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Study of *in-vitro* micropropagation of a medicinally important plant *Adhatoda Vasica* Nees by auxillary-bud culture

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Abstract

The experiment was carried out at plant tissue culture laboratory, Department of Biotechnology, E.R.R.Govt Sci. Auto. P.G. College, Bilaspur, Chhattisgarh, during the period of Nov.08 2010 to May 09 2011. An efficient protocol was devised for rapid in vitro propagation through shoot bud culture of *Adhatoda vasica*. In present investigation the proliferating auxiliary shoot cultures were established on MS medium & Gamborg B5 medium supplemented with different concentrations of BAP, NAA, Kinetin & 2, 4-D using nodal explants from the field grown mature healthy plant of *Adhatoda vasica* after 30 days of inoculation. In cultures raised from nodal explants of *Adhatoda vasica* maximum number of shoots was produced on MS medium supplemented with 1.0mg\l BAP. These explants developed greater than two shoots per nodes while in all other concentration of kn, NAA & 2,4-D developed either two or less than two shoots\explants. Highest frequencies of shoot formation and maximum number of shoots per explants were obtained on MS medium supplemented with 1mg\l IBA.

***Key Words*:** *Adhatoda vasica;* Micropropagation; MS medium; Nodal explants; Shoot bud culture.

Introduction

Adhatoda vasica, also known as Malabar nut tree is part of the Acanthaceae plant family. It is a common small evergreen, sub-herbaceous bush distributed throughout India, especially in the lower Himalayas (up to 1300 meters above sea level), India, Sri Lanka, Burma and Malaysia. In Ayurveda, the ancient system of Indian medicine it is commonly known as vasa (Chopra, 1982) which is a well known plant in indigenous system of medicine. It grows to about a height of 1.5-2.0m with leaves about 10-15cm long & 5.0cm wide & white or purple flowers & 4-seeded fruits. The leaves are of dark green colors above & pale yellow below. Flowers are typical, white arranged in pendunculated spike. Adhatoda leaves have been used extensively in Ayurvedic Medicine for over 2000 years primarily for respiratory disorders. It is propagated by tender stem cuttings. Stem cuttings of 15-20cm long & 3-4 nodes are ideal for planting. Adhatoda is obtained from commercial sources or collected from open fields. Propagation is primarily by means of seeds, can also propagate from cuttings in springs early summer with hardwoods cuttings (Prajapati *et al*., 2003).

Medicinal plants play a key role in world health care systems (Bjaj and Williams, 1995). Charaka Samhita has classified the drug under mucolytic & expecatorant drug. The roots, leaves & flowers are active principles of the plant possess a number of pharmacological properties & are used in cough, chronic bronchitis, rheumatism, asthma & bronchial asthma. Majority of medicinal plant species are rich in biomolecules contents which can cope with health hazards and recently, antibacterial activity of many plant species have been reported (Pandey and Mishra, 2010). The leaves & roots contain several alkaloids (chief principle being quinazoline alkaloid, vasicine & vasicinone, vasicinolone & vasicol), which may have a bronchodilator effect of the bronchii. These alkaloids are said to exist in combination with an acid that has been named adhatodic acid. It acts as a sedative, expectorant, antispasmodic, anthelmintic, bronchial antiseptic and bronchodilator. The leaf extract has been used for the treatment of bronchitis and asthma for many centuries. It relieves cough and breathlessness. It is also prescribed commonly for bleeding due to idiopathic thrombocytopenic purpura, local bleeding due to peptic ulcer, piles, menorrhagia etc. Its local use gives relief in pyorrhea and in bleeding gums. (Doshi *et al*.,1983). The fresh flowers are used n ophthalmia, lessen strangury and jaundice (Kirtikar and Basu, 1994). As the alkaloid content of plant varies with genotype therefore, it is recommended to propagate *A. vasica* plant using vegetative method (Dastur, 1985). The

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objective of this present study was to establish a reproducible protocol for mass propagation and preservation of this valuable medicinal plant resource.

