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MICROPROPAGATION OF *JUSTICIA ADHATODA*: MEDICINAL PLANT OF FAMILY ACANTHACEAE.

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ABSTRACT

Expanding global use of traditional medicines is leading to fast depletion of medicinal plants from the nature. Their cultivation in many areas has not been successful and there is concern about genetic fidelity. Cultivation has also reduced their secondary metabolite contents as compared to wild type. Plant tissue culture offers an easy and reproducible method of micro propagation of medicinal plants on large scale. The present paper describes micro propagation of *Justicia adhatoda* and possible efforts on metabolic engineering.

KEYWORDS: Metabolic engineering, secondary metabolites, Justicia, Acanthaceae.

INTRODUCTION

Plant tissue culture is an important method of obtaining valuable single plant or a hybrid produced through protoplast culture, anther culture, or genetic recombination.This can provide easy method of clonal propagation of selected plants. However chance occurring of "ploidy chimeras" as late Professor Neumann has called in his publication (Neumann *et al*., 2009) may be a problem. This can provide easy method of clonal propagation of selected plants. Recently molecular characterization of tissue culture produced plants has been attempted to check and monitor clonal fidelity (Kumar and Shekhawat 2009; Kumar, 2010). This has been of great importance in case of woody plants and fruit trees with immense economical potential besides horticultural crops and medicinal plants. To confirm the efficacy of the technique and genetic fidelity of the regenerants to make them commercially

viable, different molecular markers have been employed. *In vitro* cultivation, cryopreservation and micropropagation through tissue culture may also aid the preservation of medicinal plants and provide the complimentary conservation option (Benson *et al*., 2000). Recent developments in plant cell and tissue culture technique have provided an alternative to whole plant cultivation for the production of plant derived chemicals i.e. secondary metabolites (Staba, 1980; Bhojwani and Razdan, 1983; Kumar and Shekhawat, 2009 and Sharma *et al*., 2013). The application of different elicitors, cell immobilization as well as genetic transformation results in significant production of secondary metabolites from plant tissue cultures. There is a considerable demand of plants in India and this demand is met from the natural habitat. This leads to rapid depletion of plant material due to over exploitation of important plants.

Justicia adhatoda syn. *Adhatoda vasica* Nees. (Acanthaceae), a perennial woody shrub is listed under the top 36 medicinal plant species which are consumed in volumes exceeding 100 Mt. per year (Ved and Goraya, 2007). *A. vasica* is also listed under 22 major medicinal plant species exported from India. There is a considerable demand of this plant in India and this demand is met from the natural habitat. This plant shows low seed germination and slow conventional propagation through cutting. This leads to rapid depletion of plant material due to over exploitation of this important plant. Plant tissue and cell culture system are being exploited for the accumulation of the variety of natural products (Komaraiah *et al*., 2003). The tissue culture systems for a number of medicinal plants have been established and this enables the analysis of callus and suspension for the presence of the various secondary metabolites (Rao and Ravishankar, 2002).

However, *A. vasica* is widely exploited for extraction of an alkaloid, vasicine, which is used in the preparation of 'vasaka', a well known drug in the Ayurvedic system of medicine.*Justicia adhatoda* is a potential candidate for investigations aimed at controlling resurgence of multidrug resistant tuberculosis (MDR-TB) and the recent pandemichuman immunodeficiency virus (HIV) (Singh *et al*., 2010).

In *Adhatoda vasica* Nees.previously *in vitro* propagation has been reported by some workers using different types of media and combination of PGRs and also using different explant sources (Jaiswal *et al.*, 1989; Azad *et al.*, 2003; Rahman *et al.*, 2004; Sangeeta and Alak, 2005; Abhyankar and Reddy, 2007; Khalikuzzaman, 2008; Maurya and Singh, 2010b and Lone *et al.*, 2013). However, limited number of plantlets were produced in all the cases.

The present investigation was under taken to devise an effective *in vitro* protocol for high frequency regeneration of *Justicia adhatoda* Nees. using different types of explants, changed basal media and PGRs (especially TDZ) to promote indigenous production to meet ever increasing pharmaceutical demand which can be helpful in its conservation strategy and develop a protocol for induction of organogenesis for further use in biotechnological investigation.

MATERIALS AND METHODS

Explants of *Justicia adhatoda* Nees. were collected from healthy plants. For callus initiation from leaf of *J. adhatoda*, explants were cultured on Murashige and Skoog (MS) medium (Murashige and Skoog, 1962). Young leaves, seeds, nodal segments and roots from plants were collected and washed under running tap water. Explants were immersed in a detergent for 5 min, washed in water once, and then surface sterilized with mercuric chloride (0.01%) for 4-5 minutes, and then explants were washed thrice with double distilled water. The surface sterilized leaves were cut into small pieces. The cut segments were then cultured individually on MS medium containing different concentration (1, 1.5, 2, 2.5 ppm) of Indole Acetic Acid (IAA), 6-Benzyladenine (BA), Kinetin (Kn), 2,4-Dichlorophenoxyacetic Acid (2,4-D). Phytohormones were added in the MS basal medium and then pH of the medium was adjusted to 5.7 to 5.8. The agar is added in the medium before autoclaving at 121°C for 20 minutes at 15 psi.

