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IN VITRO MICROPROPAGATION OF *BALIOSPERMUM AXILLARE* BLUME

KAMALENDRA SINGH* AND M.S. SUDARSHANA

Department of Botany, University of Mysore 570 006, Karnataka

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SUMMARY

An efficient method of direct plantlet regeneration using nodal explants of mature plants of *Baliospermum axillare* has been developed for the first time. Nodes were cultured on Murashige and Skoog medium (MS) containing indole-3-butyric acid (IBA) at 1 and 2 mg/l in combination with 6-benzylaminopurine (BAP) at 1, 2 and 5 mg/l. A maximum number of 15 multiple shoots per explant were obtained on MS medium supplemented with 2 mg/l BAP and 1 mg/l IBA. The regenerated shoots rooted in MS basal media. The plantlets were maintained in vermiculite and soil mixture and were successfully transferred to soil.

Key words: ***Baliospermum axillare*, micropropagation.**

INTRODUCTION

Baliospermum axillare (Syn : *Baliospermum montanum*, Euphorbiaceae) is a leafy shrub with herbaceous branches. The plant is used in traditional Indian medicine. Leaf-decoction is used in curing asthma and an extract prepared from it is used in the treatment of abdominal tumors, seed oil is used in rheumatism, as a purgative and hydrogogue cathartic, root is used in the treatment of dropsy, anasarca and jaundice (Chopra *et al.* 1956 and Bakshi *et al.* 1999). Ethanolic extracts of the root are anticancer in nature (Oqura *et al.* 1978). Commercial exploitation and elimination of natural habitats, consequence of urbanization, has led to gradual extinction of several medicinal plants. Micropropagation is one of the effective approach to conserve such valuable germplasm. Hence, the present study was undertaken to establish a micropropagation protocol for *Baliospermum axillare*.

MATERIALS AND METHODS

One year old plants collected from the wild and maintained in the departmental garden served as the source of explants. Explants were washed thoroughly in running tap water, dipped in 70% ethanol for 30 sec. followed by 0.1% (w/v) $HgCl_2$ (3 min) solution and rinsed thrice in sterile distilled water. Following disinfection the nodal explants of 1 cm length were cut aseptically and placed in *vitro* on Murashige and Skoog (Murashige and Skoog 1962) medium containing 3% sucrose and supplemented with 1, 2 and 5 mg/l BAP in combination with indole butyric acid at 1 and 2 mg/l in 150 ml Erlenmayer flasks and culture tubes. All the media were solidified with 0.8% agar (HIMEDIA) after adjusting the pH to 5.8 and autoclaved at 15 psi for 15 min. Twelve replicates were maintained for each treatment and all the experiments were repeated thrice. For *in vitro* rooting, MS basal medium half and full strength was used, napthalne acetic acid (NAA), indole-3-acetic acid (IAA) and IBA supplemented media at 0.5-1 mg/l were used. The cultures were incubated at 25 ± 2°C with 16 h photoperiod provided by fluorescent tube lights of 1.5 K lux. After 4 weeks, the explants were transferred to a fresh medium having the same composition. The data were recorded after 4 weeks of culture. Micro-shoots of 1.5 to 2 cm length were cultured on media as described above. Rooted micro-shoots were washed free of medium and placed in pots filled with potting of mix (1 soil : 1 vermiculite v/v). Pots were covered with polythene bags for two weeks and watered initially with half-strength MS basal medium for a week and later with tap water. The rooted micro-shoots were kept under shade for two weeks before transferring to the soil.

RESULTS AND DISCUSSION

Shoot buds were observed 3 weeks after culture. The number of shoots produced per explant varied with the concentration and combinations of growth regulators used in the medium. Bud break was achieved in almost all the cases. The role of BAP in stimulating multiple shoot formation in many plants has been studied extensively (Balachandran *et al.* 1992, Berger and Schaffner 1995, Emmanuel *et al.* 2000, Venkateswarlu *et al.* 2001). BAP alone could induce multiple shoots (Fig. 1), addition of IBA along with BAP had a marked effect of the number of shoots produced. Maximum number of 15.2 shoots were produced per explant (Fig. 2, table 1) on MS medium containing 2 mg/l BAP and 1 mg/l IBA. Similar results have been reported in *Actinidia deliciosa* by Monnette (1986)., *Leucopogon obtectus* by Bunn and Dixon (1989), *Gardenia jasminoides* (George *et al.* 1983) and in *Leontochir ovallei* (Lu *et al.* 1995).

Table 1. Morphogenetic response of nodal segments with various combinations of BAP and IBA.

Growth regulator (mg/l) BAP	IBA	No. of shoots/explant	Shoot length (cm)	Basal callusing
1	0	1.5±1.0	3.2±1.1	-
1	1	2.2±1.7	4.1±1.2	+
1	2	3.5±1.4	5.4±1.4	+
2	0	4.1±1.5	3.6±1.2	++
2	1	15.2±1.6	5.3±1.0	++
2	2	6.9±1.7	5.1±1.3	++
5	0	3.1±1.4	3.4±1.0	+++
5	1	2.0±1.2	3.1±1.2	+++
5	2	1.8±0.7	2.7±1.0	+++

Mean data of 12 replicates ± SD.

