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In vitro Propagation of *Solanum capsicoides* All. – An Important Therapeutic Agent 'Kantakari'

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Abstract

Shoot tip explants from *in vitro* germinated seedlings of *Solanum capsicoides* All. inoculated on MS containing 2 mg/l BA produced maximum shoot induction response (26 shoots per explant). Rooting of the microshoots (19.4 roots per explant) was obtained better in half strength of MS supplemented with NAA (0.5 mg/l). Well rooted plantlets were successfully hardened with 80 per cent survival rate.

Introduction

Solanum capsicoides All. (Syn. *S. acculaticimum*) commonly known as Devils' Apple or Cockroach berry is a medicinal shrub distributed throughout the tropics in degraded forests and waste lands. Its roots, fruits and occasionally the whole plant are used as medicine. The plant is used as an important therapeutic agent 'kantakari' in some parts of Kerala, India (Sivarajan and Balachandran 1994). Kantakari ghrtham, kanakasavam, putikaranjasavam, suranadi leham etc. are some of the important formulations prepared using the plant. 'Kantakari' is used in the treatment of cough, asthma, bronchitis, enteric fever, bladder stones, rheumatism, vomiting and skin diseases (Nadkarni 1954). Presence of steroidal alkaloides is reported from the leaves, stems and fruits of *S. acculaticimum* (Kadkade et al. 1979). Aculeatiside A and B are the two steroidal saponins present in the roots (Saijo et al. 1983). Previous *in vitro* studies in *S. acculaticimum* revealed the production of various phytochemicals from callus or suspension cultures or hairy root cultures (Kadkade et al. 1979, Nabeta 1985, Ikenaga et al. 2000). There is no previous report on the *in vitro* propagation of this species. Natural regeneration of the species is through seeds. But, unfortunately, the germination percentage is very low. It is very urgent to develop an *in vitro* method for the rapid multiplication of this important medicinal plant because of the various threats, habitat loss, over exploitation etc. encountered by the species.

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Materials and Methods

Mature fruits of *Solanum capsicoides* All. were collected from the herbal garden of the institute. The fruits were aseptically surface sterilized in 0.1% $HgCl_2$ for 10 min and rinsed three to four times in sterile distilled water. Seeds were dissected out and inoculated on half strength MS supplemented with 1.5% sucrose. The pH of the medium was adjusted to 5.7, gelled with 0.7% agar and dispensed in 35-40 ml aliquots into 60 × 100 mm culture vials. The medium was sterilized by autoclaving at 121ºC for 15 min at 1.5 kg/cm^2. The cultures were incubated at 25 ± 2ºC with a photoperiod of 16 hr per day with a light intensity of 2500 lux. The seeds were germinated after 8 - 12 days and the explants like hypocotyle segments (5 - 7 mm long) cotyledonary leaves and shoot tips (6 - 8 mm) were isolated from 12 - 15 days. MS (30 g/l sucrose) supplemented with 0.5 - 5.0 mg/l BA and 0.5 - 5.0 mg/l Kn either singly or in combination with 0.1 - 1.0 mg/l IAA were used for testing the interaction of various explants on morphogenesis. After 60 days of incubation, the number of regenerated shoots was noted and individual shoots were isolated for *in vitro* rooting. The shoots (2.5 - 3 cm) were placed on filter paper wicks wetted with half strength or full strength MS liquid medium supplemented with and without different concentrations (0.1 and 0.5 mg/l) of IBA and NAA. The rooted plantlets were hardened in vermiculite wetted with half strength of MS liquid medium and the survival rate was noticed. After three weeks, the plantlets were transferred to the field.

Numerical data regarding the number of shoots and roots in each of the 8 replicas were recorded after 60 days of culture. Mean and standard errors were calculated. One way analysis of variance was performed ($p < 0.05$) and the means were compared using DMRT.

Results and Discussion

Type of explant and growth regulator regime influenced considerably in the *in vitro* regeneration of *S. capsicoides* (Table 1). Shoot tip explants on MS containing BA (2 mg/l) was found to be the optimal condition for rapid *in vitro* regeneration of the species. Here, it produced 26 shoots per explant after 60 days of culture (Fig. 1C). Regeneration of multiple shoots from the shoot tip explants has been reported in various *Solanum* spp. (Anish et al. 2010). However, hypocotyl explants produced better response (19 shoots/explant) in a combination of BA (1 mg/l) and IAA (0.5 mg/l). Significant variation was found in the *in vitro* morphogenesis of *S. capsicoides* with respect to the type of explant and growth regulator regime. On the other hand, no considerable morphogenetic response was induced on medium devoid of growth regulators.

Table 1. Effect of growth regulators and explants on *in vitro* regeneration of *Solanum capsicoides* after 60 days of culture in MS.

