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In vitro Micropropagation of *Adhatoda vasica*., an Important Multipurpose Medicinal plant of India

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ABSTRACT: The paper deals with in vitro micropropagation of *Adhatoda vasica* (Family: Acanthaceae) is an important multipurpose medicinal plant has been used intraditional medicine for thousands of years to threat respiratory disorder, bronchitis, tuberculosis, leprosy, blood disorders, heart troubles, thirst, asthma, fever, vomiting, leukoderma, jaundice, mouth troubles and gonorrhea. Low percentage of seed germination and low survival rate by stem cutting restricts its mass propagation. Therefore, a protocol has been developed for rapid in vitro multiplications of this plant using nodal and internodal region as explants. The induction of shoots and root was tried on various media composition in laboratory. The optimum response of callus induction was achieved on MS Medium supplemented with BAP (1mg/l) alone. Multiple shoot induction was achieved on MS Medium supplemented with combination of BAP (1mg/l) and NAA (0.2mg/l) and BAP (1.5mg/l) alone. The root induction observed on MS Medium supplemented with IBA (1mg/l).

KEYWORDS: Propagation, Conservation, Callus, Shoot multiplication, MS Medium, Micropropagation

ABBREVIATION: MS: Murashige and Skoog, BAP: 6- Benzyl amino purine, IBA: 3-Indole butyric acid, NAA: 1-Naphthalene acetic acid.

I. INTRODUCTION

Plant cell have the potential to differentiate on division and develop organs and complete plants (Murashige, 1974). The term plant tissue culture is a misnomer because plant micropropagation is concerned with the whole plantlet and not just isolated tissue, though the explant may be a particular tissue (Kumar *et.al*, 2003). Since current treatment is associated with higher risk of drug resistance and quite expensive to common person, there is more emphasis on use of the medicinal plants for infectious disease like AIDS. (Heble, 1993). A balanced solution which served as a basic medium for a wide spectrum of plant tissue is that of Murashige-Skoog (MS) (1962), Gamborg B5 (1968).

Adhatoda vasica Nees commonly known as *vasica* of family Acanthaceae. The plant grows in plains and in lower Himalayan. It is small evergreen, sub herbaceous and bush. The plant having broad, lanceolate leaves, the leaves become greenish brown when dried and smell similar to that of strong tea. The leaves are rich in vitamin, carotene and essential oil. The shrub is also source of the drug, well known in indigenous systems of medicine particularly in bronchitis, asthma, whooping cough, almost all part of the plants is used to cure asthma in ayurvedic medicine, leaves are used as fungicide and insecticide (Naik, 1998)

II. MATERIAL AND METHODS

Percentage of seed germination of *Adhatoda vasica* was much low therefore, explants were taken from field grown mature plant, which must be healthy and free from any type of fungal or bacterial infection. The explant was directly collected from the field of medicinal garden of Post Graduate Teaching Department of Botany Rashtrasant Tukadoji Maharaj, Campus, Nagpur University Nagpur.

For the surface sterilization, the explants were washed in running tap water to remove surface adherents for 30 minutes, then treated with 0.1% (W/V) Bavistin for 5 minutes, then washed in running tap water for 25-30 minutes. The further surface sterilization was carried out on laminar air flow bench. The explants selected were initially trimmed into appropriate size of 1-1.5 cm and then were washed with sterile double distilled water 3 times for 5 minutes each, later they were treated with 0.1% (W/V) HgCl₂ single wash for 3-5 minutes, followed by 2 washes of 70% alcohol for 3 minutes each and at last the explant were washed vigorously with sterile double distilled water thrice for 5 minutes each. Surface sterilized explants were inoculated in culture tubes on Murashige and Skoog (MS) basal medium. The culture medium consisted of different concentration of BAP with the combination of NAA in mg/l. After that 0.3% of sucrose were added, the pH of the medium was adjusted to 5.8, then 0.8% (W/V) agar were added and autoclaved at 121°C for 20 minutes. The cultures were kept under 16 hours of photoperiod at 25±2°C temperature.

