International Journal of Advanced Research in Biological Sciences
ISSN: 2348-8069
www.ijarbs.com
DOI: 10.22192/ijarbs Coden: IJARQG(USA) Volume 4, Issue 10 - 2017

Research Article

DOI: http://dx.doi.org/10.22192/ijarbs.2017.04.10.026

Effect of Plant Growth Regulators on Micropropagation of *Adhatoda vasica*

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Abstract

A study was conducted for the standardisation on tissue culture medium for the medicinal plant *Adhatoda vasica*. The apical meristem of the plant was used as explants. The explants were first disinfected, with tween 20, bauestin which is a fungicide under non-aseptic conditions, using distilled water. Then the explants are surface sterilized using 0.1%HgCl3. Two types of medium namely Murashige & Skoog and Mitra medium were utilized for the study. MS medium utilized for the study. MS medium showed results in different hormone concentrations. Callus was observed in MS medium BA 5mg + IBA 2mg/l concentration. After two-weeks of incubation multi shoot formation was recorded in the medium with hormone can BA 5Mg/l + NAA 1mg/l and also in BA 6mg/l. Due to the depletion of nutrients and accumulation of toxic products the media sub culturing was done. After two and half weeks the fresh media contain MS Medium with hormones necessary for indirect orhanogenesis. In Mitra medium *Adhatoda vasica* does not shows any response.

Keywords: *Adhatoda vasica* – MS medium – White medium - Plant Growth regulators

Introduction

In bio-diversity plant has important role as herbal medicine. In world's 75-90% rural people were use herbal medicine as before going to a medicinal practitioner. In the world health care system medicinal plants play a critical role (Bajaj and Williams, 1995). *Adhatoda vasica* commonly called as Vasaka or Arusha and belongs to the medicinal family Acanthaceae and is found in India, mostly in the rural areas (K.Jayapaul *et al.*, 2005). Vasaka is well known drug in the Ayurvedic system of medicine and used extensively 2000 years as recommended drugs for different ranges ofaffictionvia, fever, sore eyes, bilious vomiting, bronchitis, asthma, jaundice, disease of respiratory system, diphtheria, gonorrhea and it is used as antiseptic, antiperiodic and anathematic (Kirtika and Basu, 1994). All parts of the plant like leaves, roots, flower, stem and bank having medicinal value particularly the leaves are endorsed with insecticidal and parasiticidal properties and it also contain several alkaloids (vasicinol, adhatodine, adhotonine, adhavasinone, anisotine and peganine), betaine, steroids, carbohydrate and alkanes. In the flower triterpines (a-amirine), flavonoids (Apigenin, astragalin, kaempferol, quercetin, and vitexin) have found (Kokate.C.K *et al.*, 2003; Lahiri.P.K *et al.*, 1964; Atal.C.K *et al.*, 1980). The flower, fruits and roots are extensively used for treating cold, cough, whooping cough and chronic bronchitis and asthma as sedative expectorant, antispasmodic and as anthelmintic (Siddiqui and Husain, 1994).Vasaka is a bitter quinazoline alkaloid; the major alkaloids are vasicine and vasicinone which is present in all parts of the plant (Patel.P.K *et al.*,1984; Chakraborty. A *et al.*, 2001; Wakhloo.R.L *et al.*,1980). This leads to rapid

depletion of plant material due to over exploited for the accumulation of the variety of natural products (Kishore.K *et al.*, 1987; Komaraiah.P *et al*., 2003). The objective of the study is to standardize micro propagation of *Adhatoda vasica.*

Materials and Methods

Plant material

Plants of *Adhatoda vasica* were collected from ORARS Kayamkulam. Any parts of the plant can be used for micropropagation. Apical meristems, leaves, seeds and nodal segments were used as explants. These explants were washed thoroughly with running tap water for 30 mins, surface sterilization of the material to remove loose contaminates attached to explants. Then the explants were washed with soap solution and then the explants were allowed to stand for 20 min in a solution of boustin and washed with water. Subsequently the materials were transferred to running laminar airflow hood. Then the explants were treated in a solution 0.1% mercuric chloride of different time arrangement to ensure contamination free culture. After through rinsing in autoclaved, the explants were transferred to culture medium.