BA (mg/l)	Kn (mg/l)	IAA (mg/l)	Mean shoot No.		
			Shoot tip	Cotyledon	Hypocotyl
0	0	0	0.6 ± 0.12 a	0.7 ± 1.0 a	0.6 ± 1.02 a
0.5	-	-	11.2 ± 1.20 mn	2.9 ± 0.69 bcd	8.3 ± 0.49 efg
1.0	-	-	12.8 ± 0.86 no	8.3 ± 2.11 ghi	14.0 ± 1.34 kl
2.0	-	-	26.3 ± 0.37 p	12.9 ± 2.04 j	12.4 ± 0.86 ijk
3.0	-	-	14.4 ± 0.75 o	19.8 ± 1.37 k	16.2 ± 2.0 l
4.0	-	-	7.3 ± 0.76 ijk	8.4 ± 1.05 ghi	7.3 ± 0.74 def
5.0	-		5.9 ± 0.94 ijk	9.2 ± 0.80 hi	5.6 ± 0.85 cde
-	0.5	-	1.5 ± 0.50 abc	1.5 ± 0.76 abc	5.5 ± 1.04 cde
-	1.0	-	1.1 ± 0.13 a	0.6 ± 0.37 ab	4.5 ± 0.70 abc
-	2.0	-	2.8 ± 0.97 def	2.9 ± 0.77 bcd	6.6 ± 1.16 cde
-	3.0	-	4.0 ± 1.36 efg	6.3 ± 1.23 ghi	8.2 ± 0.46 efg
-	4.0	-	1.8 ± 0.26 bcd	5.8 ± 0.59 efg	12.5 ± 1.36 jk
-	5.0	-	1.1 ± 0.14 a	5.6 ± 0.78 efg	7.0 ± 0.69 def
0.5	-	0.1	2.1 ± 0.30 bcd	2.6 ± 0.58 abc	5.2 ± 0.61 abc
0.5	-	0.5	2.1 ± 0.35 bcd	4.0 ± 0.71 def	9.8 ± 0.92 ghi
0.5	-	1.0	1.1 ± 0.40 a	3.3 ± 0.56 cde	5.3 ± 0.88 bcd
1.0	-	0.1	7.4 ± 1.0 ijk	8.4 ± 1.64 ghi	11.3 ± 1.17 hij
1.0	-	0.5	14.0 ± 1.67 o	9.2 ± 0.41 i	18.9 ± 1.17 m
1.0	-	1.0	8.1 ± 0.61jkl	14.8 ± 1.16 j	9.5 ± 0.80 fgh
2.0	-	0.1	5.0 ± 0.40 ghi	6.1 ± 0.25 efg	5.8 ± 0.33 cde
2.0	-	0.5	4.7 ± 1.0 ghi	6.8 ± 1.16 ghi	4.9 ± 0.44 abc
2.0	-	1.0	4.2 ± 0.71 efg	8.4 ± 0.72 ghi	5.3 ± 0.53 bcd
-	0.5	0.1	1.4 ± 0.20 ab	1.0 ± 0.33 a	6.0 ± 1.03 cde
-	0.5	0.5	4.4 ± 0.37 fgh	1.1 ± 0.25 a	3.9 ± 0.48 ab
-	0.5	1.0	1.0 ± 0.22 a	1.0 ± 0.45 a	3.1 ± 0.69 a
-	1.0	0.1	5.4 ± 0.84 ghi	1.2 ± 0.56 ab	5.9 ± 1.47 cde
-	1.0	0.5	8.6 ± 1.27 kl	1.3 ± 0.64 abc	6.7 ± 0.71 cde
-	1.0	1.0	8.3 ± 0.99 jkl	3.0 ± 1.25 bcd	8.1 ± 0.1efg
-	2.0	0.1	10.1 ± 1.06 lm	2.1 ± 0.82 abc	10.4 ± 0.75 hij
-	2.0	0.5	8.6 ± 1.02 kl	2.3 ± 0.81 abc	5.4 ± 0.37 cde
-	2.0	1.0	7.1 ± 1.34 ijk	2.6 ± 0.81 abc	6.3 ± 0.42 cde

Data represent the means ± SE of 8 replicates. Means followed by the same letter do not differ statistically at p = 0.05 according to DMRT.

Shoot tip explants produced shoots from the cotyledonary node as well as from the calli developed from the basal cut end. Small black protuberances developed on the calli were modified in to shoot buds after 20 days of culture. Proliferation of more amount of callus retarded the shoot regeneration response in Kn or combinations of BA or Kn with IAA supplemented cultures. Whereas, in BA alone treated cultures, numerous shoot buds developed on the basal calli and showed an enhanced shoot regeneration response. Similarly, the efficacy of BA

on multiple shoot regeneration in two varieties of eggplant was already proved by Sarker et al. (2006). The concentration of BA or Kn also had profound influence in shoot induction response. At high concentration (5.0 mg/l) vitrified shoots were developed in all explants.

