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Micropropagation of *Withania somnifera* from germinating seeds and shoot tips

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Abstract

Shoot multiplication was achieved in vitro from shoot tips of aseptically germinated seedlings of *Withania somnifera* L. using low concentrations of 6-benzyladenine (BA), viz. 2.2, 4.4 and 8.9 μM. Maximum number of shoots were obtained when 2.3 μM 2,4-dichlorophenoxyacetic acid (2,4-D) or 2.5 μM indolebutyric acid (IBA) was added to medium containing 4.4 μM BA during initiation of shoot multiplication, but not when added later. Direct multiple shoot initiation was also obtained from germinating seeds in the presence of BA alone. Rooting was successful in excised shoots grown on growth regulator-free MS medium. Rooted shoots were successfully established in soil in a greenhouse.

Introduction

Collection of medicinal plants on a mass scale from natural habitats is leading to a depletion of plant resources. For the conservation of these valuable genotypes, micropropagation is of special use. Shoot culture, which is often utilized to maintain clonal fidelity, would be of special advantage in this respect.

Withania somnifera L. is used in the traditional system of medicine due to the presence of a group of steroids with cytotoxic activity called withanolides. Shoots of *W. somnifera* grown in culture contain a different pattern of withanolides than that in the leaves of the plants of this species (Heble 1985; Nittala & Lavie 1981). Since this species propagates by seeds but does not have natural ability for vegetative propagation, the variability generated by sexual recombination is difficult to fix. We report here micropropagation of *Withania somnifera* from seed and from seedling shoot tips.

Materials and methods

Berries of a high-yielding strain of *W. somnifera* were collected from the Experimental Garden in the Department of Botany at the University of Calcutta and were sun dried for 1–2 days. Seeds were soaked in water for 24 h, washed with 5% Teepol solution for 5 min, disinfested with 0.1% $HgCl_2$ for 12–13 min, rinsed 3 times with sterile distilled water, and inoculated on a basal medium of half-strength Murashige & Skoog (1962) (MS) salts and vitamins with 1% sucrose. Seeds were incubated at 22°C and 55% relative humidity (RH) in the dark or under 40 μmol m^{-2} s^{-1} cool white fluorescent lights for 16 h daily.

For shoot-tip multiplication, both disinfested seeds and 5- to 8-mm-long shoot tips with bifid cotyledons from aseptically germinated seedlings were cultured on 20 ml of MS medium in plugged culture tubes with 2.2, 4.4, 8.9, 22 or 44 μM BA or 2.3, 4.6, 9.3, 23 or 46 μM kinetin sol-

idified with 0.6% BDH agar. The influence of 2.3 μM 2,4-D, 2.7 μM naphthaleneacetic acid (NAA), 2.9 μM indoleacetic acid (IAA) or 2.5 μM IBA added to medium containing 4.4 μM BA or 4.6 μM kinetin were also tested for shoot proliferation. The media were adjusted to pH 5.6–5.8 before autoclaving at 105 kPa for 15 min. The cultures were maintained at 22 ± 1°C with 55–60% RH and a 16-h photoperiod with 40 μmol m^{-2} s^{-1} provided by cool-white fluorescent light and subcultured at 20 days intervals. Treatments were replicated 10 times and each experiment was done twice. The number of shoots longer than 1 cm produced per explant was recorded after 20, 40 and 60 days of culture.

The multiple shoots were excised at the base and placed in liquid full-strength or half-strength MS medium with or without 2.5 μM IBA. Fifty rooted shoots were maintained on half-strength MS liquid medium for 40–60 days before transfer to potting soil and acclimatization.

Results and discussion

Eighty per cent of the seeds kept in the dark germinated within 20 days, whereas 60% germinated within 20–25 days when incubated under a 16-h photoperiod.

