INTERNATIONAL JOURNAL OF PLANT, ANIMAL AND ENVIRONMENTAL SCIENCES
IJPAES
Volume-6, Issue-4 Oct-Dec-2016 Coden: IJPAJX-CAS-USA, Copyrights@2015ISSN-2231-4490
Received: 1st Oct-2016 Revised: 19th Oct-2016 Accepted: 20th Oct-2016
DOI: 10.21276/Ijpaes http://dx.doi.org/10.21276/ijpaes Research article

IN VITRO CALLOGENESIS AND RHIZOGENESIS IN *AZADIRACHTA INDICA* A.JUSS.

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ABSTRACT: *Azadirachta indica* is typically grown in tropical and semitropical regions all over India. The importance of neem tree has been recognized from time immemorial. The tree is highly medicinal and biopesticidal. As an ayurvedic herb, neem has antifungal and anti bacterial properties and is used to cure diabetes. Neem is an effective alternative to synthetic pesticides. To overcome the problems faced in conventional breeding due to prevalent heterozygosity and perennial nature of the tree, plant tissue culture offers an alternative for quick propagation of neem trees. In *Azadirachta indica* NAA was found more favorable for callus induction and proliferation. In MS + NAA (2mgl/l) +BAP (0.5mg/l) leaf callus proliferated and developed roots *in vitro*, within 20-25 days. Along with roots embryogenic masses were also observed. Rhizogenesis and embryonic masses indicated the possible morphogenic potential of leaf callus.
Key words: *Azadirachta indica*, biopesticidal, plant tissue culture, Rhizogenesis, callus induction

Abbreviations: MS- Murashige and Skoog, NAA- Naphthalene acetic acid, 2,4-D: 2,4-Dichorophenoxy acetic acid, BAP: Benzyl Amino Purine

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INTRODUCTION

Azadirachta indica is typically grown in tropical and semitropical regions and all over India, comes under the Order Sapindales, and family Meliaceae. The importance of neem tree has been recognized by US National Academy of Science, which published a report in 1992 entitled Neem – a tree for solving global problems [10]. The Sanskrit name of neem tree is Arishtha meaning 'reliever of sickness' and hence is considered as 'sarvaroganivarini'. Neem bark and leaf extracts have been therapeutically used as folk medicine to control leprosy, in terminal helminthiasis, respiratory disorders, constipation and also as a general health promoter. Its use for the treatment of rheumatism, chronic syphilis, sores and ulcer has also been effective. Neem finds use to control various skin infections. Bark, leaf, root, flower and fruit together cure blood morbidity, biliary afflictions, itching, skin ulcers [1,3]. Roots are also used, in toothpastes and tooth powders as the antibacterial and germicidal properties help to keep dental hygiene and prevent diseases, it also helps to strengthen the gums [4,5]. Neem is also used as a biopesticide. In agriculture neem oil, fruit and the different by products such as seed cake are used as biopesticides and fungicides [12].

Neem contains Azadirachtin is a tetranortriterpenoid constituent of neem that interrupts metamorphosis in insects, causing pesticidal effects [6, 11]. Azadirachtin (extract of neem seed) repellent for a broad spectrum of agricultural and household insects. The tree is a garden ornamental. Protein-rich stock feed is obtained by chemical processing of neem cake. Timber is used for timber and a fuel source. The biologically active fraction separated from neem kernels shows antiviral activity against certain viruses and has blood-sugar lowering and antimicrobial properties. Compounds derived from various parts of the neem tree are used to treat fevers, thirst, nausea, vomiting, some skin diseases, heat rash and boils [5]. The components of neem oil are reported to have contraceptive properties. Neem leaves and twigs can be used as mulch and fertilizer. Neem seed cake is an organic manure with insecticidal properties and relatively high nitrogen content. Neem oil replaces edible vegetable oil used in making soap which has medicinal properties.

Plant tissue culture is the technique of growing plant cells, tissues and organs in an artificial nutrient medium, solid or liquid under aseptic conditions. Callus, which shows specific characteristics under specific conditions after subculture through many successive passages, is a suitable material for cytodifferentiation.

