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Micropropagation of *Adhatoda beddomei* Using Nodal Explant

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Abstract:

Adhatoda beddomei is an important medicinal plant of Acanthaceae family, traditional Indian medicinal shrub widely used in the treatment of many medications in India. Its allied species A. vasica is known for its important drug popularly known as "Vasaca". A. beddomei is now included in the IUCN Threatened Plants List of India. The leaves & roots contain several alkaloids. A protocol was established for micropropagation of Adhatoda beddomei using nodal explants. Nodal explants were inoculated onto Murashige and Skoog – MS medium supplemented with nodal segment were cultured in full strength MS medium supplemented with different conc. hormones namely: BAP (0.6 to 1.5) mg/l. Among the various conc. of BAP hormone used for multiple shoot formation the best response was produced with MS medium containing 0.9 mg/l of BAP with an average of 6.5±0.3 cm shoots/explants. In vitro raised shoots were successfully rooted on MS medium containing combination of 1.3 mg/l of IBA and 2.2 mg/l of NAA with an average of 7.1±0.78 roots of with an average of 0.64±0.05cm length.

Key words: *Adhatoda beddomei*, micropropagation, BAP, IBA, NAA

Introduction

Adhatoda beddomei and *Adhatoda vasica* belongs to the Acanthaceae Family and is an important medicinal plant. Acanthaceae is a large panatropical family of about 250 genera and 2,500 species. It's a rare evergreen shrub having restricted distribution at elevation of 1000 m in the hills of southern India. In Hindi, it is known as Adusa, Arusa .Mature plants are the important source of drug vasaca and it is considered therapeutically important to the allied species *Adhatoda vasica. Adhatoda vasica* is a well-known plant drug in Ayurvedic and Unani medicine. Adhatoda leaves have been used extensively in Ayurvedic Medicine primarily for respiratory disorders. This plant pacifies vitiated pitta, cough, bronchitis, asthma, inflammation, haemorrhage, haemorrhoids, bleeding of diarrhoea, and disease of eyes. *Adhatoda beddomei* is a rare evergreen shrub with restricted distribution at an elevation of 1000 m in the hills of South Travancore in southern India. Mature plants are an important source of the drug vasica in the indigenous of medicine (Aiyer and Kolammal 1963).Where it is considered therapeutically superior to the allied species, *A. vasica*.

This plant has medicinal uses, mainly antispasmodic, fever reducer, anti-inflammatory, anti-bleeding, branchodilator, antidibetic, antihelminthic, disinfectant, anti-jaundice, antiseptic, oxitocic and expectorant and has many other medicinal applications (P.K. Patel and P.H. Bhatt (1984), A. Chakraborty and A.H. Brantner (2001), and R.L. Wakhloo *et al*. 1980). There is a considerable demand of this plant in India and this demand is met from the natural habitat. This plant show low seed germination and conventional propagation through cutting is slow (*The wealth of India* (1985) and A.S. Mathew and K.N. Patel, 1998). This leads to rapid depletion of plant material due to over exploitation of this important plant. Pant tissue and cell culture system are being exploited for the

accumulation of the variety of natural products K. Kishor (1987), P. Komaraiah *et al* (2007). 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 (S.R. Rao and G.A. Ravishankar (2002).

Generally, the plant is propagated by seed as well as stem cutting but very low seed germination and labour intensive vegetative propagation by stem cutting are constrains for large scale production of *A. vasica.* Attempts have been made in the past for regeneration of *A. vasica* through tissue culture (Jaiswal *et al*., 1989; Azad *et al*., 2003; Abhyankar and Reddy, 2007; Khalekuzzaman *et al*., 2008; Kumar *et al*., 2007). The alkaloid content of the plant has been reported to vary with the genotype and environment, so clonal propagation has been recommended (Duster, 1985).

The young juvenile nodal explants of *Adhatoda vasica* cultured on Murashige and Skoog's (MS) medium supplemented with CW (15% v/v) + BAP (5 mg/l) started proliferating multiple shoot buds (14±2) in 3 to 4 weeks and the shoots became 3 cm long in 6 to 8 weeks. The regenerated plants produced well developed roots on transfer to MS + IBA 1 mg/l in 10 to 12 days. The rooted plantlets were acclimatized before transfer to soil and 80% of the transferred plants survived (Raageeva Bimal and M.d. Shahnawaz, 2011). Earlier not much work has been studied in *A. beddomei* plant. In this context, our work mainly deals establishment of a plant tissue culture technique which can provide numerous plant production within a stipulated time period that to disease free and at any season.