Table. 2. Effect of various media on root production in *Baliospermum axillare* regenerated shoots.

MS Medium	Growth Regulator (mg/l)	Rooting %	Mean no. of roots/shoot	Mean root length (cm)	Basal callusing
Full	-	86	4.0±1.1	2.9±1.2	-
Half	-	81	3.4±0.6	2.7±1.0	-
Half	0.5 NAA	94	5.9±1.3	2.8±0.8	+
Half	0.5 IAA	100	6.5±1.4	3.6±0.9	+
Half	0.5 IBA	100	7.5±1.3	3.8±0.6	+
Half	1 NAA	100	7.9±1.5	3.3±1.4	++
Half	1 IAA	88	6.3±1.4	3.4±1.0	++
Half	1 IBA	92	8.6±1.6	3.0±0.6	++

Mean data of 12 replicates ± SD.

Higher concentrations of the auxin and the cytokinin led to a decrease in the number of shoots per explant and shoot length, which could be attributed to enhanced basal callusing. Addition of IBA, IAA and NAA (0.5 to 1 mg/l) led to an increase in number of roots but it also caused basal callusing (Fig. 3, Table 2). The shoots rooted on MS basal medium (Fig. 4, Table 2). Exogenous auxins may not be required for rooting of *Balisopermum axillare* micro-shoots. Similar observations have been reported in *Euphoribia fulgens* (Zhang *et al.* 1987), *Jatropha integrrima* (Sujatha and Dhingra 1993) *Croton sublyratus* (Catapan *et al.* 2000), *Phyllanthus caroliniensis* (Shibata *et al.* 1996) and *Lippia alba* (Gupta *et al.* 2001).

Plantlets obtained in the present study were subsequently acclimatized and showed a survival rate of 71% (Fig. 5). The regenerated plants were morphologically similar to their mother plants *in vivo*. This reproducible protocol could be utilized to facilitate large scale micropropagation of *Baliospermum axillare*.