Materials and Methods

Explant collection

For this experiment actively growing and healthy shoot material of *A. vasica*, with three to four nodes were collected from mature plant growing in the Botanical garden, Govt.E.R.R.Sci.Auto.P.G.College, Bilaspur, Chhattisgarh.

Explant sterilization

After removing the leaves, the shoots were thoroughly washed under running tap water for 20 min. and were treated with detergent labolene (Merck, India) for 5 min., followed by distilled water. The shoots were surface sterilized with 0.1% (w/v) mercuric chloride for 10 min. and rinsed with sterile distilled water for 5-6 times.

Inoculation on MS medium

The shoots were cut into pieces 0.5-1.0 cm containing a single node with dormant auxiliary bud and inoculated on to establishment medium to study the effect of MS and B5 medium to establish suitable medium for shoot induction and effect of different concentration of BAP, kinetin and NAA used individually on explants establishment. Established micro shoots were cut and placed vertically on rooting medium. Full strength and half strength MS medium was used for rooting.

Hardening

Hardening of in vitro regenerated plant was carried out in two stages. The first stage consist of liquid hardening for 3-4 weeks, followed by transfer in root trainers containing pre-sterile sand, soil rite and soil (2:1:1) plantlets with well-developed adventitious root systems were washed with sterile distilled water and dipped in 0.2% (w/v) solution of fungicide bavistin (BASF, India) for 30 min. these plantlets were then dipped in IBA solution for 10 min. and planted in the root trainers containing sterile sand, soil rite and garden soil (2:1:1). Humidity was maintained by covering each pot with transparent polythene bags.

Result and Discussion

Table 1. Effect of different levels of BAP (in MS medium) on auxillary shoot growth from nodal explant of *Adhatoda vasica* after 30 days of incubation

Growth Regulator conc. (BAP) (mg/l)	Shoots/ Explant	Average Shoot Length (cm)	Nodes/ Shoot	Leaves/ Shoot	Bud Break (%)
0.00	1.2±0.2	0.3±0.2	Nil	Nil	50
0.10	1.5±0.1	0.6±0.1	1.0±0.1	7.2±0.9	80
0.25	1.6±0.2	2.2±0.2	3.2±0.3	13.9±0.4	100
0.50	2.0±0.3	2.3±0.4	2.9±0.3	13.2±0.2	90
0.75	2.2±1.0	3.5±0.4	3.0±0.2	18.3±1.8	100
1.0	2.5±0.2	6.2±0.3	6.0±0.2	30.2±1.5	100

All the values are mean ± standard deviation of three determinations

Table 2. Effect of different levels of BAP (in B5 medium) on auxillary shoot growth from nodal explants of *A. vasica* after 30 days of incubation

Growth Regulator conc. (BAP) (mg/l)	Shoots/ Explant	Average Shoot Length (cm)	Nodes/ Shoot	Leaves/ Shoot	Bud Break (%)
0.25	0.6±0.08	0.6±0.09	1.3±0.02	1.2±0.16	80
0.50	0.8±0.09	1.0±0.08	1.6±0.18	1.0±0.18	80
0.75	0.6±0.09	0.8±0.18	1.2±0.06	1.0±0.09	80
1.00	1.0±0.02	2.0±0.20	3.0±0.18	2.2±0.16	100
1.50	0.8±0.10	0.6±0.10	0.0±0.00	0.0±0.00	80

All the values are mean ± standard deviation of three determinations

The segments showed bud break response on both the basal mediums (Table 1 & 2). In *A. vasica* the formulation of Murashige and Skoog (1962) basal medium was found more suitable than B5 medium for establishment of nodal explants. Shoots per explants, average shoot length and nodes per shoot were more on MS medium as compared to B5 medium. The MS medium supplemented with 1.0mg/L BAP was most suitable medium for in vitro establishment of nodal explants. The plant growth regulators play a key role in directing the organogenesis response of any plant tissue\organ under in vitro conditions (Che, *et al*., 2002; Sugiyama and Imamura, 2006). On this medium, the nodal segments showed bud break 100%, 2.5±0.2 shoots per explants, 6.2±0.3cm average shoot length and 6.0±0.2 nodes per shoot

