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IN VITRO PROPAGATION OF *RHINACANTHUS NASUTUS* - A MEDICINAL HERB

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Rhinacanthus nasutus is an important medicinal plant which posses anticancer, antifungal, antiviral properties. A regeneration protocol has been developed for *R. nasutus* using leaf and shoot tip nodal explants. Calli were induced on MS media supplemented with 2, 4-D (0.5 mg/l). The calli were cultured on MS medium with different concentrations of KIN in combination with GA_3. KIN at 1.25 mg/l was effective for shoot regeneration and elongation of shoots was noticed on MS media supplemented with 1.30µM GA_3 within two weeks. The developed shoots were transferred to half strength MS medium containing IBA (1 mg/l) for rooting. The rooted plantlets were successfully established (100%) in the pots containing sand, peat moss and humus (1:1:1) for a period of two weeks.

Keywords : Acclimatization; Callus; *Rhinacanthus nasutus;* Shoot elongation; Shoot tip and leaf explants.

Introduction

The plant tissue culture had attained fundamental and practical relevance. One aspect of its potential use is the production of medicinally important substances of plant origin like secondary metabolites. Tissues of a number of pharmaceutical plants have therefore been cultured *in vitro* with success in productivity. Plant based remedies have always been an integral part of traditional medicine through out the world. The increasing demand for herbal medicines in recent years due to their fewer side effects in comparision to synthetic drugs and antibiotics, have highlighted the need for germplasm conservation and propagation of medicinal plants .

Rhinacanthus nasutus (Acanthaceae) is an important medicinal plant used in several ayurvedic preparations[1]. This plant is widely distributed in some parts of the sub-continent of India, China and some parts of South East Asia. It is reported to posses anticancer[2], antifungal[3,4] and antiviral[5] properties. It is used in poultice for treatment of eczema and ringworm. The plant contains different types of Rhinacanthin A, D, G. N etc., a quinol, 4-acetonyl-3, 5-dimethoxy-p-quinol and napthoquinone esters from roots amongst other compounds. Compounds rhinacanthin-G to -N, belongs to a class of 2-hydroxy-3-(3-hydroxy-2, 2-dimethylpropyl)-1, 4-naphthoquinone esters, have been isolated so far from this plant[6,7]. The requirement of *R. nasutus* is now met from the natural population, leading to their gradual depletion. Tissue culture techniques could play an important role in the multiplication of their elite clones and possibly, their germplasm conservation.

Furthermore, there is a wide scope for application of biotechnology tools for improvement of this important medicinal plant for which standardization of tissue culture technique is a pre-requisite. The present paper reports for the first time, a simple protocol for the *in vitro* multiplication through indirect regeneration of *Rhinacanthus nasutus* from leaf and shoot tip explants.

Material and Methods

Plants were collected from Alagar Hills and maintained in the Department Garden. The leaf explants were excised from cleaned cuttings and surface sterilized using Tween 20 for15 min. The leaf explants were placed in 70% ethanol for 60 sec followed by rinse with sterile distilled water for 3 times. Explants were cut using a sterile knife aseptically and dried on a sterile filter paper. It was cultured on modified MS basal medium[8,9] containing MS salts with iron source, supplemented with 3%(w/v) sucrose and 0.8% agar (Hi- media) and different concentrations of 2,4-D for callusing, KIN and GA_3 for shooting, and IBA for rooting. The pH of the medium was adjusted to 5.7 - 5.8 before addition of agar.

For each experiment 15 replicates were used and all experiments were repeated at least thrice. The cultures were incubated at 25°C with 65% RH under cool white fluorescent light (12h/day) with 1200 lux. The explants

Table 1. Influence of KIN on plant regeneration from the nodal explants in MS medium.

KIN(mg/l)	No. of Regenerated shoots	Regeneration response
0.50	---	---
0.75	21.4 ± 1.83*	+
1.00	28.6 ± 1.32	++
1.25	34.1 ± 2.34	+++
1.50	27.3 ± 1.56	++

* Each value is the SD of three experiments with fifteen replications per experiment.
- No growth; +Meager growth response; ++less growth response; +++more growth response.