III.RESULT AND DISCUSSION

Explants were inoculated on the MS medium supplemented with BAP (1 mg/l) and NAA (0.2 mg/l) for callus initiation. The node was found to be the best responding explant, followed by internode and leaf. The nodal explant when inoculated on the MS medium supplemented with BAP (1mg/l) callus induction was observed. Whereas, explants inoculated on MS medium supplemented with the combination of 2,4-D (1-2.5 mg/l) and Kinetin (0.5 mg/l) did not shows the response at all (Table A). Similar type of hormones combination was also used by Pande, 1996 in the plant *Ammi majus* L. (Apiaceae/Umbeliferae), Ramashree *et al.*, (1997) in *Aristolochia indica*, Johri *et al.*, (1996) in *Piper betel* from shoot tip, Bhat *et al.*, (1992) in *Piper longam*, Nataraja and Neelambika, (1996) in *punica granatum*. Callus on further subculturing on MS medium having BAP (1.5mg/l) shows Organogenesis within 30 days of subculture (Table B2). The nature of the callus was greenish yellow in color, compact and hard to cut (Table A, Plate 1A). The callus derived from the nodal and internodal explants were subcultured on MS medium with BAP (1.5mg/l) was initially turned yellowish green in color and then multiple shoots were regenerated within 30 days of subculture (Table B2, Plate 1 F&G).

Various types of explants were used to obtain direct multiple shoot induction like node, internode, and leaf. The explants were inoculated on MS medium supplemented with BAP (1-10mg/l) alone and also in combination with NAA (0.1-0.5mg/l). Best medium for multiple shoot regeneration and callus induction were MS medium supplemented with BAP (1mg/l) and BAP (1.5 mg/l) alone. Whereas, BAP (10mg/l) show only shoot bud induction without callus formation. In case of nodal explant shoot initiation was obtained within 13-15 days after the day of inoculation. Therefore, node as an explant was found to be the best explant for regeneration of multiple shoots (Table B1, Plate 1 D & E). Many experimenters had utilized different combination of BAP and NAA for multiple shoot induction like, Mustafa and Hariharan, (1997) in *Alpinia galanga*, Mujib *et al.*, (1995) in *Catharanthus roseus*, Sharma *et al.*, (1993) in *Gentiana kurroo*, Korochet *et al.*, (1997) in *Hedeomamultiflorum*. Many researchers had also used BAP alone in MS medium for multiple shoot induction like, Usha and Swamy, (1998) in *Artimisiaannua*.

The regenerated shoots were separated from parent culture aseptically with the help of sterile scalpel under laminar air flow chamber. The healthy shoot was inoculated on MS medium with various combination of IBA (0.1-1mg/l). The root induction was found within 15 days of inoculation. MS medium supplemented with IBA (1 mg/l) was found to be the best root induction medium (Table C, Plate 1 H). Many scientists found root regeneration using IBA like, Usha and Swamy *et al.*, (1998) in *Artemisia annua*.

Table A; Induction of the callus on MS medium supplemented with different hormones concentration in *Adhatoda vasica*;

Type of explant	Media + Hormone conc.(mg/l)	No.of tube inoculated	% Response \pm SE	Duration of Response (days)	Nature of callus
Node	MS+ BAP(1)	10	95 \pm 0.5	15	Greenish yellow, hard, Compact.
Internode		10	85 \pm 1.0	25	
Leaf		10	75 \pm 1.5	30	
Node	MS+ BAP(1)+ NAA(0.2)	10	80 \pm 1.50	25	White, hard, compact
Internode		10	-	-	
Leaf		10	-	-	
Node	MS+ BAP(1)+ NAA(1)	10	85 \pm 1.0	20	White, hard, compact
Internode		10	-	-	
leaf		10	-	-	
Node	MS+2,4D(2) + Kn (0.5)	10	-	-	
Internode		10	-	-	
leaf		10	-	-	

Table B; Induction of multiple shoots in *Adhatoda vasica* on MS medium supplemented with different hormones concentration.

