BIOLOGIA PLANTARUM 45 (4): 581-584, 2002

BRIEF COMMUNICATION

Micropropagation of *Spilanthes acmella* Murr.

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Abstract

Multiple shoots of *Spilanthes acmella* Murr. were induced from hypocotyl segments obtained from 1-week-old seedlings on Murashige and Skoog's (MS) medium containing benzyladenine (BA), isopentenyl adenine, and naphthaleneacetic acid (NAA). High frequency shoot proliferation (95 %) and maximum number of shoots per explant (10 ± 0.6) were recorded with 0.5 mg dm^{-3} BA in combination with 0.1 mg dm^{-3} NAA. A proliferation was achieved by repeatedly subculturing the nodal segments on shoot multiplication medium. About 95 % of the *in vitro* shoots developed roots after transfer to half strength MS medium containing indole-3-butyric acid (1.0 mg dm^{-3}). 95 % of the plantlets were successfully acclimatized and established in soil. Transplanted plantlets showed normal flowering without any morphological variation.

Additional key words: direct regeneration, growth regulators, medicinal plant, tissue culture.

Spilanthes acmella (marati mogga) is a medicinal plant belonging to the family *Asteraceae*. It is a perennial herb grown in tropics and subtropics. The antiseptic activity is mainly due to the presence of an alkaloid spilanthol (N-isobutyl-2,6,8-decatrienamide (Khadir *et al.* 1989). Eighteen compounds were characterized in the essential oil of *S. acmella*. The major constituents identified were β-caryophyllene (30.2 %), γ-cadinene (30.3 %), and thymol (18.3 %) (Lemos *et al.* 1992). In view of various medicinal properties and increased demand in the pharmaceutical sector, there is a demand for large scale propagation of *S. acmella*. So far, there have been no reports on *in vitro* studies on this species. In the present investigation a rapid high frequency shoot regeneration system is developed for pilot production of this highly valuable medicinal plant.

Seeds of *Spilanthes acmella* Murr. were collected during March and April from plants growing in the medicinal plant garden, Sri Venkateswara Field Research Station, Tirupati, Andhra Pradesh, India. Seeds were surface sterilized by 1 % *Teepol* for 15 min followed by immersion in 70 % ethanol for 1 min and in 0.1 % mercuric chloride for 10 min, and then rinsed thoroughly with sterile distilled water. Disinfected seeds were inoculated on Murashige and Skoog's (1962) medium without growth hormones to raise aseptic seedlings. Depending on the experiment the basal medium was further supplemented with benzyladenine (BA) or isopentenyl adenine (2-ip) alone and in combination with naphthaleneacetic acid (NAA) at various concentrations (Table 1). The pH of the media was adjusted to 5.8 before autoclaving. All media were autoclaved at 1.06 kg cm^{-2} pressure and 121 °C for 15 min. For all experiments 20 replicate cultures were established and each experiment was repeated thrice. The cultures were incubated in growth room at temperature of 24 ± 2 °C, relative humidity 55 ± 5 %, a 16-h photoperiod and irradiance of 70 μmol m^{-2} s^{-1} (cool, fluorescent tubes).

Multiple shoot cultures were established and single nodes were regularly subcultured every four weeks on

Received 26 January 2001, *accepted* 4 December 2001.

Abbreviations: BA - 6-benzyladenine, IBA - indole-3-butyric acid, NAA - α-naphthaleneacetic acid, MS medium - Murashige and Skoog's (1962) medium, 2-ip - isopentenyl adenine.

Acknowledgement: The authors wish to acknowledge to Department of Forest and Environment, Government of Andhra Pradesh, India for necessary laboratory facilities.