The most effective cytokinin in promoting shoot proliferation from shoot tips was BA (Table 1). Although 50–100% of explants underwent swelling within 10–15 days in the presence of different BA concentrations, only 20–80% of the explants exhibited a similar response to kinetin. With 2.2, 4.4 and 8.9 μM BA, shoot multiplication rates were higher than at greater BA concentrations; kinetin was less effective than BA in inducing shoot multiplication. Similar results have been obtained with other species (Lundergan & Janick 1980; Rahaman & Blake 1988).

Inclusion of 2,4-D or IBA in MS medium containing 4.4 μM BA increased shoot proliferation more than the inclusion of NAA or IAA (Table 2). This effect was more enhanced when auxin was omitted from the culture after 40 days. Finally, after 60 days, approximately 100–150 shoots were obtained on both these sets. Such a phenomenon was not evident when no auxins were included in the medium (Table 1). Under similar conditions, approximately 60 shoots/explant were obtained in the set with 4.4 μM BA alone.

Multiple shoot formation from germinating seeds of *W. somnifera* was evaluated on MS medium containing 0.44, 2.2, 4.4 and 8.9 μM of BA. Multiple shoots were observed only in the treatment with 4.4 μM BA, in which 15% of the seeds were observed to form 4–10 shoots. Seeds with multiple shoots were transferred to MS medium with 4.4 μM BA and 2.3 μM 2,4-D,

Table 1. The effect of different concentrations of BA on multiplication of shoots. Average of 2 experiments with 10 replications.

Cytokinin	No. of shoots >1 cm with S.D.		
BA (μM)	Days		
	20	40	60
0	2 ± 1.1	2 ± 0.8	3 ± 0.6
2.2	5 ± 1.2	15 ± 2.8	50 ± 8.2
4.4	8 ± 2.1	20 ± 3.1	60 ± 9.1
8.9	5 ± 0.9	18 ± 2.1	62 ± 11.1
22	5 ± 2.2	10 ± 3.2	5 ± 3.1
44	2 ± 1.0	2 ± 0.8	2 ± 0.9
Kinetin (μM)			
2.3	2 ± 1.0	4 ± 1.2	4 ± 0.9
4.6	2 ± 0.6	2 ± 0.6	3 ± 1.1
9.3	1 ± 0.3	2 ± 0.8	2 ± 0.9
23	10 ± 2.9	15 + 3.1	15 ± 3.6
46	1 ± 0.8	10 ± 2.2	10 ± 4.1

Table 2. *Effect of cytokinins along with auxins** on multiplication of shoots.

Auxin	Concentration (μM)	4.4 μM BA			4.6 μM Kinetin		
		days			days		
		20	40	60	20	40	60
		% cultures responding					
2,4-D	2.3	30	80	80	100	–	–
NAA	2.7	100	70	70	60	5	–
IAA	2.9	100	100	–	40	40	–
IBA	2.5	90	90	90	100	–	–
		Number of shoots >1 cm with S.D.					
2,4-D	2.3	15 ± 3	90 ± 10	120 ± 16	2 ± 1	–	–
NAA	2.7	2 ± 1	10 ± 1	10 ± 2	2 ± 1	4 ± 1	–
IAA	2.9	2 ± 1	5 ± 1	–	2 ± 1	6 ± 1	–
IBA	2.5	15 ± 1	105 ± 20	145 ± 22	–	–	–

– No response as single plantlet/explant
* Experiments were repeated twice with 10 replications.
** Auxin was omitted after 40 days.

which favored shoot multiplication as well as elongation. This multiple shoot formation seems to be due to enhanced auxillary branching as reported in other species (Hisajima 1982a, 1982b).

Shoots excised from shoot clusters rooted readily (80%) on growth regulator-free liquid MS medium within 30 days. Rooted plants were transferred to media with half-strength MS for 30 days, followed by transfer to sterile distilled water and ultimately to potting soil. Only 10 plants out of 50 transferred to potting soil survived. The rest showed wilting.

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