Table 2. Effect of GA_3 on shoot elongation of *Rhinucanthus nasutus* in MS medium.

GA_3 (μM)	No. of Regenerated Shoots	Regeneration response
1.0	0.4	---
1.1	3.5	+
1.2	6.3	++
1.3	8.0	+++
1.4	6.7	++

- No growth; +Meager growth response; ++less growth response; +++more growth response.

Table 3. Effect of IBA in root formation of shoots.

IBA (mg/l)	No. of Roots per Shoot	Rooting response
1.0	0.4	---
1.1	3.5	+
1.2	6.3	++
1.3	8.0	+++
1.4	6.7	++

* Each value is the SD of three experiments with fifteen replications per experiments.

-No rooting; +Meager growth rooting response; ++less rooting response; +++more rooting response.

were sub-cultured once in 15 days. The nodal segments were isolated from the *in vitro* plantlets obtained from the primary cultures and transferred to fresh medium for further multiplication. The regenerated shoots were transferred to MS media containing different concentration of IBA for rooting. The rooted shoots, from which the agar-based medium had been removed under running tap water, were individually transferred to 10 cm plastic cups containing sand, peat moss and humus in 1:1:1 ratio. In order to prevent fungal infection, plants were watered with 0.5 g/l bavistin solution, after that each pot was covered with polythene bags which were progressively opened over a 2 week period. Subsequently, well-established plants were shifted to field.

Results and Discussion

After two weeks of culture the leaf and nodal explants induced callus on MS medium containing different concentrations of 2, 4-D. The well-developed calli were placed on MS medium with different concentrations of KIN (Table 1), (Fig. 1) for shoot regeneration. Several

