Research Article

ISSN Print 2231 – 3648
Online 2231 – 3656

Available Online at: www.ijpir.com

International Journal of Pharmacy and Industrial Research

EFFECT OF PLANT GROWTH REGULATORS ON *IN VITRO* PROPAGATION OF *RHINACANTHUS NASUTUS* L. (ACANTHACEAE)

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Abstract

The purpose of this study was to develop a new micropropagation system for *Rhinacanthus nasutus*" (Acanthaceae) an important medical plant in India. In this present study a standard protocol for micro propagation has been raised by the use of various explants inoculated on MS media fortified with different concentrations and combinations of cytokinin (BAP). The maximum number of 2.8 ± 1.2 shoots were proliferated per explants on MS media supplemented with BAP (0.1, 0.5, 1, 1.5, 2.0 mg/l). Multiple shoots were observed on media supplemented with 2.0 mg/l from the explants. The nodal explants were inoculated on MS media fortified with different concentrations and combinations of Auxin (IBA). Maximum numbers of roots were proliferated per explant, supplemented with IBA 1.5mg/l. White nodular callus pronounced from the leaf explants supplemented, when they were subjected to 2,4-D at different concentrations.

Keywords: *Rhinacanthus nasutus*, Acanthaceae, Micropropagation, Explants.

Introduction

The use of medicinal plants is increasing worldwide. According to the World Health Organization (WHO), approximately 80% of the world's population currently uses herbal medicines directly as teas, decoctions or extracts with easily accessible liquids such as water, milk, or alcohol[1]. The top 10 ranked plants that have received greatest interest in the USA and Europe over the past 30 years[2]. It also has listed 20,000 medicinal plants globally India's contribution is 15–20%[3]. Biotechnological tools are important for multiplication and genetic enhancement of the medicinal plants by adopting techniques such as *in vitro* regeneration and genetic transformations[4].

This paper reviews the achievements and advances in the application of tissue culture and genetic engineering for the *in vitro* regeneration of medicinal plants from various explants. *In vitro* propagation of plants holds tremendous potential for the production of high-quality plant-based medicines[5]. This can be achieved through different methods including micro propagation. Micro - propagation has many advantages over conventional methods of vegetative propagation, which suffer from several limitations[6]. With micropropagation, the multiplication rate is greatly increased. Micro propagation of various plant species including many medicinal plants has been

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reported[7]. Propagation from existing meristems yields plants that are genetically identical with the donor plants[8]. Micropropagation can be used to produces disease-free plants. Micropropagation produces rooted plantlets ready for growth, saving time for the grower when seeds or cuttings are slow to establish. In-vitro regeneration holds tremendous potential for the production of high-quality plant-based medicine[9]. It can have an extraordinarily high fecundity rate, producing thousands of propagules while conventional techniques might only produce a fraction of this a number. Numerous factors are reported to influence the success of in-vitro propagation of different medicinal plants[10]. *Rhinacanthus nasutus* (Acanthacae) is widely distributed in some part of the sub-continent India, in the region of south East Asia and china. An Under shrub up to 1.5 m in height with obscurely angled spreading stems and branches; leaves simple, opposite, elliptic-lanceolate, entire, acute, nearly glabrous, main nerves 8-10 pairs; flowers white, solitary or 2-3 together on the divaricate branches of very large lax terminal panicles; fruits narrow, pointed, velvety pubescent,4-seeded capsules, seeds tuberculate, glabrous, black. Mature shoots grayish white in color and fresh shoots emerald in color. Experiments under laboratory and semi-field conditions showed that the activity of the extracts persisted for up to 10 days[11].

Materials and methods

Materials

The plant material, *Rhinacanthus nasutus* was collected from the green house established at Sathyabama University Chennai. Explants like nodal segments, shoot tips and leaf lamina were used in this present investigation to study the regeneration capacity. All other chemicals used were of analytical grade and purchased locally.

In Vitro Inoculation Methods

Standardization of sterilization technique

In vitro cultures were initiated from nodal segments, petiolar segments and leaf lamina. The surface sterilization was carried out with 0.1% HgCl2 in the following time intervals. 1. 0.1% HgCl2 for 3 minutes, 2. 0.1% HgCl2 for 5 minutes, 3. 0.1% HgCl2 for 10 minutes

Growth regulators

The growth regulators used were 6 - Benzyl Amino Purine (BAP), α - Naphthalene Acetic Acid (NAA), Indole Butyric Acid (IBA) and 2, 4-Dichlorophenoxy acetic acid (2,4 D). The prepared solutions were stored in the refrigerator at 4 - 5º C.