Conventional breeding programmes for qualitative and quantitative improvements have been rendered inefficient due to prevalent heterozygosity and perennial nature of the tree. In this regard, plant tissue culture offers an alternative for quick propagation of neem trees [13].

MATERIALS AND METHODS

The explants of *Azadirachta indica* were collected from healthy plants from the Zamorin's Guruvayurappan College campus. Tender leaves (especially 3^{rd} – 4^{th} from the apex) and stem fragments (1-2 cm long) were taken from actively growing plants. The leaf explants were prepared by cutting the leaves in to 1-3 cm^2 including the midrib.

The explants were initially washed with running tap water for 1-2 hour and treated with Teepol prior to its treatment with disinfectant solution. These were then surface sterilized with mercuric chloride (0.1%w/v) for 5-10 minutes inside in the laminar air flow chamber and later thoroughly washed 4-5 times in sterile distilled water.

Culture Medium

For the induction of callus, MS medium [8] was used as the culture medium. Double distilled water is used for the preparation of stock solutions, and culture medium. The concentrations of Auxins and Cytokinin used for leaf explants were 2,4-D or NAA (2.0 mg/l), and BAP (0.5mg/l) respectively. The pH of the prepared media was adjusted to 5.8 ± 0.1. The nutrient media was sterilized by autoclaving at 121°C and 16 psi for 15-20 minutes.

RESULTS AND DISCUSSION

Two explants selected in *Azadirachta indica* leaf and shoot fragments, showed different responses in two different media. When 2,4-D was used the stem explant showed very slow and weak response and in NAA, callus was induced within 15 days after inoculation. In leaf explants a slow growing callus masses were observed at the leaf surfaces with 2,4-D. In MS+ NAA callus induction in leaf happened within 10 days after inoculation.

In stem 2, 4-D didn't result in any significant change in fresh weight. In NAA medium, fresh weight increased from an initial fresh weight 0.344g to 0.3994g. The growth index was calculated as 16.1%.

In leaf explants the initial fresh weight of explants 0.237g was increased to 0.945g in NAA and growth index was calculated as 298.73%. Compared to NAA, 2,4-D presented a low increase in fresh weight in leaf explants from the initial fresh weight 0.237g the fresh weight increased to 0.3744g. The growth index was calculated as 57.97%.

Rhizogenesis

In MS + NAA (2mgl/l) leaf callus developed roots *in vitro*, within 20-25 days. Along with roots embryogenic masses were also observed. The rhizogenesis and the embryogenic masses indicated morphogenic potential of the leaf callus. Among the two auxins NAA was more favorable for callus induction and proliferation. Roots were white or later slightly brown, with root hairs. Along with roots, embryogenic masses were also observed in the same culture. The ability to induce roots from callus provides an exclusive system for possible root-specific secondary metabolite production without the need for transgenic technologies (Figure-1, table-1).