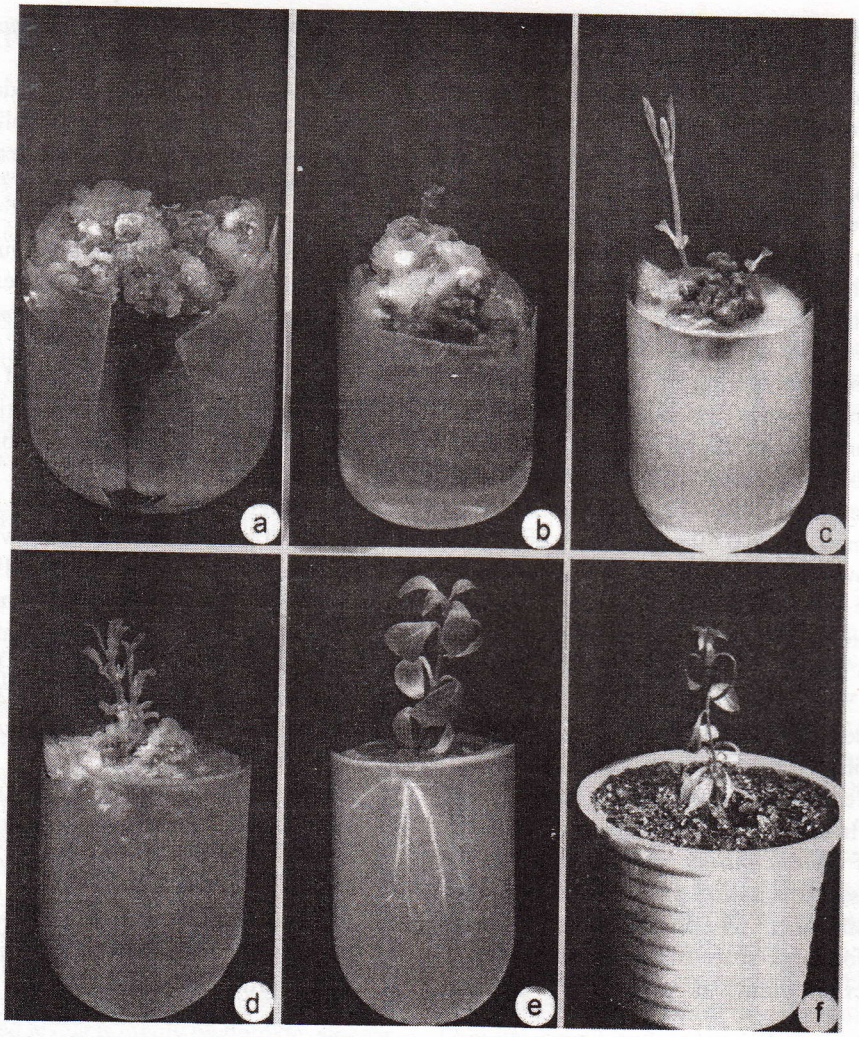

Fig.1. Indirect regeneration from nodal explant; a) Induction of orgonogenic callus from nodal explant; b) Regeneration of shoots from leaf callus; c) High frequency of shoot formation; d) Elongation of shoots; e) Rooting of elongated shoot; f) Hardened plantlet.

shoots were induced but failed to elongate. When the shoot tip explants in MS media supplemented with KIN initiated the regeneration of shoots only, but it did not favour for shoot elongation. Elongation of shoots was not favoured in the shoot multiplication medium even after 6-8 weeks of incubation in *Ophiorrhiza mungo*[10]. In order to improve the growth of regenerated shoot, the shoot tip and nodal explants were inoculated on MS medium containing GA_3 (1.30 µM) and it shows maximum elongation (Table 2). After 4 weeks, shoots attained the average height of 8 cm with 6-7 nodes. The proliferation of shoots on medium with KIN and GA_3 was noticed after 8 days of culture. Incorporation of KIN alone did not show any response except callusing from the base of both nodal and shoot tip explants. Several workers in other plant species have observed positive effect of cytokinin for shoot differentiation[11]. No growth occurred when KIN alone was present in the medium. When the concentration of the KIN was increased from 0.25 to 0.50 mg/l, the regeneration of nodal explants occurred. Whereas at 1.25mg/l KIN, the frequency of shoot regeneration was maximum with *in vitro* flowering. Frequency of shoot regeneration gets reduced gradually with an increase in concentration of KIN (1.5 mg/l). Further, adventitious shoots were subcultured on MS medium with GA_3 (1.30 µM) for normal elongation (Table 2). Approximately 85% of the shoots were elongated up to 5-7 cm after 15 days of culture. There are number of earlier report that affirms the

stimulatory effect of GA_3 on shoot elongation[12]. The shoot elongation had declined at higher concentrations of GA_3. However higher concentration of GA_3 seems to affect the growth of the developed shoots[13]. Negative effect of KIN on elongation of axillary buds indicated that high cytokinin was only needed for shoot initiation and varying ratio of GA_3 may be required to nullify the suppressive effect of high concentration on shoot elongation and to restore normal shoot growth[14]. For the induction of rooting *in vitro* a low salt medium has been found satisfactory in most of the plant species, *i.e.,* MS salt concentration diluted half strength was effective in rooting of many herbaceous plants. IBA is generally known to induce effective rooting in plant cuttings at *in vitro* conditions. Regenerated shoots were transferred to rooting medium containing IBA at different concentrations (Table 3) and no rooting was noticed with 0.25 mg/l, but at 0.50 mg/l rooting occurred in less number. However, best rooting was obtained at 1 mg/l IBA. Further increase of IBA (1 .25 mg/l) reduces the frequency of rooting of the regenerated shoots. Earlier workers have also suggested superior effect of IBA on rooting behaviour[15,16].

Rooted shoots were transferred to sterile soil with MS salts for hardening (Data not shown) and subsequently they were transferred to soil. Almost 70% of the plantlets survived on soil. Induction of multiple shoots through shoot cultures of *Rhinucanthus nasutus* is a useful technique for mass propagation as followed in many important medicinal plants[17]. The protocol described here could be useful for large-scale multiplication of this important medicinal plant, as well as it's *ex situ* conservation.

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