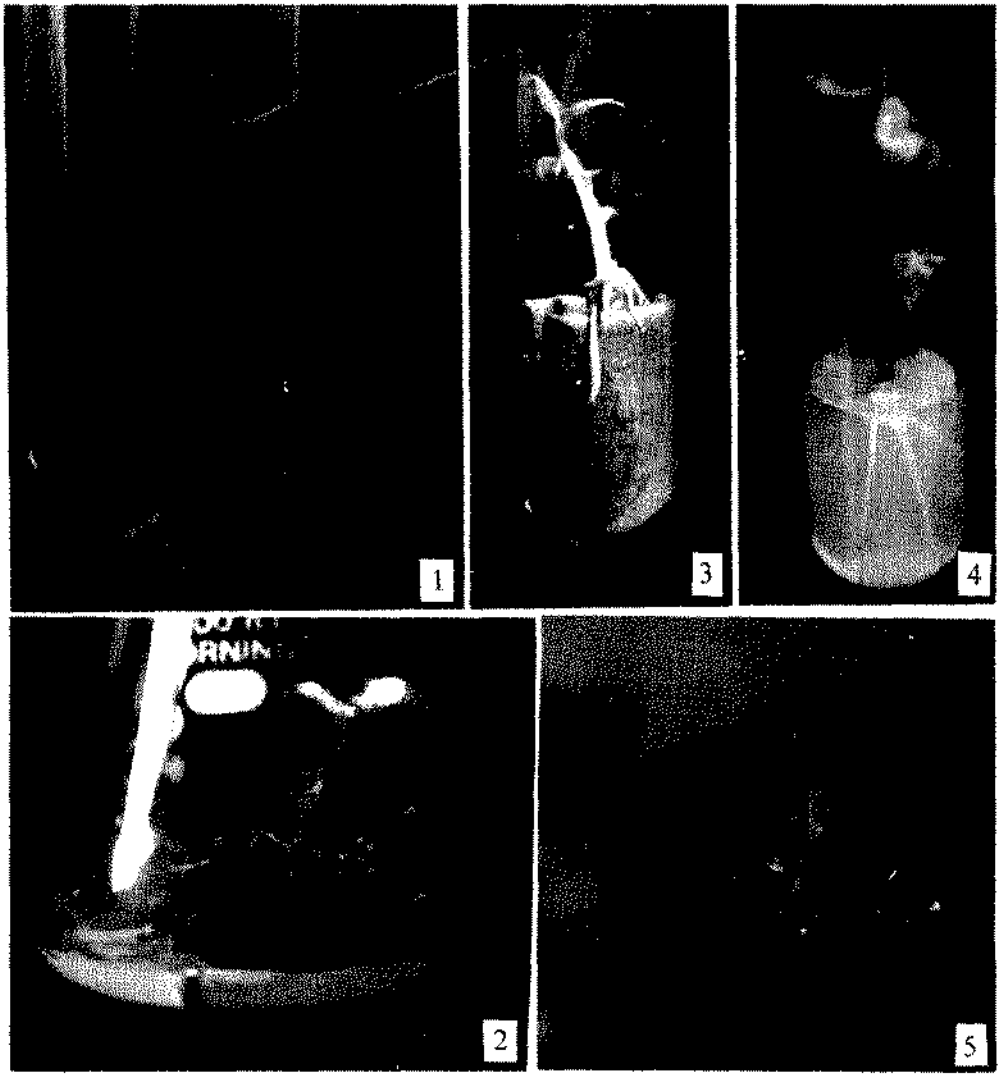

Fig. 1. Multiple shoot induction from nodal explant on medium with 1 mg/l BAP.
Fig. 2. Multiple shoot induction on medium with 2 mg/l BAP and 1 mg/l IBA.
Fig. 3. Micro-shoot rooting on MS + 0.5 mg/l NAA.
Fig. 4. Micro-shoot rooting on MS basal medium.
Fig. 5. A plantlet established in potting mix.

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REFERENCES

Bakshi, G.D.N., Sensarma, P. and Pal, D.C. (1999). A Lexicon of Medicinal Plants in India. Vol. I. Naya Prakash. Calcutta.

Balachandran, S.M., Bhat, S.R. and Chandel, K.P.S. (1992). *In vitro* clonal propagation of turmeric (*Curcuma* sp.) and Ginger (*Zingiber officinale* Rosc). *Plant Cell Rep.* **8:** 521-524.

Berger, K. and Schaffer, W. (1995). *In vitro* propagation of leguminous tree *Swatzia madgascarensis*. *Plant Cell Tissue. Org. Cult.* **40:** 289-291.

Bunn, E. and Dixon, K.W. (1989). In *vitro* propagation of *Leucopogon obtectus* Benth (Epiceridaceae). *Plant Cell Tissue Org. Cult.* **19:** 77-84.

Catapan, E., Otuku, M.F. and Viana, A.M. (2000). *In vitro* culture of *Phyllanthus caroliniensis* (Euphobiaceae). *Plant Cell Tissue Org. Cult.* **62:** 195-202.

Chopra, R.N., Nayar, S.L. and Chopra, I.C. (1956). Glossary of Indian Medicinal plants. CSIR. New Delhi, pp 32.

Emmanuel, S., Ignacimuthu, S. and Kathiravan, K. (2000). Micropropagation of *Wedelia calendulacea*, a medicinal plant. *Phytomorph.* **50:** 195-200.

George, P.S., Ravishankar, G.A. and Venkataram, L.V. (1993). Clonal multiplication of *Gardenia jasminoides* Ellis through axillary bud culture. *Plant Cell Rep.* **13:** 59-62.

Gupta, S.K., Khanuja, S.P.S. and Kumar, S. (2001). *In vitro* micropropagation of *Lippia alba*. *Curr. Sci.* **81:** 206-210.

Lu, C., Ruan, Y. and Brigden M. (1995). Micropropagation procedures for *Leontochir ovallei*. *Plant Cell Tiss. Org. Cult.* **42:** 219-221.

Monnette, P.L. (1986). Micropropagation of kiwi fruit using non-axenic shoot tips. *Plant Cell Tissue. Org. Cult.* **6:** 73-82.

Murashige, T. and Skoog, F. (1962). A revised medium for rapid growth and bioassays with tobasceo tissue cultures. *Physiol. Plant.* **15:** 467-497.

Oqura, M., Koike, K., Cordell, G.A. and Farnswarth, N.R. (1978). Potential anticancer agents VIII constituents of *Baliospermum montanum* (Euphorbiaceae). *Plant Med.* **33:** 128-143.

Shibata, W., Murai, F., Akiyama, T., Siriphol, M., Matsunaga, E. and Morimoto H. (1996). Micropropagation of *Croton sublyratus* Kurz-a tropical tree of medicinal importance. *Plant Cell Rep.* **16:** 147-152.

Sujatha, M. and Dhingra, M. (1993). Rapid plant regeneration from various explants of *Jatropha integerrima*. *Plant Cell Tiss. Org. Cult.* **35:** 293-296.

Venkateswarlu, B., Mukhopadhay, J., Sreenivasan, E. and Kumar, V.M. (2001). Micropropagation of *Paulownia fortunei* through *in vitro* axillary shoot proliferation. *Indian J. Exptl. Biol.* **39:** 594-599.

Zhang, B., Stolz, L.P. and Snyder, J.C. (1987). *In vitro* propagation of *Euphorbia fulgens*. *Hort. Sci.* **22:** 486-488.