Culture medium containing media and conditions

MS (Murashige and Skoog) medium with normal salts has been modified for achieving the normal growth and development. The medium containing inorganic nutrients (N, P, Ca, Mg, Fe, Co, Zn, Br, Mb), organic nutrients (Carbon source- glucose, fructose, sucrose). Nitrogen source amino-acid, vitamin B1, B2, B4, B5 and B6) and growth regulators (auxin). The medium used for root induction was supplemented with various concentration of IAA (Indole Acetic Acid) and IBA (Indole Butyric Acid), and the medium used for shoot induction supplemented with various concentration of NAA (Napthalene Acetic Acid) and cytokines (BAP, Kinetin, Zeatin). The media pH was adjusted to 5.7 ± 0.1 before autoclaving at 121ºC under 151lbs pressure for 20 min. in order for the development of the culture suitable temperature wall adjusted to about 25±2ºC with a light intensity of 1500 lux provided during 16h photoperiod.

Shoot differentiation and proliferation

Callus incubation is observed within 7 days of incubation. Callus transferred to MS media along with hormones for shoot development. Shoot proliferation achieved in 2 weeks of inoculation. The shoots are then transferred to medium. The callus induced wa ssubcultured at an interval of 20 days on the same medium.

Establishment in pots

After 2 weeks, the rooted micro shoots were transferred to full strength MS liquid for a week followed by transfer to half strength MS liquid medium for acclimatization for another week. The plantlets were then planted in pots containing sterilized mixture of sand and soil, irrigated and kept under florescent light (16hrs) at 25±2ºC. These plants were kept covered with polythene bags to maintain humidity for a week before transfer to the field.

Results

The present study the epical meristem portion of *Adhatoda vasica* was used. Two basal media were used namely (Murashige and Skoog) and mitra medium with various hormone concentrations. For the multiple shoot and proliferation obtained at high frequency from shoot tips, apical meristem and nodal segments were used. The hormones used include NAA, IBA, BA kinetin etc., influence the various concentration showed the shooting, organogenesis, callusing.

The experiment consisted 12 treatments (MS medium with different hormonal treatments), 4 subculturing treatments and one control (MS medium without hormones). At different concentration the explants were capable of directly developing multiply shoot on MS media by combinations of cytokinin and auxin. But when the MS medium supplement to both explants at various concentration of KIN (0.5-4.0mg/l), single healthy shoots were produced in all media composition. These was the result reported in earlier in *Momordica charantia* (Sikdar *et al.* 2003); in *Elipta alba* (Neeti and Kothari 2005) and in *Vanasushava pedata* (Kauppusamy *et al*. 2006).Different concentrations of BA (5, 4 & 2mg/l), NAA(4, 4 & 1 mg/l) and IBA(2, 2 & 1 mg/l) were tested for shoot induction/proliferation studies. Meristem explants placed in mitra medium supplied with hormone BA 4mg/l + NAA 5mg/l the explants showed no response in table.1. In liquid medium of MS medium which was supplemented with BA 2mg/l NAA 5mg/l proliferation of tissues were observed in table 1. Direct organogenesis was also observed on MS medium supplement with BA 4mg/l. Whereas MS media with BA 4mg/l showed no response in table – 1.

From media supplemented with BA 5mg/l and NAA 1mg/l multiple shoot formation was observed through indirect organogenesis. Shooting was desired of MS media with BA 5mg/l and IBA 1mg/l. Whereas MS media with concentrations BA 5mg/l + IBA mg/l showed callusing in table – 1. Multiple callusing was observed on media with BA 6mg/l and NAA 1mg/l in table -1. Micropropagation if *Adhatoda vasica* through callus of apical meristametic portion was activated on the basal MS medium. Supplemented with BA 2mg/l + IBA 2mg/l and BA 6mg/l + NAA 1mg/l in plate I & II the explants classified as week and after another 2 weeks differentiated into shoot. Auxin compound are often distilled in combination with cytokines. Auxin has an ethanol rod in shoot induction and plant regenerations. Adventitious shoot buds were induced from apical meristametic explants of *Adhatoda vasica* on basal medium supplemented with BA + NAA in table.

Table 1. Effect of shoot formation in different MS and Mitra in *Adhadota vasica* by using nodal segment and apical meristem.

