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BRIEF COMMUNICATION

Rapid axillary bud proliferation and *ex vitro* rooting of *Eupatorium triplinerve*

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Abstract

Effective protocol was established for micropropagation of the medicinal plant *Eupatorium triplinerve* Vahl through rapid axillary bud proliferation and *ex vitro* rooting. Murashige and Skoog (MS) medium fortified with 8.87 μM benzylaminopurine (BAP) and 2.46 μM indole-3-butyric acid (IBA) was the best for axillary bud proliferation and developed a mean of 8.1 shoots per node. Excision and culture of the node segments of the *in vitro* shoots on medium supplemented with the same concentration of growth regulators developed more than 30 shoots within 40 d. Shoot multiplication did not exhibit decrease in the number of shoots even at 7th subculture. Dipping of the basal end of shoots in 2.46 μM IBA solution for 10 d induced roots and its transfer to small pots facilitated the survival of all rooted shoots (100 %). *Ex vitro* rooting by direct transfer of the shoots from multiplication medium showed 92 % survival.

Additional key words: ayapana, benzylaminopurine, clonal propagation, indole-3-butyric acid.

Eupatorium triplinerve Vahl (Syn. *E. ayapana* Vent.) belonging to the family *Asteraceae*, commonly known as ayapana, is a native of America. Ayapana is compared to chamomile in its medicinal properties. Micropropagation through axillary bud multiplication has been reported in many medicinal plants (Abrie and van Staden 2001, Dias *et al.* 2002, Salvi *et al.* 2002). No *in vitro* propagation of this valuable medicinal plant has been reported so far. In the present study, clonal propagation of *Eupatorium triplinerve* through axillary bud multiplication using tap water and commercial sugar and *ex vitro* rooting was accomplished.

Young shoots (with 2 - 4 nodes) of *Eupatorium triplinerve* Vahl were collected from flowering mature plants grown in Calicut University Botanical Garden. They were washed under running tap water followed by a detergent *Extran* (5 % v/v) for 5 min. After repeated wash with double distilled water, young shoots were surface sterilized using 0.5 % (m/v) mercuric chloride solution for 7 - 10 min. After thorough washing with sterile double distilled water, single node segments of 1.0 - 1.5 cm were cultured on sterile medium consisting of the salts and vitamins of Murashige and Skoog (1962, MS). Commercial-grade sucrose at 3 % was used as the carbon source. The medium was gelled with 0.6 % agar. Basal medium was supplemented with different growth regulators at different concentrations either singly or in combination (Table 1). *In vitro* root induction was carried out on full strength growth regulator-free or on half strength MS solid medium without or with auxins (Table 2). All the media were prepared using tap water. The pH of the medium on all cases was adjusted to 5.8 before autoclaving at a pressure of 1.06 kg cm^{-2}. The cultures were incubated at 25 ± 2 °C with 16-h photoperiod under irradiance 25 μmol m^{-2} s^{-1}(white fluorescent tubes). All the experiments were set up in a completely randomised design. Twenty cultures were raised for each treatment and all experiments were repeated twice (total of 60 cultures). Means were compared using Duncan's multiplication range test (Duncan 1955).

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Abbreviations: BAP - 6-benzylaminopurine; IAA - indole-3-acetic acid; IBA - indole-3-butyric acid; Kn - kinetin; MS - Murashige and Skoog; NAA - α-naphthaleneacetic acid.

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For *ex vitro* rooting, the well-grown shoots from the shoot multiplication medium were directly transferred to small pots containing soil and sand (1:1) and also by transferring to pots after dipping the basal end of shoots in IBA solution for 10 d.

Growth regulator-free MS medium facilitated the initiation of axillary buds of the node explants. MS medium with different concentrations of 6-benzylaminopurine (BAP) and kinetin (Kn) individually or in combination and also in combination with auxins [α-naphthaleneacetic acid (NAA), indole-3-butyric acid (IBA), and indole-3-acetic acid (IAA)] also resulted in initiation of axillary buds (Table 1). BAP at 8.87 μM in combination with IBA at 2.46 μM was the best for axillary bud multiplication. On this medium, both the axillary buds were initiated within 10 d and developed mean of 8.1 shoots per node explant within 40 d (Table 1). The developed shoots attained more than 6 cm height within 40 d. Axillary buds at the nodes of the *in vitro* developed shoots also underwent initiation and growth. These shoots also attained growth sufficient (more than 3 cm) for the transfer to root induction. Upon BAP and IBA containing medium, the shoots were healthier longer than that on BAP alone. Present investigation was in agreement with the earlier reports that low concentrations of an auxins can positively modify the shoot induction frequency in combination with cytokinins. Synergistic effect of BAP and an auxin in multiplication and growth of shoots has been exemplified on medicinal plants viz. *Hemidesmus indicus* (Sreekumar *et al.* 2000), *Holostemma ada-kodien* (Martin 2002) and turmeric (Salvi *et al.* 2002). Excision and culture of the node segments from the *in vitro* derived shoots on MS medium supplemented with the same concentrations of BAP (8.87 μM) and IBA (2.46 μM) developed more than 30 shoots within 40 d. Subsequent culture increased the rate of shoot multiplication and no decline of shoots was observed up to 7th subculture.