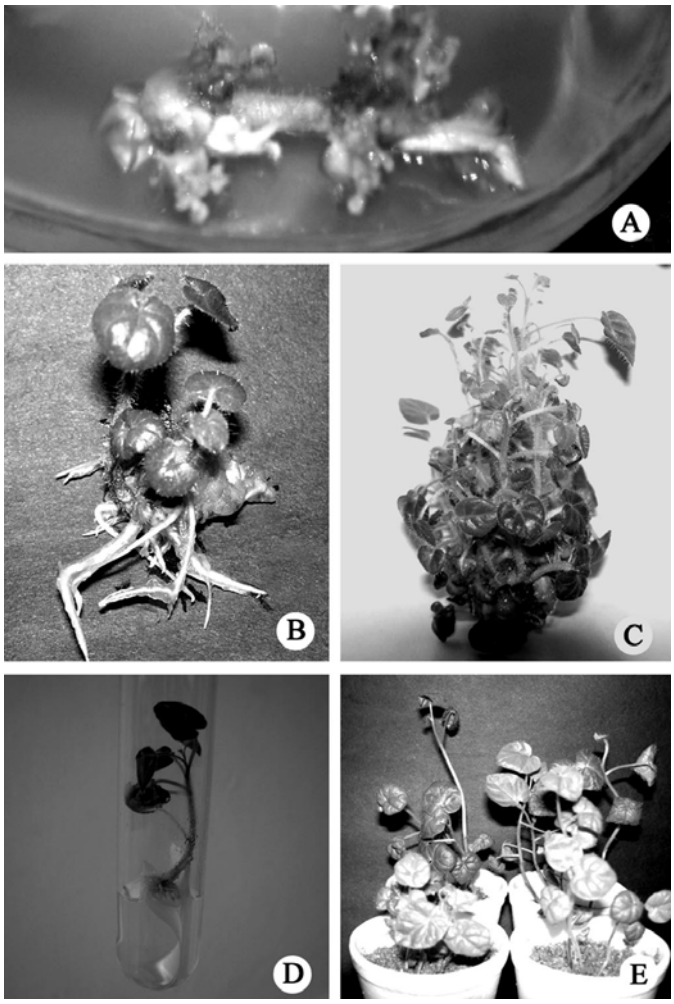

Fig. 1. *In vitro* propagation of *Solanum capsicoides* All. A. Regeneration of shoot buds from hypocotyl explants on MS supplemented with 1 mg/l BA and 0.5 mg/l IAA after 30 days. B. Response of cotyledonary leaves on 0.5 mg/l BA after 45 days. C. Multiple shoots regenerated from shoot tip explants in 2 mg/l BA after 60 days. D. Rooting of excised shoots on half MS fortified with 0.5 mg/l NAA after 20 days. E. Hardened plants of *S. capsicoides*.

Cotyledonary leaves showed initial culture response as the enlargement of lamina along with the development of calli from the basal cut end. Shoot buds directly regenerated in the cultures of BA supplemented media (Fig. 1B). In other

treatments callusing and subsequent development of shoot buds were noticed. But the rate of shoot production was low compared to shoot tip explants. However, Rajesh et al. (2010) and Sarker et al. (2006) reported the effectiveness of cotyledonary leaves on the *in vitro* multiplication of *Solanum macrocarpum* and *S. melongena,* respectively.

Hypocotyl explants showed polarity in initial response. Combination of BA (1.0 mg/l) and IAA (0.5 mg/l) was found to be the best growth regulator regime for shoot production using hypocotyl explants (Fig. 1A). The calli were initiated from the cut ends of the explants and eventually extended all over the explants. Regeneration of dark green shoot buds occurred in these calli after three weeks.

Table 2. Influence of basal media and auxins on the *in vitro* rooting of *Solanum capsicoides* after 30 days of incubation.

Basal medium	IBA (mg/l)	NAA (mg/l)	Mean root No.
½MS	-		8.3 ± 0.90 a
"	0.1		6.5 ± 0.57 a
"	0.5		8.5 ± 0.57 a
"		0.1	17.3 ± 2.69 bc
"		0.5	19.4 ± 2.98 c
Full MS	-		6.8 ± 0.70 a
"	0.1		8.5 ± 0.65 a
"	0.5		6.8 ± 0.62 a
"		0.1	13.2 ± 1.16 b
"		0.5	16.5 ± 1.16 bc

Data represent the means ± SE of 8 replicates. Means followed by the same letter do not differ statistically at p = 0.05 according to DMRT.

Shoots of 2.5 - 3 cm were excised and used for *in vitro* rooting. Rhizogenesis was maximum (19.4 roots/explant) in NAA (0.5 mg/l) supplemented half strength MS (Table 2, Fig. 1D). Root initiation was observed after 10 - 12 days in all the cultures. Even though, the number of roots were more in NAA supplemented medium the length of roots were high in IBA supplemented cultures. The rooted plantlets showed an average survival rate of 80% during hardening (Fig. 1E). The regenerants established in the field were morphologically similar and showed normal flowering and fruiting.

The report presents an efficient protocol for the mass multiplication of *S. capsicoides*. The technique will be useful for the mass production of this over-exploited medicinal plant for its conservation and utilization.

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