S.No	No. of Tubes	Media + Hormone composition			Nodel segment	Apical Meristem	Response	Remarks
		BA	NAA	IBA				
1	6	5	1	1	4.7 ± 0.78	2.3 ± 0.38	---	No growth is occurred (shooting)
2	8	4	5	1	5.0 ± 0.62	2.8 ± 0.35	---	No growth is occurred
3	8	2	4	1	1.8 ± 0.22	2.5 ± 0.31	---	No growth is occurred
4	8	2	4	2	4.0 ± 0.5	2.3 ± 0.28	++	Callus shooting
5	9	1	1	1	1.7 ± 0.56	2.7 ± 0.3	---	No growth
6	7	2	5	2	14.5 ± 1.07	7.5 ± 1.07	++	Indirect 0rganogenesis
7	3	4	1	2	9.0 ± 1.1	4.4 ± 1.1	+++	Direct Organogenesis
8	8	4	4	2	1.7 ± 0.56	2.2 ± 0.73	--	No growth is occurred
9	4	5	1	1	2.3 ± 0.28	8.6 ± 1.07	++	Multiple shoot formation
10	7	5	5	1	3.5 ± 0.5	2.4 ± 0.6	--	No response
11	8	5	1	2	3.5 ± 0.5	2.9 ± 0.41	++	Callusing
12	9	6	1	1	15.3 ± 0.34	14.2 ± 0.03	+++	Callusing multiple shooting
					Sub culturing			
13	13	1	1	3	3.5 ± 0.26	3.6 ± 0.24	++	Shooting is observed
14	14	1	2	5	4.0 ± 0.28	3.6 ± 0.24	++	Shooting is observed
15	15	1	2	2	1.8 ± 0.12	1.8 ± 0.11	--	No response
16	16	1	3	2	19.6 ± 0.33	17.4 ± 0.03	++	Shoot Proliferation Observed

Further sub culturing treatments at various concentration of BA + NAA + IBA (1+1+3mg/l and 1+2+5mg/l) were tested for shoot induction and found to be best concentration for shoot induction but in (1+2+2mg/l) the explants showed no result and in (1+3+2mg/l) the explants show shoot proliferation (Table 1).

Discussion

Traditional medicine has been studies in several ways for humans and veterinary (Sharma *et al.,* 2005; Sharma and Kumar, 2007; Sharma and Kumar 2012). Various countries were well known Medicinal appications over 9,000 people as cultures (Farnsworth, 1988; Kumar, 2008). For the veterinary and human ailments 89 plant species were recorded and frequently applied in that *Adhatoda vasica,* was the most cited species (43%).

Mass micro propagation system has been developed for *Adhatoda vasica.* Several explants were observed during *in vitro* propagation of *Adhatoda vasica,* it shows best result in nodal segment and apical meristem. An agreement with result reported by Khalekuzzaman *et al.*, (2008). A range of cytokines have been investigated for multiple shoot induction with apical meristametic portion as explants. Optimum adventitious shoot buds were produced when meristametic portion was taken 21 days incubation. After sub culturing, the callus shooting, and rooting was also obtained. Multiple shoots were observed in basal MS medium supplement with BH 5mg/l + NAA 1mg/l, BA 6mg/l + NA 1mg/l. In *A. vasica* the development and proliferation were found to more effective in BA than Kn.

Callus formation in explants tissue involved the development of progressively more random planes of cell division. Then frequent specialization of cells and loss of organized progressively more random plants of cell division, less frequent specialization of cells and loss of organized structures are seen. From the above observation available for multiple shoot generation. After 21 days of growth on MS medium, the calluses were transferred to fresh medium. The sub culturing done for multiple shoot generation and initiation. On sub culturing the MS media with IBA 3mg/l showed shoot proliferation and callus formation.

Sakthinarayan suggested that *in vitro* grown shoot for nodel explants were cultured on MS medium with different concentration of BA, NAA and IBA and selecting best BA-NAA and BA-IBA combinations. The nodel segments in the relatives amount and ratios of BA and NAA were remarkably influenced the axillary shoot. The formation of callus was recorded in 4mg/l of IBA and followed by media supplemented with 1mg/l of IBA. The decreased rate of NAA in normal shoots development and simultaneously increased the callus formation of the explants. At these combinations of NAA and BA callus began to form within 2 weeks of culture and the nodel explants BA-NAA combination for proliferation of shoot were found to be be better result in other combination of explants produced only axillary shoot but no roots. On the proliferation medium of shoot, comparatively higher concentrations of BA (6mg/l) along with lower concentration of NAA (1mg/l) showed best result.

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DOI:10.22192/ijarbs.2017.04.10.026	

How to cite this article:
Abdhul. K., P. Bandana Devi, Balagopal. C.P. (2017). Effect of Plant Growth Regulators on Micropropagation of *Adhatoda vasica*. Int. J. Adv. Res. Biol. Sci. 4(10): 204-208.
DOI: http://dx.doi.org/10.22192/ijarbs.2017.04.10.026