Inoculation

The sterilized MS media (Murashige and Skoog, 1962) were used for inoculation. Hence, the nodal explants were inoculated vertically on the MS medium containing different combinations and concentrations of growth regulators. Twenty culture tubes with single explants having different concentrations of growth regulators were assigned to each inoculation. The cultures were kept under 16 hrs light / day (2400 Lux) photoperiod at 25 ± 2ºC.

Callus induction

Leaf lamina was inoculated on MS media fortified with different concentrations of 2, 4-D (0.5, 1, 1.5, 2mg/l).Observations were made on the following aspects i.e., number of days for callus formation and percentage of explant response. The quality of the callus implies whether the callus produced was compact, friable or watery and whether the patches were necrotic or not.

Indirect regeneration

Indirect regeneration from leaf lamina petioles was initiated by inoculating the explants on MS media supplements with growth regulators in following concentrations.

1. Basal media, 2.BAP 0.5mg, 3. BAP 1mg, 4. BAP 2mg, 5. BAP 0.5mg/l + NAA 0.1mg/l
6. BAP 1mg/l + NAA 0.1mg/l, 7. BAP 2mg/l + NAA 0.1mg/1. Explants – Nodal and Shoot tip parts.

Shoot Elongation

From the inoculated cultures, the highly proliferated multiple shoots were taken into small cultures of 2 - 3 shoots. They were sub cultured on shoot elongation medium containing BAP (0.5 - 1.0 mg / l) individually along with IBA (0.5-1.0 mg / l).The cultures were incubated at 25 ± 2ºC under 16 hrs light / day photo period. After two weeks, shoots longer than 2 cm were counted and transferred to rooting medium.

Rooting and Hardening

Well developed shoots [2 cm and above in length] were excised from the culture tube and sub cultured into the MS medium supplemented with 0.5 to 2.0

mg / l IBA. *In vitro* regenerated shoots were transferred to half strength MS basal media supplemented with growth regulator in the following concentrations.

1. IBA 0.1mg/l + ½ MS,
2.IBA 0.5mg/l + ½ MS,
3. IBA 1.0mg/l + ½ MS

Observations were recorded on percentage of rooting, average number of roots and mean length of roots. The cultures were kept under 16 hrs light / day photo period, the rooted plantlets were removed from the culture tubes and washed in running tap water. The numbers of roots in each plantlet were counted and they were transplanted into plastic pots containing sterile soil, sand and vermiculite [1:1:2]. The pots were covered with transparent plastic bag to maintain humidity under 16 hrs. The well grown plants were transferred to larger pots containing 2 liters of the soil mixture [soil – 1; sand – 1; vermiculite- 1] and placed in the green house. The plants growing in field were observed for their growth and survival after 20 days. Each and every stage of the differentiation was noted and photographs were taken with macro lens. Rooted plants were established at 75% success rate in pots after hardening[12].

Results and discussion

Standardization of sterilization technique

Sterilization procedures for obtaining the aseptic cultures of were done using explants rhinacanthus nasutus collected from the fields. Sterilization procedures were carried for nodal, shoot tip, petiolar segments and leaf lamina. The percentage of contamination, mortality and response were recorded and tabulated in Table No 1.

Table No. 01: Effect of Mercuric chloride on the establishment of Explants

Duration Of $HgCl_2$ Treatment (min)	Percentage of Contamination		Percentage of Mortality		Percentage of Survivability	
	Nodal & Shoot tips	Leaf lamina	Nodal & Shoot tips	Leaf lamina	Nodal & Shoot tips	Leaf lamina
3	12.65	18.66	6.71	19.13	80.59	62.21
5	7.60	6.87	14.54	36.77	77.86	56.36
10	0.00	0.00	99.60	99.33	0.40	0.67

Callus induction

Leaf lamina and petiole was inoculated on MS medium containing different concentrations of 2, 4-D and the number of days of callus formation, percentage of explants response, types of callus and relative amount of callus was recorded and tabulated in Table No -2

