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# Micropropagation of *Baliospermum montanum* (Willd.) Muell.-Arg.-A Red Listed Medicinal Plant

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**Abstract:** A protocol has been standardized for mass *in vitro* propagation of *Baliospermum montanum*. Among the cytokinins, BA was superior to kinetin in terms of the number of shoots produced per explant. All the media containing cytokinins and auxins in combination showed superior results than the media with cytokinins alone. Murashige and Skoog (MS) medium containing 1.0 mg $L^{-1}$ BA in combination with 0.1 mg $L^{-1}$ NAA was superior for shoot multiplication. In this medium an optimum number of 9.25 shoots per explant was produced after six weeks of incubation. Among the auxins tried for root induction, NAA and IAA were the most and least effective in root induction, respectively. MS medium containing 0.2 mg $L^{-1}$ NAA was superior for root induction with an average number of 6 roots per shoot. Rooted plantlets on transfer to moist sand, got established successfully in one week and were planted out to pots containing sand, soil and cow dung in 1:1:1 ratio. The micropropagated plants were successfully established with 90 percent success.

**Key words:** *Baliospermum montanum,* micropropagation, *in vitro* rooting, medicinal plant, red listed

## INTRODUCTION

*Baliospermum montanum* is a vulnerable medicinal plant belonging to the family Euphorbiaceae. The species is distributed throughout the sub-Himalayan tracts and Peninsular India. Root, leaf and seed of the plant are used medicinally. The root contains phorbol esters belonging to diterpene hydrocarbon viz. montanin, baliospermin, 12-deoxyphorbol-13-palmitate, 12-deoxy-5B-hydroxyphorbol-13-myristate and 12-deoxy-16-hydroxy phorbol-13-palmitate. Leaves contain 8-sitosterol 8-D-glucoside and hexacosanol. The presence of steroids, terpoids and flavanoids is also reported from the plant (Sharma *et al*., 2000). The root is acrid, thermogenic, purgative, anti-helmintic, carminative and anti-inflammatory. They are useful in abdominal pain, constipation, calculus, piles, helminthic manifestations, scabies, skin disorders, wounds and jaundice (Warrier *et al*., 1995). Root paste is applied to painful swellings and piles. Leaves cure asthma and bronchitis. They are purgative and also used for dropsy. Seeds are drastic purgative, rubifacient, hydragogue and stimulant and are useful in inflammations and flatulence. Seeds are also used in snakebite (Sivarajan and Balachandran, 1994). The plant is used for the treatment of abdominal tumours and cancer (Anonymous, 1988).

The multiple use of this important herb has led to its indiscriminate collection. Over exploitation from the wild sources due to its high demand resulted in the depletion of this important medicinal plant and this necessitates other alternative methods for propagation and conservation. Micropropagation of *Baliospermum montanum* was reported by Singh *et al*. (2003) and Johnson and Manickam (2003). This study describes the results of the study taken up for developing an effective, reproducible and simple protocol for clonal multiplication and conservation of *B. montanum*.

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## MATERIALS AND METHODS

The field grown plants from Herbal Garden of Arya Vaidya Sala, Kottakkal, Kerala, India were used as the source of explants. Explants were washed in tap water; swabbed with wet cotton containing a wetting agent, Tween-20 and then kept under running tap water for 30min. Explants were treated with 0.1% Mercuric chloride ($HgCl_2$) and Tween-20 (2 drops per 100 mL) and kept on continuous agitation for 5 min followed by thorough washing in distilled water. The explants were then treated with 0.1% $HgCl_2$ for 4min under aseptic conditions and washed with sterile distilled water. The explants were then cut into single node segments and again treated with 0.1% $HgCl_2$ for min followed by washing with sterile distilled water. Surface sterilized explants were inoculated aseptically into the culture initiation medium. Sufficient stock of contamination free cultures raised in culture initiation medium was used to study the effect of various growth regulators such as Nb-Ben-2yl adenine (BA), Kinetin (Kin), α-Naphthalene acetic acid (NAA) and Indole 3-acetic acid (IAA) on shoot multiplication and rooting. Murashige and Skoog (1962) basal medium with 3% (w/v) sucrose and 0.8% (w/v) agar, further supplemented with various concentrations and combinations of growth regulators were used.

The rooted plantlets were carefully removed from the culture tube; washed thoroughly in running tap water. These were planted in small thermocol cups filled with moist sand and kept in humid chamber for hardening. Plantlets after hardening for one week were planted out in polythene bags and pots containing sand, soil and cow dung in 1:1:1 ratio.