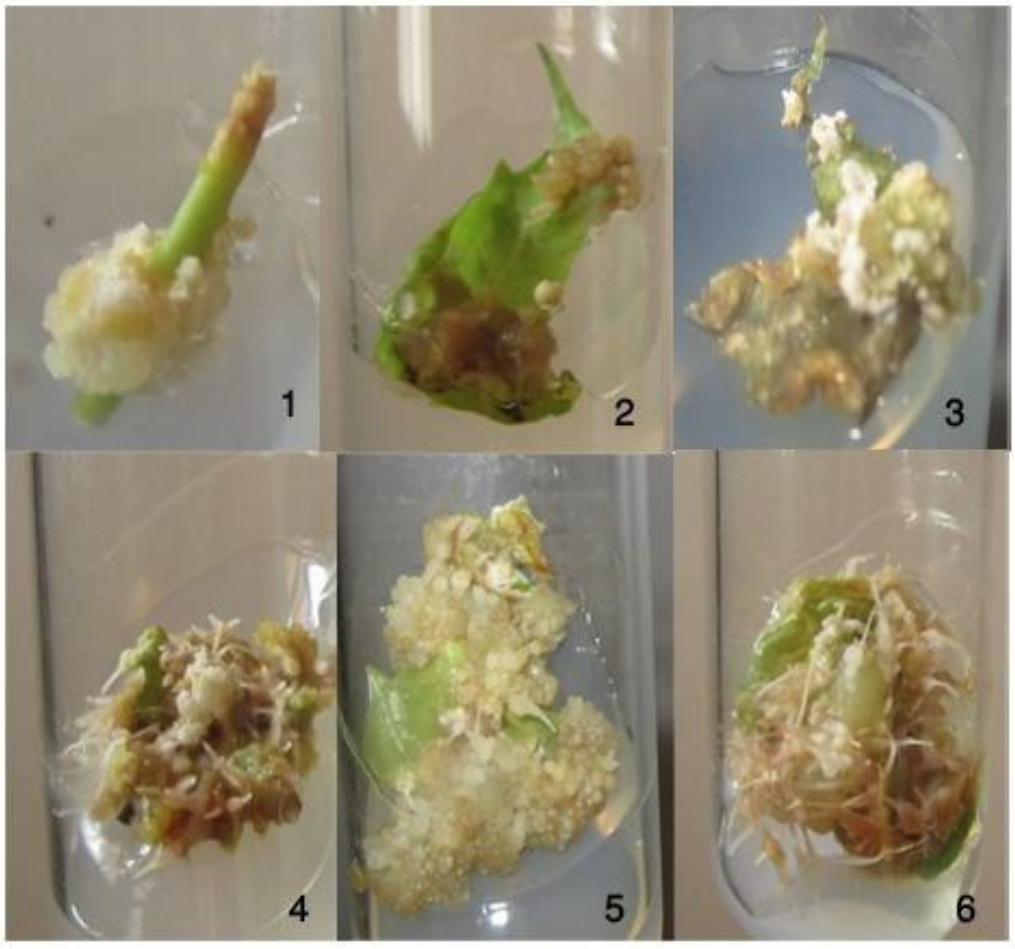
The earlier reports of *Azadirachta indica* in axillary shoot culture showed callus proliferation in medium containing IAA and BA [7,9], regeneration in 2,4-D and BAP [2]. In the present work a good callus proliferation and rhizogenesis was obtained with 2,4-D (2mg/l) and BAP (0.5mg/l).

*Growth Index= Final fresh weight (g) - Initial fresh weight
---------- X 100
Initial fresh weight

Table-1: Effect of NAA, 2, 4-D and BAP on *in vitro* callogenesis and rhizogenesis of stem and leaf explants *Azadirachta indica*

Explant	Plant Growth Regulators (mg/l)	Fresh weights (g)		Growth index (%)*	Root initiation. %	No.of roots initiated
		Initial	Final**			
Leaf	2,4-D 2.0 + BAP 0.5	0.237	0.3744±0.05	57.97	0	0
	NAA 2.0 + BAP 0.5	0.237	0.945±0.04	298.73	82.6%	7.67±0.06
Stem	2,4-D 2.0 + BAP 0.5	0.344	-	-	0	0
	NAA 2.0 + BAP 0.5	0.344	0.3994±0.06	16.1	0	0

**Mean of 25 tubes ± SD



***In Vitro* Response of *Azadirahcta indica* A.Juss explants in various media**
Fig.1. Stem callus 25 days after inoculation in MS + NAA 2.0 mg/l + BAP 0.5 mg/l
2,3. Leaf callus 15 and 20 days after inoculation in MS + 2,4-D 2.0 mg/l + BAP 0.5 mg/l
4,5,6. Leaf callus at various stages in MS + NAA 2.0 mg/l + BAP 0.5 mg/l

CONCLUSIONS

1. In *Azadirachta indica* NAA was found more favorable for callus induction and proliferation than 2,4-D.
2. Rhizogenesis and embryonic masses indicated the possible morphogenic potential of leaf callus.
3. Callogenesis and rhizogenesis can be exploited for the production of several commercially valuable secondary metabolites through *in vitro* culture technique. Normal methods of production of biochemical compound may require destruction of large number of field plants. This protocol also promises conservation of valuable germplasm through *in vitro* methods.

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ISSN 2231-4490

International Journal of Plant, Animal and Environmental Sciences