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EFFECT GROWTH REGULATORS AND STATE OF MEDIUM ON MICROPROPAGATION OF *ADHATODA VASICA* (NEES.)

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Abstract: The experiment was conducted at Plant Biotechnology Laboratory of Khulna University, Khulna, Bangladesh to study the effect of auxin and cytokinin on shoot induction and subsequent rooting from shoot tip of *Adhatoda vasica*. For shoot proliferation, MS medium was supplemented with BA and Kinetin, ranging from 0.1-2.0 mgL^{-1} and for root induction, both solid and liquid MS media fortified with NAA or IBA 0.1-2.0 mgL^{-1} were employed. Maximum number of usable shoots was found on the medium containing 0.5 mgL^{-1} BA (1.86±0.52) and 1.5 mgL^{-1} Kinetin (1.18±0.34). Root induction on solid medium showed that only one concentration of NAA (0.1 mgL^{-1}) and 1.0, 1.5 and 2.0 mg/l of IBA responded in rooting, but the highest number of roots (6.22±1.79) with the highest average root length (0.88±0.25 cm) were observed on solid medium supplemented with IBA 1.0 mgL^{-1}. All the concentrations of IBA and NAA responded in rooting on liquid medium with maximum number at the concentration of 1.5 mgL^{-1} NAA (16.79±5.3) and 1.0 mgL^{-1} IBA (13.1±3.78). The healthy roots were observed in liquid medium at the concentration of 0.1 mg/l NAA. *In vitro* regenerated plantlets were transferred to sterile soil containing 75% soil and 25% sand and then successfully established under natural condition.

Key words: Solid medium; liquid medium; auxins; cytokinin; shoot and root

Introduction

Adhatoda vasica Nees belongs to the genus *Adhatoda* under a large family Acanthaceae. This plant produces less number of seeds. It is an evergreen bushy shrub of 1.2-2.4 feet in height with many long opposite branches. The species has important medicinal value. Leaves and roots of this plant contain quinqzoline alkaloids, vasicine, anisotine, adhatodine, kaempferol, quercetin, b-sitosterol and essential oil. Seeds also contain some chemicals like behenic, lignocerin, cerotic, oleic and linoleic acids (Ghani, 1998), which are also essential in medicine industries. Peoples are using this plant as a drug of various chest ailments including cough, asthma, bronchitis, pneumonia and phthisis. Alcoholic extract of leaves is hypotensive and has been using in ague.

Many growth-active substances, phytohormones as well as other types of compounds, have included in the culture medium to manipulate organogenesis *in vitro* (Thrope 1980; Chandler and Thrope, 1986). A large number of plant species respond to suitable auxin or cytokinin balance by forming shoots and roots. A high level of auxin to cytokinin is root promoting, whereas, the opposite condition i.e., an increased concentration of cytokinin and low auxin favors shoot

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induction. Shoot induction by rising auxin concentration and its inhibition by the addition of cytokinin have been shown in a particular variety of *Armoracia rusticana* (Sastri, 1963). Use of liquid medium has some advantages such as better oxygen supply; tissues are able to fragment in liquid media producing a greater surface area with a resulting improvement in uptake of material from the medium (Pierik, 1987). In this study, suitable concentrations of growth regulators for clonal propagation of *A. vasica* and performance of liquid media for *in vitro* rooting over solid medium were investigated.

Materials and Methods

About 10 cm long shoots of *A. vasica* were collected from mature plants. Shoots were cut into pieces of about 1 to 2 cm long with scalpel and washed in running tap water. The cut pieces were then rinsed with 1% savlon and subsequently surface sterilized with 0.1% $HgCl_2$ for 5 min with continuous agitation in a laminar hood. Treated shoot pieces were rinsed several times using sterilized distilled water and then cut into small ones (0.5 cm) with sterile scalpel and inoculated aseptically onto shoot proliferation media.

For shoot proliferation MS (Murashige and Skoog, 1962) basal solid medium supplemented with BA and kinetin at varying concentrations (0.1-2.0 mg/l) was prepared. Both solid and liquid MS media were prepared for rooting. Rooting mediawere supplemented with various concentrations (0.1 – 2.0 $mgL^{-}1$) of auxins (IBA and NAA). A support (bridge) was provided with filter papers for standing the micro shoots on liquid medium. The pH of all media was adjusted to 5.7 and to solidify the media, 0.7% agar was added. Prepared culture media were dispensed in the test tubes and sterilized at 121°C for 15 minutes.

