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MICROPROPAGATION OF *ACMELLA CALVA* (DC) R. K. JANSEN FROM NODE EXPLANTS

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SUMMARY

Efficient method for plant regeneration from nodal explants of *Acmella calva* (DC) R. K. Jansen has been developed. The nodal explants were cultured on Murashige and Skoog's (MS) medium fortified with different concentrations (0.5 – 5.0 mg l^{-1}) of benzyldenine (BA), and adenine sulphate (Ads). BA was found to be the effective cytokinin, which when used at 3.0 mg l^{-1}, produced 15 shoots/explant. The well developed shoots were excised and rooted on ½ and full strength MS medium containing different concentrations (0.5 – 5.0 mg l^{-1}) of IAA and NAA. The rooted plantlets were transferred to plastic cups containing vermiculite for hardening and then to the soil condition. After one month the *in vitro* derived plantlets started to produce flowers.

Key words **: *Acmella calva*, medicinal plant, micropropagation**

INTRODUCTION

Acmella calva (DC) R. K. Jansen (Asteraceae) is a medicinal plant. The flower heads are chewed to relieve toothache, affection of throat, gum and paralysis of the tongue. By producing irritation of the gums and salivation relieves the pain. The herb is boiled in water and the decoction as well as the solid material is given in dysentery. The root is used as a purgative (Singh and Jain 1997). In the present study, an attempt has been made to multiply this medicinal plant using node explant and to evolve a protocol for successful micropropagation.

MATERIALS AND METHODS

The shoot of the experimental material under study was collected from garden grown plants and kept in running tap water for 30 minutes. These shoots were surface sterilized with 0.1 % bavistin for 15 min. and then in 0.1 % $HgCl_2$ for 3 min., washed 4 or 5 times with sterile distilled water. These surface sterilized shoots were cut into appropriate size of about 5 mm length containing single node, implanted into the agarified (0.8 % agar agar) MS (Murashige and Skoog's 1962) medium with 3 % sucrose, containing different concentrations (0.5, 1.0, 2.0, 3.0, 4.0 and 5.0 mg l^{-1}) of cytokinins like benzyladenine (BA), and adenine sulphate (Ads) for multiple shoot formation.

The cultures were incubated at 24 ± 2 ° C under 2000 lux intensity provided by white fluorescent lamp for 16 hr photoperiod. In all experiments 20 replicates were used and each experiment was repeated thrice. All the results were statistically analyzed. Subculturing was carried out regularly at 2 weeks interval. The microshoots were excised and transferred to half and full strength MS medium fortified with different concentrations (0.5, 1.0, 2.0 and 3.0 mg l^{-1}) of auxins like NAA and IAA to study the effect on rooting. The rooted plantlets were transferred to plastic cups containing vermiculite for hardening and then to the soil condition. The acclimatized plantlets produced flowers in the soil condition.

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RESULTS AND DISCUSSION

Node explant of *Acmella culva* inoculated on multiple shooting medium showed shoot bud initiation after 3 days of inoculation (Fig. 1a). A maximum of 15 shoots was observed at 3.0 mg l^{-1} BA (Fig. 1b) followed by 1.0 mg l^{-1} Ads, from which 9 shoots were obtained (Table 1). From this observation it is inferred that, the BA plays a key role in multiple shoot formation, when compared to other cytokinin tried. Similar findings were observed in *Sterculia urens* (Purohit and Dave 1996), *Artemisia annua* (Usha and Swamy 1998), *Tridax procumbens* (Sahoo and Chand 1998) and *Houttuynia cordata* (Handique and Bora 1999). The positive effect of cytokinin on regeneration decreased at higher concentrations and it failed to show any effect at 5.0 mg l^{-1} and above. The regenerated shoot attained a length of 3-5 cm within 20 days on inoculation on multiple shooting medium. In our study a single cytokinin (BA) alone was enough for higher shoot multiplication. Some authors suggested that the combination of two cytokinins were needed for producing multiple shoots on *Aristolochia bracteolata* (Rameshree *et al*. 1994), *Lavandula* species (Jordan *et al*. 1998), *Canavalia virosa* (Kathiravan and Ignacimuthu, 1999) and *Enicostemma littorale* (Shanthi and Anne Xavier 2003).