All cultures were maintained under cool white, fluorescent light with a 16 h photoperiod, the intensity of lights was 3000 lux, at 24±2°C temperature. The response of these inoculated explants was observed after 3 weeks of culture. The present frequency of callus induction was determined by counting the number of explants producing callus as the percentage of the total number of explants. The callus induced was subcultured at an interval of 21 days on the same medium. They were monitored developed and maintained on the same medium. Callus was healthy over a period of time on this medium. Callus growth was measured after 35 days of culture in term of fresh weight and dry weight. The dry weight was used for alkaloid analysis.

RESULTS

Induction of callus

Induction of callus from different plant parts like leaf, stem, root and nodal segments was attempted. Best callus induction (80.0%) was observed in the nodal segment. based on

average of 3 replicates (Table-1.1.)

Table-1.1. Induction of callus from different parts of *Justicia adhatoda.*

S.No.	Plant Part	Callus Induction
1.	Leaf	8.0
2.	Stem	12.0
3.	Root	4.0
4.	Nodal segment	80.0

Effect of different growth regulators on callus formation

The experiment consisted of 6 treatments five(MS medium with different growth regulators) and one control (MS medium without growth regulators).

Results are depicted in Table-1.2. The best result was obtained in MS medium supported with NAA (10 mg/l), BA (0.1mg/l), Kn (0.1 mg/l) and 2,4-D (2.0 mg/l).

Table-1.2. Effect of different growth regulators in MS medium on callus induction from nodal segment of *Justicia adhatoda*

S.No.	NAA (mg/l)	BA (mg/l)	Kn (mg/l)	2,4-D (mg/l)	Percent callus induction
1	00	00	00	00	Nil
2	1.0	0.1	0.1	0.1	11.0
3	5.0	0.1	0.1	0.1	59.0
4	10.0	0.1	0.1	0.1	78.0
5	10.0	0.1	0.1	2.0	81.0
6	10.0	0.1	0.1	4.0	40.0

Effect of different growth regulators on shooting

The experiment consisted of three treatments with different combination of growth regulators and one control without any growth regulators using nodal segments.

Results are shown in Table-1.3. The best shooting was observed in MS medium supported with IAA(1.0 mg/l), BA (1.0mg/l) and Kn (1.0 mg/l).

Table-1.3. Effect of different growth regulators in MS medium on shooting in callus of *Justicia adhatoda.*

S.No.	Concentration mg/l			No.of shoots
	IAA	BA	Kn	
1.	00	00	00	No shooting
2.	0.5	1.0	1.0	2.0
3.	1.0	1.0	1.0	4.0
4.	1.5	1.0	1.0	2.0

DISCUSSION

Several studies have been conducted on use of plants in traditional medicine for humans and veterinary (Sharma *et a*l., 2005; Sharma and Kumar, 2007; Sharma and Kumar 2012).Recent estimates suggest that over 9,000 plants have been known to have medicinal applications in all cultures of various countries (Farnsworth, 1988; Kumar, 2008 and Bhansali *et al*.,2011).Of the 89 recorded plant species, frequently applied plant species against veterinary and human ailments included: *Adhatoda vasica*, was the most cited species (43%).

Thar desert of Rajasthan is also highly rich in medicinal plant diversity. Some very important medicinal plants of potent medical value have been discovered through ethnobotanical survey of Thar desert in Rajasthan (Sharma and Kumar 2007; Bopana and Saxena 2007; Sharma and Kumar 2011 and Pareek and Kumar, 2013). With increasing use of traditional medicines globally, attempts are also underway to discover the cure of HIV in the traditional medical system (Kumar, 2000; Kotia and Kumar 2001 and Sharma and Kumar2012). Modern pharmacopoeia still contains at least 25% drugs derived from plants and many others which are synthetic analogues built on prototype compounds isolated from plants (Sharma *et al*.,2005). Much of the recent efforts have focused on finding out the traditional uses of medicines and establish their scientific explanations based on biochemical pharmacological and biotechnological methods. A large number of old remedies have provided basis for the modern medicine.Extracts of the leaves of *Adhatoda vasica* are extensively used in cough, asthma, bronchitis, tuberculosis, inflammation and allergy.The plant extract has been reported to contain a number of alkaloids(Chakraborty and Brantner, 2001).Vasicine is a major alkaloid of vasaka present in the concentration of 1.3%. Minor alkaloids include adhatonine, vasicinol and vasicinolone. Vasicinone formed by oxidation of vasicine at C-8 position, is one of the major alkaloids of J. adhatoda and is known to possess interesting biological activities. This includes respiratory, immunostimulant, bronchodilator and hypotensive activities. The leaves contain several alkaloids (vasicinone, vasicinol, adhatodine, adhatonine, adhavasinone, anisotine and peganine), betaine, steroids, carbohydrates and alkanes.In the flowers triterpines (α-amirine), flavonoids (Apigenin, astragalin, kaempferol, quercetin and vitexin) are found (Laakso *et al.*,1990 and Suthar *et al.*, 2009).Plant tissue and cell culture system are being exploited for the accumulation of the variety of natural products. In view of interesting biological activities associated with vasicine despite the extensive use of vasaka, tissue culture studies for production of vasicine have been limited. The present study was

therefore, focused on initiation and establishment of tissue cultures of *A. vasica* to compare the potential of *in vitro* cultures for production of secondary metabolites.

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