For shoot proliferation, inoculated test tubes were places immediately under light (3000 lux) provided by white cool fluorescence tubes. Micro shoots produced in shoot proliferating media inoculated onto the rooting media aseptically. Inoculated test tubes were then placed in dark for seven days. After that the test tubes were replaced in a condition provided by 14 hours light and 10 hours dark. The temperature and humidity of the culture room were maintained 26±2°C and 65%, respectively.

Visual observation of culture was made every week. Data on shoot proliferation and root induction were recorded after six weeks of inoculation and used for calculation. For each treatment 25 explants were used for shoot proliferation and 25 shoots were used for rooting. All the treatments were repeated trice. Data were analyzed as means ± SE according to Mian and Mian (1984). Plantlets were transferred in pot containing 75% soil and 25% sand. For gradual hardening pots were kept under shade for seven days and exposed to sunlight. The pots were irrigated by tap water whenever necessary. Then the hardened plantlets were transferred to soil.

Results

Effect of cytokinins on shoot formation of A. vasica: Different concentrations of BA and kinetin ranged from 0.1-2.0 mgL^{-1} were employed for shoot proliferation. A control treatment was also carried out (Table 1). The highest percentage of response of the inoculated shoot tips (100%) as well as number of usable shoots (1.86±0.52) were observed on the medium supplemented with BA 0.5 mgL^{-1} (Fig. 1), while 85% explants formed shoots at higher concentration (2.0 mgL^{-1}) of kinetin.

Effect of Auxins on root formation onto solid and liquid medium: Five treatments viz. 0.1, 0.5, 1.0, 1.5 and 2.0 mgL^{-1} were employed using NAA and IBA. No roots or basal callus were produced on the solid medium containing IBA 0.1 and 0.5 mg/l onto the cut end of the micro shoots. But in higher concentrations of IBA (1.0, 1.5 and 2.0 mgL^{-1}) roots were formed (Table 2). Maximum number of roots (6.22±1.79) and root length (0.88±0.25 cm) were found at 1.0 mgL^{-1} of IBA (Fig. 2). Solid medium containing NAA 0.5, 1.0, 1.5, 2.0 mgL^{-1} did not produce root, but large basal calli were found at the cut end of the shoot (Table 2 and Fig. 3). In liquid medium IBA 1.0 mgL^{-1} produced maximum number of roots (13.1±3.78) as well as maximum root length (0.66±0.19 cm) (Table 2). Whereas, NAA 1.5 mgL^{-1} and 0.1 mgL^{-1} produced amximum number number of roots (16.79±5.30) and root length (1.02±0.32) respectively (Table 2 and Fig. 2). No callus was found in any of the treatments using liquid media.

Table 1. Effect of BA and Kinetin on shoot proliferation of *A. vasica*.

Concentratio (mgL^{-1})	% explant responded	Average No. of usable sgoot/explan	Average shoot length (cm)
BA			
00	20	0.36±0.1	0.43±0.12
0.1	60	-	0.36±0.01
0.5	100	1.86±0.52	1.96±0.33
1.0	95	0.95±0.26	0.63±0.18
1.5	85	1.70±0.47	0.53±0.15
2.0	70	1.44±0.43	0.15±0.14
Kn			
0.1	50	-	0.28±0.08
0.5	70	0.31±0.1	0.40±0.13
1.0	70	0.63±0.17	0.24±0.07
1.5	80	1.79±0.34	0.23±0.07
2.0	85	1.78±0.56	0.24±0.07

In comparison between liquid and solid media, both IBA 1.0 mgL^{-1} and NAA 1.5 mgL^{-1} produced maximum number of roots (13.1±3.78 and 16.79±5.30 respectively) in liquid media. On the other hand, IBA 0.1 mgL^{-1} produced 2.83±0.82 and NAA 1.0 mL^{-1} produced 6.22±1.79 roots, respectively in solid media. Thus, liquid medium found superior to solid medium. Complete plantlets were taken out from the test tubes and rinsed with tap water to remove the medium. Plantlets were then transferred to pot containing 25% sand and 75% soil. Some plantlets died due to fungal, bacterial contamination and dehydration. However, 65% plants survived and hardened properly and then transferred to soil.