Table No. 02: Effect of 2, 4- D on callus induction from the leaf explants of Rhinacanthus nasutus

Explant	2,4-D (mg/l)	No of days For Callus formation	Percentage of explant response	Type of callus	Relative Amount of callus
Leaf	0.5	10	68	Green&compact	++
Leaf	1	15	74	Green&Compact	++
Leaf	1.5	20	95	Green&Compact	+++
Leaf	2.0	25	97	Green	++++

0 : No callus, + : Poor callus (1/4th of the explant is covered by callus}, ++ : Moderate callus (1/2 of the explant is covered by callus), +++ : Good callus (3/4th of the explant is covered by callus), ++++ : Very good callus (full explant is covered by callus). MS media complemented with 2 mg/l 2, 4-D showed 98% response of cultures followed by 0.5mg/l 2,4-D and 1 mg/l 2,4-D. The minimum number of days taken for initiation of callus in 2mg/l 2,4-D is followed by 1mg/l in 2,4-D. The type of callus formed was compact and green.

Effect of 2, 4- D on callus induction from the leaf explants of Rhinacanthus nasutus

Indirect regeneration

The explants were inoculated MS media supplemented with different concentrations of BAP and Basal media. The regeneration was observed after 20 days. The percentage of response, number of shoots per explant was recorded at regular time interval. The maximum numbers of shoots were recorded in the medium containing 1 mg/l of BAP supplemented with 0.1mg/ NAA. Minimum numbers of shoots were recorded in 2mg/l of BAP

in combination with 0.1mg/l NAA. Nodal and shoot tips were opted as source of explant for multiple shoot production as their multiplication rate was high. It was observed that the best medium for multiple shoot production was MS basal medium supplemented with 1mg/l of BAP and 0.1mg/l NAA.

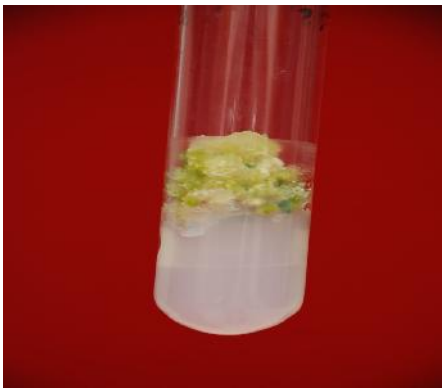

Fig. 01

Fig. 02

Effect of cytokinins on shoot proliferation from the nodal explants of Rhinacanthus nasutus

Table No. 03: Effect of cytokinins on shoot proliferation from the nodal explants of Rhinacanthus nasutus

Sl No.	Concentration and combinations of hormones	Percentage of shoot proliferation	Number of shoot/ explant	Average shoot length (cm)
1.	Basal	60	1.2 ± 0.2	5.6 ± 0.4
2.	BAP 0.5	30	0.3 ± 0.1	0.8 ± 0.2
3.	BAP 1.0	30	0.4 ± 0.1	0.8 ± 0.1
4.	BAP 2.0	40	0.6 ± 0.2	1 ± 0.2
5.	BAP 0.5 + NAA 0.1	40	0.6 ± 0.2	1 ± 0.1
6.	BAP 1.0 + NAA 0.1	92	1.2 ± 0.4	5.6 ± 0.2
7.	BAP 2.0 + NAA 0.1	70	1.2 ± 0.1	4 ± 0.3

Rooting of Plantlets

Half strength MS media supplemented with 0.5mg/l IBA showed maximum number of shoots gave roots .The average root length was found to be 1.7 cm.

Table No. 04: Effect of auxins on root formation from the in vitro micro shoots of Rhinacanthus nasutus

Treatments(mg/l)	Percentage of Shoots developing in to roots	Average No. of Roots	Mean length of Roots (cm)
½ MS + 0.1 IBA	20	5	1.5
½ MS + 0.5 IBA	28	10	1.7
½ MS + 1.0 IBA	56	12	2

In vitro regeneration is a technique used for crop improvement and rapid multiplication of plants. The present study was carried out with an idea to standardize suitable protocols for sterilization, direct and indirect regeneration of Rhinacanthus nasutus. The experiments were conducted at the Plant Tissue Culture laboratory, Sathyabama University, Chennai. Inductions of multiple shoots were best achieved with MS basal medium supplemented with 1mg/l BAP and 0.1mg/l NAA. At this concentration of BAP maximum number of multiple shoots and highest percentage of explant response were obtained. Callus initiation was best inferred in leaf lamina at 2mg/l 2,4-D. *In vitro* rooting of multiple shoots obtained through direct regeneration was best noticed in half strength MS media supplemented with 0.5mg/l IBA. The average number of roots and mean length of roots were maximum in this concentration.