The well developed elongated shoots were excised from shoot clumps and transferred to half and full strength MS medium containing different concentrations (0.5 – 3.0 mg l^{-1}) of auxins like NAA and IAA. The roots were initiated within 5 days of inoculation. Of the different auxins tried, maximum (30 roots/shoot) rooting was observed at 1.0 mg l^{-1} NAA on half strength medium (Fig. 1c). The roots are pale white, linear with an average of 3 – 4 cm in length (Fig. 1d). It is followed by 2.0 mg/l NAA which produced an average of 28 roots/shoot, associated with bulging of the shoot base (Table 2). Of the two different strength of media tried, the maximum results on rooting were obtained on half strength medium than in full strength medium. Similar results were reported by Satykala *et al*. (1995) in

Table 1. Multiple shoot formation from node explants of *Acmella calva* on MS medium containing different concentrations (0.5 – 5.0 mg l^{-1}) of BA and adenine sulphate (Ads).

Hormone	Hormone concentrations (mg l^{-1})	Response (%)	No. of shoots/explant (Mean ± SD)	Average length of shoots in cm (Mean ± SD)
BA	0.5	33	3.4 ± 1.8	3.0 ± 0.2
	1.0	52	5.8 ± 1.7	3.2 ± 0.1
	2.0	71	8.4 ± 1.5	4.0 ± 0.6
	3.0	93	15.1 ± 1.1	4.9 ± 0.9
	4.0	82	7.0 ± 1.4	3.6 ± 1.1
	5.0	43	6.4 ± 1.8	3.1 ± 0.9
Ads	0.5	57	4.4 ± 1.2	3.3 ± 0.3
	1.0	78	9.0 ± 0.8	2.9 ± 1.6
	2.0	72	6.4 ± 1.1	3.2 ± 0.5
	3.0	51	5.7 ±1.5	3.1 ± 0.3
	4.0	39	5.0 ± 1.4	3.3 ± 0.1
	5.0	31	4.5 ± 0.9	3.3 ± 0.4