## RESULTS AND DISCUSSION

Nodal segments of *B. montanum* were established in MS medium without any growth regulators with 80% success. The axillary buds elongated to an average length of 5 cm within 14 days of culture initiation (Fig. 1A). Shoot tip explants in half strength MS growth regulator free medium showed the development of axillary buds of upper nodes. The shoot multiplication tendency in growth regulator free medium can be attributed to the presence of higher amounts of endogenous hormones.

Among the cytokinins, BA was superior to kinetin in terms of the number of shoots produced per explant. In medium with BA (2.0 mg $L^{-1}$) an average of 4.0 shoots per explant was produced, whereas in kinetin (2.0 mg $L^{-1}$) containing medium, an average number of 1.6 shoots per explant was produced. The effectiveness of BA as an efficient cytokinin for axillary bud multiplication has been reported in other plants like *Artemisia annua* (Usha and Swamy, 1998). Though auxins are regarded as the only source of callus induction in certain plants, callus formation was observed in BA containing medium as reported in other plants like *Eryngium foetidum* (Arokiasami and Ignacimuthu, 1998). But there was no callus formation in kinetin containing media. Medium with 1 mg $L^{-1}$ each of BA and kinetin showed shoot multiplication together with basal callusing. There was an increase in the number of shoots per explant. The potential of two cytokinins in combination for axillary bud multiplication have been well established (Kathiravan and Ignacimuthu, 1999).

All the media containing cytokinins and auxins in combination showed superior results than the media with cytokinins alone (Table 1). Similar results of synergistic effect have already been reported in other plants (Kannan and Jesrai, 1998). Among the various combinations of cytokinins with auxins, MS medium containing 1.0 mg $L^{-1}$ BA and 0.1 mg $L^{-1}$ NAA showed better results in terms of shoot multiplication with an average number of 9.25 shoots per explant (Fig. 1B). According to George (1993) presence of cytokinin is an important factor for inducing axillary bud multiplication by suppressing the apical dominance. Increased concentrations of NAA (0.2 mg $L^{-1}$) in combination with BA 1.0 mg $L^{-1}$ showed reduced rate of multiplication with more tendency for callusing, whereas NAA in combination with kinetin showed occasional rooting along with callusing and comparatively reduced rate of multiplication. Medium containing kinetin in combination with lower concentration of NAA produced roots along with shoot multiplication. Roots developed

Table 1: Effect of growth regulators in shoot multiplication of *Baliospermum montanum*

| Growth regulators (mg $L^{-1}$) | | | | | |
|---|---|---|---|---|---|
| BA | KIN | NAA | IAA | No. of shoots± SD | Shoot length± SD |
| 0.0 | 0.0 | 0.0 | 0.0 | 3.66±0.63 | 2.38±1.16 |
| 1.0 | 0.0 | 0.0 | 0.0 | 3.80±1.86 | 3.02±0.86 |
| 2.0 | 0.0 | 0.0 | 0.0 | 4.00±1.92 | 2.83±2.13 |
| 0.0 | 1.0 | 0.0 | 0.0 | 1.40±0.98 | 1.36±2.04 |
| 0.0 | 2.0 | 0.0 | 0.0 | 1.60±0.76 | 4.24±1.89 |
| 1.0 | 0.0 | 0.1 | 0.0 | 9.25±2.31 | 4.50±1.39 |
| 1.0 | 0.0 | 0.2 | 0.0 | 2.50±1.61 | 5.63±0.12 |
| 0.0 | 1.0 | 0.1 | 0.0 | 1.75±1.19 | 2.18±1.59 |
| 0.0 | 1.0 | 0.2 | 0.0 | 2.25±0.89 | 2.25±0.79 |
| 1.0 | 0.0 | 0.0 | 0.1 | 4.80±0.23 | 2.34±0.36 |
| 0.0 | 1.0 | 0.0 | 0.1 | 1.25±1.33 | 2.12±1.66 |
| 1.0 | 1.0 | 0.0 | 0.0 | 4.60±2.18 | 2.78±1.87 |