Materials and Methods

Nodal explants were collected from Anand Agriculture University, Gujarat, India. The explants were surface sterilised with 0.1% (w/v) mercuric chloride solution for 5 min under

aseptic condition and washed 4-5 times with autoclaved distilled water. In order to initiate *in vitro* cultures of axillary shoots, surface sterilised nodal cuttings 3.5-4.0 cm with one node inoculated on Murashige and Skoog (1962) (MS) medium supplemented with different levels of cytokinins concentration (0.6-1.5 mg/l BAP) and pH of the medium adjusted to 5.8.before autoclaving. Initial explants were cultured in borosilicate glass tubes (25 × 150 mm) containing 20 ml of medium. Data concerning bud breaking (%), shoot number per initial bud and shoot length were recorded after 45 days of culture.

For shoot multiplication, single nodes from elongated shoots were excised and were regularly sub cultured at intervals of 45 days on MS medium supplemented with same concentration of BAP, and on the same time shoots (minimum 2.5 cm in length) were excised from culture and transferred to rooting medium containing mineral MS salts diluted by half, supplemented with different combination (0.1 up to 3.5 mg/l) IBA/NAA.

All the cultures were maintained in an air conditioned culture rooms at a temperature of 25 ± 4^0 C and with a relative humidity 50-60%. The source of illumination consisted of tubes with intensity of 2000 lux and a 16 hours light regime and was followed by 8 hours darkness.

For hardening and acclimatization, rooted shoots were carefully removed from the culture tubes and washed with sterile water to remove traces of gel. Then plantlets were transferred to thermo coil cup containing soil, vermiculate and organic matter (1: 1: 1) for 15 days.

All the experiments were conducted with minimum 10 replicates per treatment and repeated twice. Each replicate represents one node per culture vessel. The results are expressed as means ± SD of two experiments. The data were analyzed statistically.

Results and Discussion

Shoot multiplication could be achieved from the nodal explants of *A. beddomei* inoculated on MS medium supplemented with BAP. The ability of the nodal explants for the bud break varied depending on the concentration of BAP. Shoots emerged with all the concentrations of BAPs investigated and bud break occurred after 7-8 days of culture

The results of the concentrations of BAP effects on shoot induction and multiplication are shown in Table 1 and Fig 1(A).Among the various conc. of BAP hormone used for multiple shoot formation the best response was produced with MS medium containing 0.9 mg/l of BAP with an average of 6.5±0.3 cm shoots/explants along with an average of 2.9±1.6cm leaves/explants. As growth concern, from above result it can be stated that the endogenous hormone level is higher in the nodal segment so only low level of hormone i.e., 0.9 mg/l of BAP is required for shoot induction and multiplication. It might be due to the more endogenous cytokinins percentage in plant. Concomitantly nodal segment possess more of cytokinins meant for cell division and proliferation of leaves and certain flower synthesis.

Findings that in *Adhatoda vasica* by Nath and Buragohain (2005), average number of shoots (3.0±0.15) were more in the medium supplemented with 2mg/l BA, but 96.7% of the explants showed callus at the cut end. The medium turned brown due to the release of the phenolic exudates and the leaves turned necrotic and fell off.

The increase of shoot number due to successive transfer of cultures on fresh media may be due to suppression of apical dominance during subcultures that induce basal meristematic cells to form new shoots (Shekhawat & Shekhawat 2011, Tripathi & Kumari 2010).

Sr.no	BAP conc. (mg/l)	No of explants cultured	No of explants responding	Mean percent response	No of shoot/explants	Mean length of shoot (cm) (mean±s.d.)	Mean no of leaves/shoot (mean±s.d.)
1	0.6	10	8	80	2 – 3	3.2±0.21	2.0±0.15
2	0.7	10	10	100	2 – 3	4±0.21	2.5±0.12
3	0.8	10	7	70	2 – 3	4.3±0.23	1.9±0.16
4	**0.9**	**10**	**10**	**100**	**3 -4**	**6.5±0.3**	**2.9±0.15**
5	1	10	8	80	2 – 3	3.1±0.26	1.68±0.16
6	1.1	10	8	80	2 – 3	2.4±0.24	2.1±0.2
7	1.2	10	8	80	2 – 3	2.3±0.29	2.2±0.14
8	1.3	10	7	70	2 – 3	1.6±0.22	0.3±0.05
9	1.4	10	8	80	2 – 3	1.5±0.22	1.2±0.1
10	1.5	10	10	100	2 – 3	1.1±0.24	**2.1±0.13**

Table1. Effect of different concentrations of BAP on proliferation of shoot and formation of leaves/shoot

The results of the concentrations of IBA and NAA on root induction and multiplication are shown in Table 2 and Fig 1(B, C & D). Among the various conc. of hormones used for multiple root formation the best response was produced with MS medium containing combination of 1.3 mg/l of IBA and 2.2 mg/l of NAA with an average of 7.1±0.78 roots of with an average of 0.64±0.05cm length. In the absence of NAA (naphthalene acetic acid), there was no rooting which indicates the exogenous hormones required for morphogenesis.