Table 1. Axillary bud multiplication of *Eupatorium triplinerve* on MS medium enriched with different growth regulators. Means followed by different letters are significantly different at 5 % level. $n = 60$. Growth period 40 d.

BAP [μM]	Kn [μM]	IBA [μM]	IAA [μM]	NAA [μM]	Response [%]	Number of shoots [node^{-1}]
0.00	0.00	0.00	0.00	0.00	74.2e	1.9f
2.22					85.5cd	2.3ef
4.44					93.1b	2.9e
6.66					95.7ab	3.9cd
8.87					84.4d	4.3cd
13.20					95.2ab	2.6e
22.10					93.5b	0.9g
	2.32				75.4e	1.8f
	4.65				82.8d	2.1ef
	6.97				95.3ab	3.3d
	9.29				92.5b	3.1de
	13.90				91.6b	2.8e
8.87	0.46				96.2a	2.8e
8.87	2.32				94.6b	3.8d
8.87	4.65				89.0c	3.2d
8.87		0.49			97.3a	6.7b
8.87		2.46			98.5a	8.1a
8.87		4.90			94.2b	6.2b
8.87			0.57		93.1b	5.3c
8.87			2.85		94.4b	6.5b
8.87			5.71		97.1a	5.1c
8.87				0.54	84.7d	5.4c
8.87				2.69	93.8b	4.0cd
8.87				5.37	91.1bc	3.4d

Twenty per cent shoots spontaneously developed roots on shoot multiplication medium. Shoots without roots were transferred to full or half strength growth regulator-free medium for root induction. Half strength growth regulator-free medium was superior to full strength medium for root development. Auxins at lower concentrations facilitated better rooting (Table 2). Among the different auxins, IBA was superior to IAA and NAA. Half strength MS medium fortified with 2.46 μM IBA was the best for *in vitro* root development (Table 2). In root induction efficacy of auxins, IAA followed IBA (Table 2). NAA was inferior and favoured callus formation at the base. Efficacy of IBA at lower concentrations in *in vitro* rooting has been reported in medicinal plants like *Swainsona salsula* (Yang *et al.* 2001) and *Plumbago* spp. (Das and Rout 2002). *In vitro* rooted healthy shoots transferred directly to small pots containing soil and sand (1:1) revived growth after 15 d of transplantation and 100 % plantlets survived in field conditions.

Table 2. *In vitro* rooting of *Eupatorium triplinerve* on half-strength MS medium fortified with different auxins. Means followed by different letters are significantly different at 5 % level, $n = 60$. Growth period 30 d.

IBA [μM]	IAA [μM]	NAA [μM]	Response [%]	Number of roots [shoot^{-1}]
0.00	0.00	0.00	81.3d	4.4f
0.49			92.6b	7.5d
2.46			100.0a	11.7a
4.90			100.0a	10.3b
	0.57		100.0a	6.3e
	2.85		100.0a	10.1b
	5.71		100.0a	9.5c
		0.54	93.7b	6.2e
		2.69	87.9c	7.9d
		5.37	87.3c	6.4e

Ex vitro rooting was also tried in the present study. In the first method, the basal portion of rootless shoots was kept in different concentrations of IBA solution for 10 d.

Of the different concentrations of IBA solution, 2.46 μM was most effective and developed a mean of 12.4 roots per shoot. Lower (0.49 μM) and higher (4.9 μM) concentration of IBA proved to be less efficient (developed mean of 8.3 and 10.5 roots per shoots respectively). Shoots rooted through this method transplanted to small pots exhibited 100 % survival. Successful induction of rooting by basal dipping of *in vitro* developed shoots in IBA solution for short periods has been reported in *Decalepis hamiltonii* (Reddy *et al.* 2001), *Ceratonia siliqua* (Romano *et al.* 2002) and *Simmondsia chinensis* (Agrawal *et al.* 2002). In the second method of *ex vitro* rooting, the healthy shoots excised from the shoot multiplication medium were directly transferred to small pots containing soil and sand (1:1). Of the 150 plants transferred, 138 plants (92 %) survived. Rooting *ex vitro* has also been reported in *Gardenia jasminoides* (Economou and Spanoudaki 1985), *Actinidia deliciosa* (Pedroso *et al.* 1992), and *Stackhousia tryonii* (Bhatia *et al.* 2002). The shoots revived growth within 20 d after transfer. In view of Debergh and Maene (1981) *ex vitro* rooting accounts 35 - 75 % reduction of the total cost of plants propagated through tissue culture.

Present protocol facilitates cost effective production of more than 60 000 plantlets within 150 d starting from a single node explant using single medium and enables to meet the need in time without the annihilation of the natural resources of this valuable medicinal plant.

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