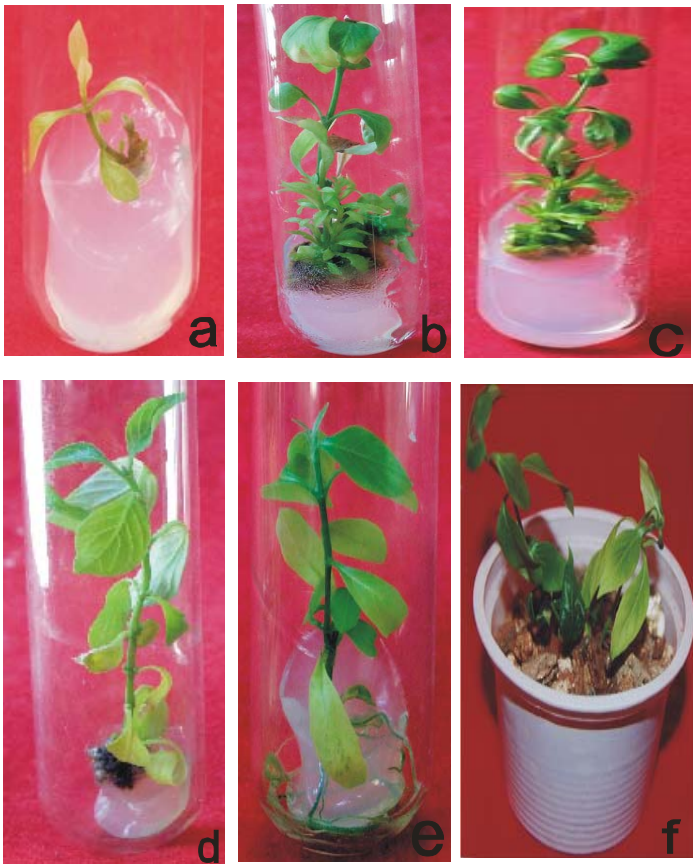

Fig -1 (a- f) In vitro plant regeneration of Rhinacanthus nasutus**.**

a) Initiation of multiple shoots from shoottip explants on MS medium fortified with BAP 1.0 mg/L after one week. b) Multiple shoot regeneration from nodal explants on MS medium supplemented with BAP 2.0 mg/L after three weeks. C) Multiple shoot regeneration from nodal explants on MS medium supplemented with KIN 2.0 mg/L after three weeks. d) Elongation of shoots on MS medium containing BAP 1.5 mg/L after 3 weeks. e) Direct rooting from regenerated shoots on MS medium fortified with IBA 2.0 mg/L after two weeks. f) Hardened plant in poly cups containing mixture garden soil, farmyard (manure) and sand (2:1:1).

ACKNOWLEDGEMENT

We place on record our deep sense of gratitude to our Hon'ble Secretary and correspondant Srimaan K.Thulasiah Vandayar, A.V.V.M Sri Pushpam College (Autonomous) Poondi, Thanjavur district, Tamilnadu, India for providing necessary facilities and encouragement.

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