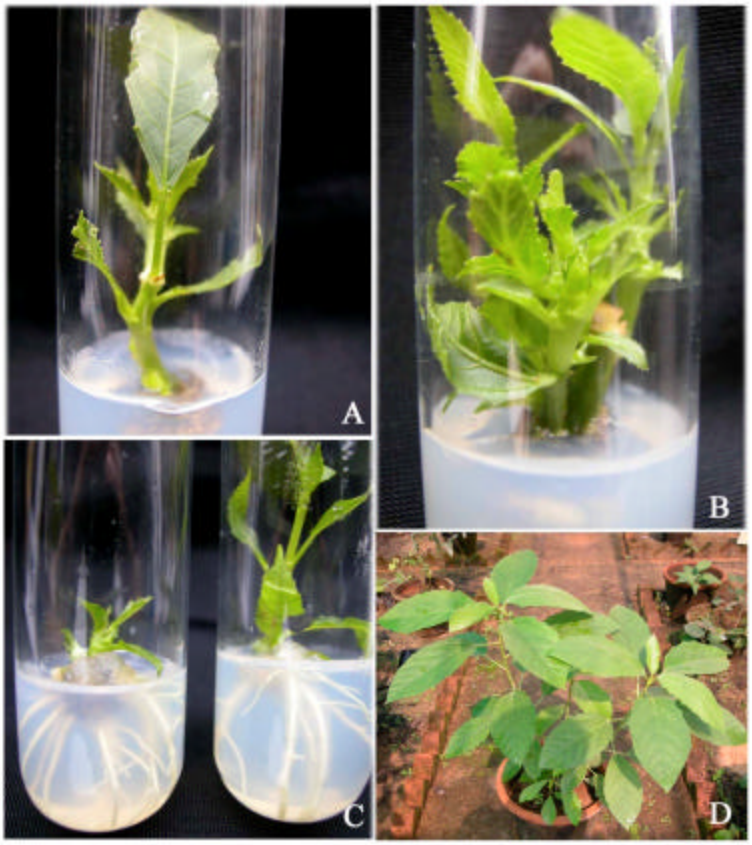


Fig. 1: A-D: Micropropagation of *Baliospermum montanum* (Willd.) Muell. -Arg. (A) Shoot initiation in MS growth regulator free medium, (B) Plants with multiple shoots in MS medium with 0.1 mg $L^{-1}$ NAA and 1.0 mg $L^{-1}$ BA, (C) Well rooted plants in MS medium with 0.2 mg $L^{-1}$ NAA and (D) Hardened plants in pots

independent of the callus. Cytokinins (BA and kinetin) in combination with IAA showed tendency for the development of axillary buds of the upper nodes along with multiplication of the basal buds and poor rate of callus formation.

Table 2: Effect of growth regulators in root induction of *Baliospermum montanum*

| Growth regulators (mg $L^{-1}$) | | | | |
|---|---|---|---|---|
| NAA | IBA | IAA | Average no. of root | Average root length |
| 0.0 | 0.1 | 0.0 | - | - |
| 0.0 | 0.2 | 0.0 | 1.2±0.64 | 2.8±0.91 |
| 0.1 | 0.0 | 0.0 | 3.2±0.58 | 3.5±0.77 |
| 0.2 | 0.0 | 0.0 | 6.0±0.72 | 6.0±0.94 |
| 0.0 | 0.0 | 0.1 | - | - |
| 0.0 | 0.0 | 0.2 | 1.0±0.45 | 3.2±0.88 |

Among the auxins tried for root induction, NAA and IAA were the most and least effective in root induction, respectively (Table 2). MS medium containing 0.2 mg $L^{-1}$ NAA was superior with an average of 6.0 roots per shoot (Fig. 1C). Lower concentrations of IBA (0.1 mg $L^{-1}$) and IAA (0.1 mg $L^{-1}$) did not show any rooting response, whereas lower concentrations of NAA (0.1 mg $L^{-1}$) produced comparatively higher rate of rooting. Medium containing 0.2 mg $L^{-1}$ IBA produced only single roots from the basal cut end of the shoots. The result obtained can be correlated to the faster uptake of NAA compared to IAA (Peeters *et al*., 1991). The present observation is in contrast with the published micropropagation reports in the plant. Singh *et al*. (2003) have reported MS basal medium as the best combination for root induction whereas, Johnson and Manickam (2003) reported half strength MS medium containing 9.84 μM IBA in combination with 5.37 μM NAA as the potent combination for root induction in this plant.

Well-rooted plantlets were transferred to thermocol cups containing moist sand and covered with polythene cover for about one week for acclimatization of the plant. The plants were successfully established (90%) and they were later transferred to pots containing soil, sand and cow dung (1:1:1) and kept in the nursery for further growth (Fig. 1D).

During the present study a simple and viable micropropagation protocol was standardized and this can be utilized for the clonal multiplication of elite genotypes and also for the conservation of this vulnerable medicinal plant.

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