Table 2. Effect of IBA and NAA on root formation of *A. vasica* into solid and Liquid medium.

Growth regulators	Conc. (mgL^{-1}	No. of explant produced roo		Average root length (cm)	
		Liquid	Solid	Liquid	Solid
NAA	0.1	2.3±0.73	-	0.48±0.15	-
	0.5	3.83±1.21	-	0.44±0.14	-
	1.0	13.10±3.78	6.22±1.79	0.66±0.19	0.88±0.25
	1.5	4.54±1.37	4.15±1.19	0.28±0.09	0.44±0.13
	2.0	3.92±1.24	5.38±1.55	0.59±0.19	0.52±0.15
IBA	0.1	4.65±1.47	2.83±0.82	1.02±0.32	1.41±0.41
	0.5	3.69±1.17	-	0.30±0.09	-
	1.0	5.17±1.49	-	0.46±0.13	-
	1.5	16.79±5.30	-	0.60±0.19	-
	2.0	7.43±0.86	-	0.48±0.15	-

Discussion

Among the kinetin concentrations, 2.0 mgL^{-1} produced maximum number of usable shoots (Table 1). Multiple shoot formation from shoot tip using cytokinin was also reported in many plant species (Pierik, 1987; Bhadra and Hossain, 2003; Bhavisha and Yogesh, 2003). Among cytokinins, BA was found the most effective for inducing the adventitious shoots (Pierik, 1987; Chawla, 2000). In this study, BA also peoformed better than that of kinetin, which is consistent with the results of Jeyakumar and Jayabalam (2002) and Rahman *et al*. (2001).

When five treatments of NAA ranging from 0.1-2.0 mgL^{-1} were employed for root induction, only one treatment that is NAA 0.1 mgL^{-1} produced roots and the average number of roots and average root length were 2.83±0.82 cm and 1.41±0.42 cm respectively (Table 2).

NAA is a synthetic and relatively more active auxin and is used at concentration of 0.001-10 mgL^{-1} (Pierik, 1987). IBA is also a synthetic and relatively less active than that of NAA, hence slightly higher concentration may be used. Auxin generally causes: cell elongation and swelling of tissues, cell division and the formation of adventitious roots. With low auxin concentrations, adventitious root formation predominates, whereas, with high auxin concentrations root formation fails to occur and callus formation takes place (Pierik, 1987; Chawla, 2000).

Hundred percent rooting was found in all types of liquid medium and NAA showed comparatively healthy and longer roots than that of IBA. In this study, the result of IBA is consistent but NAA is inconsistent with the findings of Azad and Amin (1998). Bhavisha and Yogesh (2003) also transferred multiple shoots of *Curculigo orchioides* to liquid MS basal medium containing different concentrations of NAA for root induction. They also found the suitability of liquid media over solid media. Transfer of *in vitro* multiplied shots to liquid medium with filter paper platform was necessary to ensure a healthy root system in *A.vasica*. Our results regarding root induction in liquid media is consistent with the results of Bhavisha and Yogesh (2003). Similar treatments of IBA and NAA viz 0.1,0.5,1.0,1.0 and 2.0 mgL^{-1} were also employed for root induction. All the treatments of both NAA and IBA produced root with varying root number and length without callus formation.

Fig. 1. Shoot proliferation of A. vasica from shoot tip culture on MS medium supplemented with BA 0.5 mgl^{-1}.

Fig. 2. Root induction of A. vasica on MS semi-solid medium supplemented with IBA 1.0 mgl^{-1}.

Fig. 3 Root induction on MS liquid medium supplemented with NAA 1.5 mgl^{-1}.

Conclusion:

Among various growth regulators in this experiment tested, BA 0.5 mgL^{-1} found superior to kinetin for shoot proliferation. Liquid medium also showed better response over solid medium; hence, for root induction of *A. vasica* can be suggested. Low concentration of NAA is recommended for rooting.

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