References

1. Farnsworth, N.R. The role of ethno pharmacology in drug development. Ciba Foundation Symposium 1990, 154, 2–11; discussion 11–21.
2. Farnsworth, N. Screening plants for new medicines. In: Wilson, E. (Ed.), *Biodiversity*. National Academy Press, 1988, 83–97.

3. Matthys K. Julsing, Wim J. Quax, and Oliver Kayser The Engineering of Medicinal Plants: Prospects and Limitations of Medicinal Plant Biotechnology 175-184.
4. Gupta, R. and Chadha, K. L., Medicinal and aromatic plants in India. In *Advances in Horticulture, Medicinal and Aromatic Plants* (eds Chadha, K. L. and Gupta, R.), Malhotra Publishing House, New Delhi, 1995, 1–44.
5. Murch SJ, Krishna Raj S, Saxena PK. Tryptophan is a precursor for melatonin and serotonin biosynthesis in in-vitroregenerated St. John's wort (*Hypericum perforatum* L. cv. Anthos) plants. Plant Cell Rep. 2000; 19: 698-704.
6. Singh, H. B., Alternative source for some conventional drug plants of India. In *Ethno botany and Medicinal Plants of Indian Subcontinents* (ed. Maheshwari, J. K.), Scientific Publishers, Jodhpur, India, 2000, 63–78.
7. Nehra NS, Kartha KK. Meristem and shoot tip culture: requirements and applications. In: Vasil IK, Thorpe T (eds). *Plant Cell Tissue Culture.* Kluwer Academy, 1994, 37-70.
8. Pareek, S. K., Medicinal plants in India: Present status and future prospects. In *Prospects of Medicinal Plants* (eds Gautam, P. L. *et al.*), Indian Society for Plant Genetic Resources, NBPGR Campus, New Delhi, 1996, 5- 14.
9. Murashige T. The impact of plant tissue culture on agriculture. In: Thorpe TA (ed). *Frontiers of Plant Tissue Culture,* University Offset Printing Service, 1978, 15–26.
10. Hu CY, Wang PJ. Meristem, shoot-tip and bud culture. In: Evans DA, Wang WR, Ammirato PV, Yamada Y *Handbook of Plant Cell Culture*, vol 1. New York: MacMillan, 1983, 177–277.
11. Dikshit, V. K., Export of medicinal plants from India: need for resource management. In *Biodiversity – North-east India Perspectives: People's Participation in Biodiversity Conservation* (eds Kharbuli, B., Syem, D. and Kayang, H.), NEBRC, North-Eastern Hill University, Shillong, 1999, 85–88.
12. Hussey G. In-vitropropagation. In: Ingram DS, Helgeson JP (eds). *Tissue Culture for Plant Pathologists*. Oxford: Blackwell Scientific, 1980, 51–61.
13. E. Pushpalatha and J. Muthukrishnan ,Efficacy of two tropical plant extracts for the control of mosquitoes Department of Environmental Biology, School of Biological Sciences, Madurai Kamaraj University, and Madurai India.
14. Skirvin RM, Chu MC, Young HJ. Rose. In: Ammirato PV, Evans DR, Sharp WR, Bajaj YPS (eds). *Handbook of Plant Cell Cultures*, vol. 5, New York: MacMillan, 1990, 716– 743.
15. Withers LA, Anderson PG. *Plant Tissue Culture and its Agricultural Applications.* London: Butterworths, 1986.
16. Paek KY, Yu KJ, Park SI, Sung NS, Park CH.. Micro propagation of Rehmannia glutinosa as medicinal plant by shoot tip and root segment culture. Acta Horticult. 1995; 390: 113-120.
17. Roy SK, Hossain MZ, Islam MS. Mass propagation of Rauvolfia serpentina by invitroshoot tip culture. *Plant Tissue Culture* 1994; 4: 69-75.