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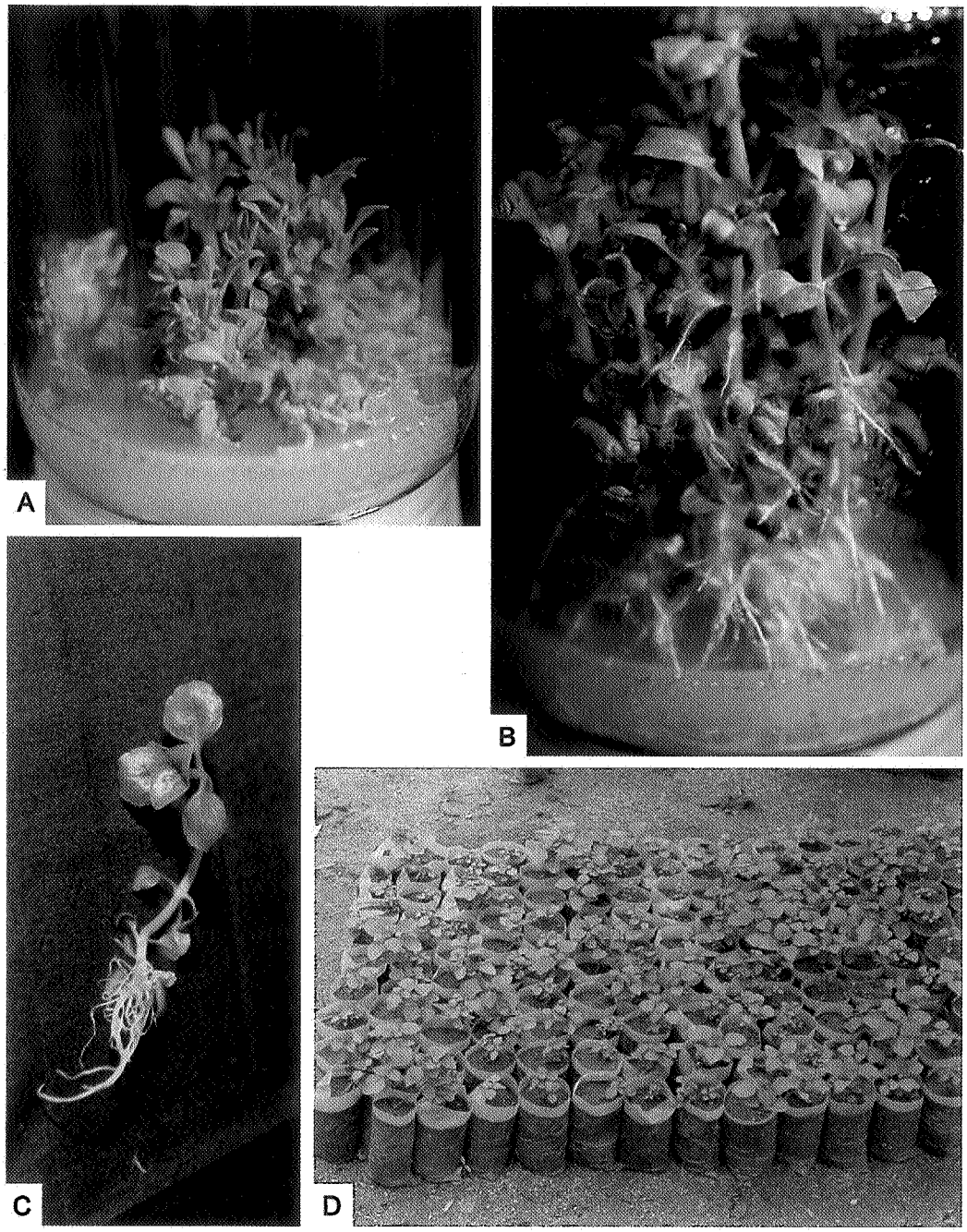

Fig. 1. Micropropagation of *Spilanthes acmella* from hypocotyl segments: *A* - formation of multiple shoots on MS medium containing 0.5 mg dm^{-3} BA and 0.1 mg dm^{-3} NAA (25 d after inoculation), *B* - multiple shoots with hairy roots formation from the nodal region on MS medium with 0.5 mg dm^{-3} 2-iP and 0.1 mg dm^{-3} NAA (30 d after inoculation), *C* - rooting of the regenerated shoot on MS medium supplemented with 0.5 mg dm^{-3} BA and 0.1 mg dm^{-3} NAA (10 d after inoculation), *D* - 1-month-old established plants in polythene bags.

shoot multiplication medium containing various concentrations of growth regulators. *In vitro* differentiated shoots 3 - 4 cm in length were excised and transferred to half-strength MS medium with different concentrations of IBA (0.1, 0.5, 1.0, 1.5, and 2.0 mg dm^{-3}). The maximum number of roots per shoot (30 ± 0.6) were recorded in half-strength MS medium containing 1.0 mg dm^{-3} IBA.

Healthy plantlets with 3 - 5 cm roots were individually removed from the culture tubes. After washing their roots carefully with tap water plantlets were transplanted into plastic pots containing autoclaved vermiculite and soil.

The plants were watered and covered with polythene bags for one week. After hardening, plants were gradually transferred to the field for developing into mature plants.