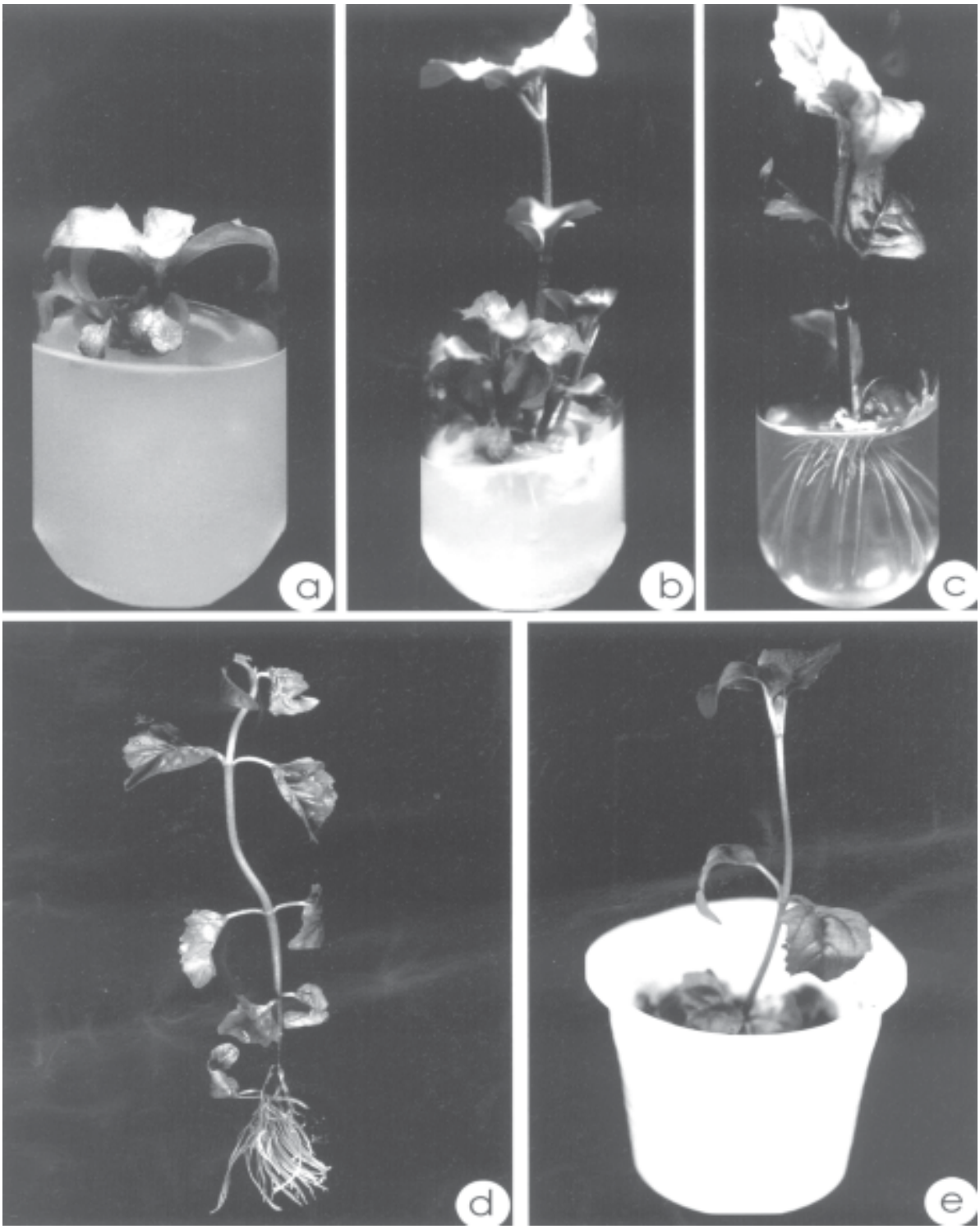

Fig. 1. Micropropagation of *Acmella calva* (DC) R.K. Jansen from node explants. (a) initiation of multiple shoots from node explant, (b) proliferation of multiple shoots from node explant, (c) rooting of regenerated shoot, (d) *in vitro* derived plantlet, (e) hardening of *in vitro* derived plantlet

Table 2. Rooting of *in vitro* derived shoots of *Acmella calva* on half strength MS medium fortified with different concentrations (0.5 – 3.0 mg l^{-1}) of NAA and IAA

Hormone	Hormone concentrations in mg l^{-1}	% of rooting	No. of roots/ shoot (Mean ± SD)	Length of roots in cm (Mean ± SD)
NAA	0.5	94	28.1 ± 1.1	3.1 ± 0.4
”	1.0	100	30.3 ± 0.9	4.0 ± 0.1
”	2.0	97	28.7 ± 1.1	3.7 ± 0.8
”	3.0	72	27.4 ± 0.6	3.2 ± 0.5
IAA	0.5	78	18.9 ± 1.2	3.0 ± 0.3
”	1.0	98	22.5 ± 1.0	3.2 ± 0.5
”	2.0	64	20.1 ± 0.8	3.7 ± 0.2
”	3.0	52	24.5 ± 0.3	3.1 ± 0.1

Pelargonium graveolens. In our study NAA (1.0 mg l^{-1}) was found to be efficient auxin for root induction. Our observations are in accordance with the results of Gangopadhyay *et al*. (1999) in *Tagetes erecta*.

The well rooted plantlets were transferred to plastic cups containing vermiculite for hardening and kept under controlled condition (Fig. 1e). After 15 days, the acclimatized plantlets were shifted to soil under greenhouse condition. The survival rate was 95 % in soil condition. After one month of acclimatization in soil, the *in vitro* derived plantlets started to produce flowers. The number of flowers *in vitro* derived plants were threefold when compared to *in vivo* grown plants. From this it is seen that, the *in vitro* technique is a valuable tool for multiplication of *Acmella calva* plants, which has very useful medicinal properties.

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