Table B1; - Direct multiple shooting.

Type of explants	Medium hormone + conc.(mg/l)	No. of tube inoculated	% Response \pm SE	Duration of Response (days)	No. of Shoots per explant
Node	MS +	10	75 \pm 1.50	25	2-4
Internode	BAP(1) +	10	00.00	-	
Leaf	NAA(0.5)	10	00.00	-	
Node	MS +	10	85 \pm 1.00	20	3-4
Internode	BAP(2) +	10	00.00	-	
Leaf	NAA(0.5)	10	00.00	-	
Node	MS +	10	90 \pm 0.50	17	6-7
Internode	BAP(10)	10	00.00	-	
Leaf		10	00.00	-	
Node	MS +BAP(1)	10	95 \pm 0.25	13	8-9
Internode		10	00.00	-	
Leaf		10	00.00	-	

Table B 2; Indirect multiple shooting.

Type of explants	Medium hormone + conc.(mg/l)	No. of tube inoculated	% Response \pm SE	Duration of Response (days)	No. of shoots
Node callus	MS +BAP(1.5)	10	85 \pm 1.0	30	7-8

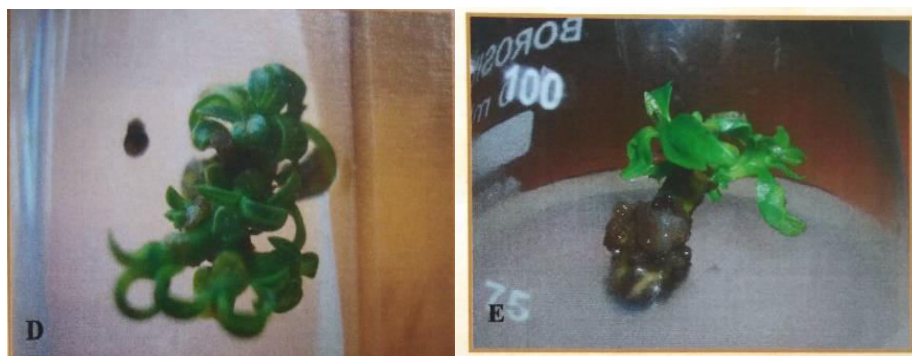
Table C; Root induction on MS medium supplemented with different concentration of IBA.

Medium + hormone conc. (mg/l)	No. of inoculation	% Response \pm SE
MS + IBA(0.1)	5	-
MS + IBA(0.2)	5	-
MS + IBA(1)	5	50 \pm 1

PLATE-1



A: Callus Induction through nodal explant, B: Callus induction through internodal explant, C: Callus induction through leaf explant



D and E: Direct multiple shoot induction through nodal explant



F and G: Indirect multiple shoot induction through nodal callus, **H:** Root induction.

IV.CONCLUSION

In *Adhatoda vasica* of all the explants node and internode were found to be the best explant for callus induction. Whereas, leaf was found to be less responsive. The MS medium supplemented with 1 mg/l BAP was the best medium for the callus induction, also the MS medium with the combination of 1 mg/l BAP and 0.2 mg/l NAA was found to be the good combination for callus induction. Only node was found to be the best explant for direct and indirect shoot induction. The MS medium supplemented with the BAP (1mg/l) alone was the best medium for direct multiple shoot induction. Whereas, MS medium with the combination of BAP (1.5mg/l) was found to be the best medium for indirect shoot induction for the root induction various concentration of IBA (0.1-1mg/l) were tried. The MS medium supplemented with the 1mg/l IBA was found to be best for the root induction. On the basis of present observation, it was concluded that only BAP is sufficient for callus induction and direct shoot induction in case of *Adhatoda vasica*, whereas high concentration of cytokinin and low concentration of auxin favors the multiple shoot induction. For root induction various concentration of only auxin (IBA) alone can promote rapid root induction without cytokinin.

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