In the preliminary screening various explants (shoot tip, nodal segments, cotyledonary nodes, epicotyl and hypocotyl segments) derived from aseptic seedlings of *S. acmella* were used for determining their ability to induce multiple shoots (data not shown). Among these the hypocotyl segments were found to be the best for multiple shoot induction. Shoot cultures of *S. acmella* were initially induced from hypocotyl segments on various concentrations of BA and 2-ip alone and in combinations with NAA. Among the various concentrations of BA and 2-ip tested, BA was found to be better than 2-ip for induction of multiple shoots measured in terms of mean shoot number and shoot length. Increasing the concentration of BA from 0.5 to 2.0 mg dm^{-3} did not show any marked increase in the mean number of multiple shoots formation. Induction of multiple shoots was also observed when media were supplemented with 2-ip. However, the shoots formed hairy roots (Fig. 1*B*) from the nodal region of the multiple shoots causing difficulty in separation of shoots during subculture. Moreover, the mean shoot number obtained was also less in presence of 2-ip. Among the various combinations tested, highest rate of shoot multiplication (Fig. 1*A*) was achieved with 0.5 mg dm^{-3} BA and 0.1 mg dm^{-3} NAA (Table 1). The nodal segments obtained from multiple shoots were continuously subcultured to promote rapid proliferation of multiple shoot culture. The results of the present study indicates a balanced content of cytokinin (BA) and auxin (NAA) is intrinsically advantageous for the production of either more number of multiple shoots or profuse callus growth from the hypocotyl segments. Higher concentration of BA than NAA is needed for the production of maximum callus growth. Large number of multiple shoots were induced in the MS medium supplemented with BA in *Artemisia annua* (Whipkey 1992) and NAA in the presence of BA induced callus formation in *Artemisia annua* (Nair *et al.* 1986). Enhanced multiple shoot production on MS medium supplemented with BA and IAA was also observed in *Artemisia annua* (Fulzele and Sipahimalani 1991) and *Cardiospermum halicacabum* (Babber *et al.* 2001).

Multiple shoots were successfully rooted on rooting media containing either IBA or BA and NAA (Fig. 1*C*). Higher percentage of rooting was obtained on medium containing IBA, more roots were produced but root length was shorter compared to shoots rooted on medium containing NAA (results not shown). Decreasing the salt concentration in MS medium from full to half strength increased the number of roots. However, half strength MS medium without any growth regulators failed in root formation in regenerated shoots even after 15 d. In the present study the shoots also developed roots on MS medium supplemented with BA in combination with NAA or 2-ip (Table 2). The root induction in *Artemisia annua* occurred in medium supplemented with NAA and kinetin (Fulzele and Sipahimalani 1991).

Rooted shoots were successfully acclimatized in vermiculite (Fig. 1*D*) under high relative humidity and gradually decreasing air humidity and 95 % of the transplanted plantlets survived and showed normal flowering within 6 weeks without any morphological variations.

Table 1. Effect of different concentrations of BA and 2-ip added to MS medium with 0.1 mg dm^{-3} NAA on multiple shoot formation from nodal segments in *S. acmella*. Means of 20 replications ± SE; + - moderate, ++ - high, +++ - intense callus formation.

Cyt.	Conc. [mg dm^{-3}]	Shoot number [segment^{-1}]	Shoot length [cm]	Callus
BA	0.1	8 ± 0.6	4 ± 0.6	-
	0.5	10 ± 0.6	4 ± 0.4	-
	1.0	5 ± 0.7	6 ± 0.6	+
	1.5	5 ± 0.7	5 ± 0.6	++
	2.0	6 ± 0.7	4 ± 0.5	+++
2-ip	0.1	2 ± 0.3	4 ± 0.6	++
	0.5	5 ± 0.5	9 ± 0.8	+
	1.0	4 ± 0.6	5 ± 0.5	+++
	1.5	3 ± 0.4	5 ± 0.5	+++
	2.0	4 ± 0.6	8 ± 0.5	++

Table 2. Effect of different concentrations of BA and 2-ip added to MS medium with 0.1 mg dm^{-3} NAA on rooting in *S. acmella*. Mean of 20 replications ± SE; + - moderate, ++ - high, +++ - intense callus formation.

Cyt.	Conc. [mg dm^{-3}]	Root number [shoot^{-1}]	Root length [cm]	Callus
BA	0.1	17 ± 0.6	3.5 ± 0.2	+
	0.5	19 ± 0.9	3.0 ± 0.4	++
	1.0	17 ± 0.8	2.0 ± 0.3	++
	1.5	14 ± 0.6	2.5 ± 0.2	+++
	2.0	12 ± 0.5	2.0 ± 0.3	+++
2-ip	0.1	15 ± 0.6	5.0 ± 0.6	++
	0.5	20 ± 0.6	5.0 ± 0.6	++
	1.0	5 ± 0.7	1.0 ± 0.3	++
	1.5	10 ± 1.0	2.5 ± 0.3	++
	2.0	30 ± 0.7	6.0 ± 0.6	+

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