S.no	Auxin concentration		% of root responding	No. of roots/explants	Mean length of roots
	IBA	NAA			
1	0.1	3.4	20	4.6±0.51	0.32±0.12
2	0.6	2.9	40	3.0±0.94	0.2±0.094
3	0.7	2.8	20	3.8±1.39	0.26±0.10
4	0.8	2.7	40	4.8±1.22	0.36±0.10
5	0.9	2.6	60	3.8±1.39	0.28±0.12
6	1	2.5	60	3.8±0.78	0.28±0.07
7	1.1	2.4	80	4±0.94	0.34±0.084
8	1.2	2.3	60	4.4±0.84	0.34±0.051
9	**1.3**	**2.2**	**100**	**7.1±0.78**	**0.64±0.051**
10	1.4	2.1	80	3.4±1.07	0.31±0.11
11	**1.5**	**2**	**100**	**6±0.66**	0.48±0.078
12	2	1.5	20	2.8±0.42	0.22±0.078
13	2.5	1	0	-	-
14	3	0.5	0	-	-
15	3.5	0	0	-	-

Table 2 Effect of different concentrations of IBA and NAA in full strength MS medium on the root development and the root length

After hardening of plantlet in soil, vermiculate and organic matter it was found that hardening of plantlets in soil: vermiculate: organic matter (1:1:1) showed higher rate of plant survival (Fig.1 E & F).

Hardening of plantlet is most important stages in micropropagation as plantlets are very soft to withstand soil environment. Hardening of plantlets in vermiculate shows high rate of survival because it having higher amount of organic supplement.

Water holding capacity of soil is crucial parameter so different soil mixture was used for acclimatization.

The effect of IBA on rooting of other plants was reported (Bernabe-Antonio *et al*. 2012, Phulwaria *et al*. 2012, Phulwaria *et al*. 2013, Rathore & Shekhawat 2013). Seventy percent of plantlets survived acclimatization in our case.

Conclusion

This work describes a simple protocol for micropropagation of *A. beddomei* for mass multiplication and conservation.

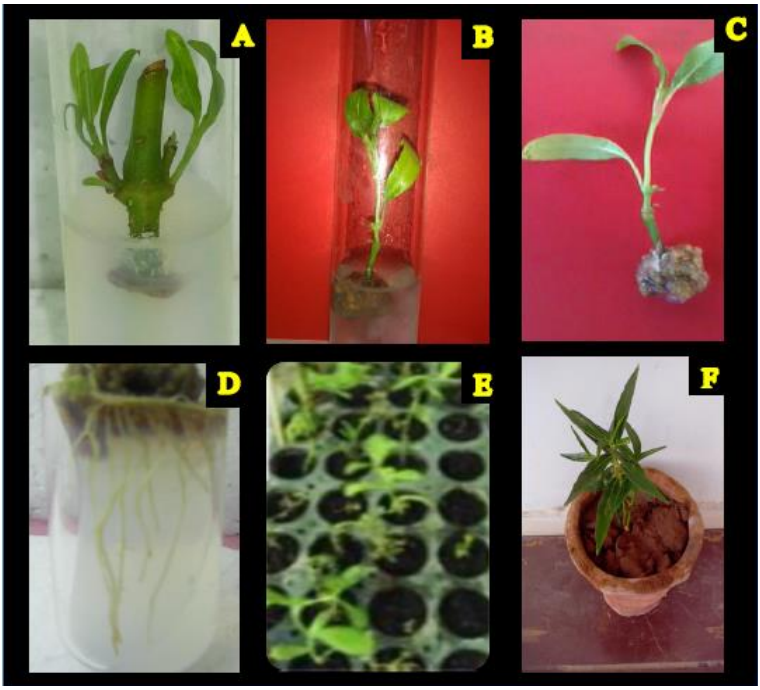

Figure: 1:- (A & B) - Shoot multiplication, (B & C) - initiation of roots, (D) - showing only multiplication of roots and (E & F) - hardening of plantlets.

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