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after 30 days of incubation (Table 1) while on B5 medium supplemented with BAP, the nodal segments induced bud break 80-100%, 1.0±0.02 shoots per explants, 2.0±0.02cm average shoot length and 3.0±0.18 nodes per shoot after 30 days of incubation (Table 2). Nodal segments when inoculated on MS medium supplemented with varying concentration of NAA showed 80-100% bud break. The maximum number of shoot per explant, average shoot length and nodes per shoot recorded were 1.4±0.1, 4.0±0.2 and 5.2±0.6 respectively on MS medium with 1.0mg\l NAA (Table 3). Higher concentrations of NAA in MS medium reduced bud break percentage, shoots per explants, average shoot length and nodes per shoot. When MS medium supplemented with kinetin, 100% bud break was recorded at all concentrations. Maximum values for shoot number per explants (1.0±0.00), average shoot length (3.2±0.1) and nodes per shoot (5.2±0.2) were recorded with 0.75mg\l kinetin (Table 4).The different concentrations of BAP in MS medium influenced shoot number, shoot length and node number. In cultures raised from nodal explants of *A. vasica* maximum number of shoots was produced on MS medium supplemented with 1.0mg/L BAP. These explants developed greater than 2 shoots per nodes while in all other concentrations of Kn, NAA and BAP developed either two or less than two shoots/explant. Some other studies reporting efficient callus induction, from different explants, on medium supplemented with cytokinins only are also available for some other plant species as well (Prasad *et al*., 2004; Osman *et al*., 2010**;** Aasim *et al*., 2008). In *A. vasica*, explants showed low percentage of shoot bud induction on cytokinin free MS medium, but addition of BAP in MS medium increased shoot bud induction percentage, shoot length and number of nodes. In the present study the shoots produced with BAP were longer and more uniform in appearance than those produced in kinetin and NAA. Higher concentration of BAP (1.0mg/L) was found suitable for establishment of explants.

Table 3. Effect of different levels of NAA (in MS medium) on auxillary shoot growth from nodal explants of *A. vasica* after 30 days of incubation

Growth Regulator conc. (NAA) (mg/l)	Shoots/ Explant	Average Shoot Length (cm)	Nodes/ Shoot	Leaves/ Shoot	Bud Break (%)
0.10	1.0±0.00	0.8±0.02	1.0±0.1	6.2±0.8	100
0.25	1.0±0.0	1.2±0.1	2.2±0.1	8.3±0.4	100
0.50	1.0±0.0	1.5±0.8	2.8±0.2	16.2±1.0	100
0.75	1.2±0.1	2.0±0.3	4.0±0.3	18.6±2.4	80
1.00	1.4±0.1	4.0±0.2	5.2±0.6	20.2±1.6	100
1.50	1.2±0.1	2.0±0.2	2.2±0.2	12.8±2.0	80

All the values are mean ± standard deviation of three determinations

Table 4. Effect of different levels of Kinetin (in MS medium) on auxillary shoot growth from nodal explants of *A. vasica* after 30 days of incubation

Growth Regulator conc. (Kn) (mg/l)	Shoots/ Explant	Average Shoot Length (cm)	Nodes/ Shoot	Leaves/ Shoot	Bud Break (%)
0.10	1.0±0.0	2.2±0.1	4.2±0.1	18.2±0.2	100
0.25	1.0±0.0	2.3±0.1	4.0±0.2	18.3±1.3	100
0.50	1.0±0.0	2.8±0.1	4.3±0.2	20.2±1.2	100
0.75	1.0±0.0	3.2±0.1	5.2±0.2	22.2±1.2	100
1.00	1.0±0.0	1.7±0.09	4.0±0.3	18.3±1.8	100
1.50	1.0±0.0	1.3±0.06	2.3±0.1	13.4±0.6	100

All the values are mean ± standard deviation of three determinations

Conclusion

From this study, it is concluded that the multiple shoots of *Adhatoda vasica* were developed from axillary bud explants on MS medium and B5 medium supplemented with BAP, NAA and kinetin at the concentration on 1mg/l. This study was efficiently developed a standard protocol to initiate multiple shoot and root culture of plant that may provide a good source of pharmacologically active plant constituents.

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