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In vitro propagation of cactus pear

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Abstract

In this investigation an elite genotype of spineless cactus pear was selected for *in vitro* propagation. The effect of different levels of BA (0, 3.0, 6.0 and 9.0 mg l^{-1}) and NAA (0, 0.1 and 1.0 mg l^{-1}) alone and in combination were evaluated for *in vitro* culture establishment, axillary bud breaking, micro-shoot formation and root initiation. Early axillary bud breaking in cladode explant was induced with 9.0 mg BA l^{-1} + 0.1 mg NAA l^{-1} in 19.47 days, whereas all NAA concentrations failed to induce bud breaking in the explants. However, root initiation was noticed with NAA concentrations of 0.1 and 1.0 mg l^{-1}. Culture establishment in terms of percentage of explant with microshoot was maximum (80%) with 6.0 mg BA l^{-1} + 0.1 mg NAA l^{-1} as compared to minimum (26.67%) with 3.0 mg BA l^{-1} and 1.0 mg NAA l^{-1} treatment. All treatments of BA alone and in combination with NAA found to induce micro-shoots in single bud/areole of cladode explant. The proliferated micro-shoots were subjected for elongation treatment in order to obtain rootable length of micro-shoots. The elongated micro-shoots were further subjected to root formation under *in vitro* condition with different concentrations of NAA (0, 2.0, 4.0 and 6.0 mg l^{-1}) and IBA (2.0, 4.0 and 6.0 mg l^{-1}). Maximum number of roots was recorded with 6.0 mg IBA l^{-1}. Further the length of roots was also maximum (3.80 cm) with 6.0 mg IBA l^{-1}. The rooted plantlets were successfully acclimatized and approximately 92 per cent success obtained in potting mixture of vermiculite and cocopeat 3: 1 (v/v)

Key words: *Cactus pear, micropropagation, acclimatization*

Introduction

The cactus pear (*Opuntia ficus-indica* (L) Mill) is native of Central American and Mexican region (Pareek *et al.*, 1998). Owing to presence of pricks on its body, cactus pear is commonly known as prickly pear and its edible fruit is called "tuna". In Mexico, the tender stems are used as vegetable (nopalitos) and sweet fruits are in demand in international market. In some countries cactus pear and their products serve various purposes such as the tender cladodes can be used to prepare vegetable, salad, pickle and as animal fodder (Singh and Felker, 1998). Use of nopales to cure diabetes has opened new vistas for its utilization in human health (Hagwood, 1990). The economic interest for cactus pear has remarkably increased during the last few years especially in the arid and semi-arid zones. However, one of the problems faced in increasing the area under cultivation of cactus pear rapidly is lack of suitable true-to-type planting material.

Propagation of cactus pear by seeds leads to several problems such as genetic segregation, a long juvenile phase, slow growth and lack of availability of true-to-type planting material and through vegetative means by cladodes produce limited plants which give poor survival in field due to foot rot disease infection.

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In view of the above, an attempt was made to multiply true to type plant material of cactus pear through tissue culture technique with an objective to develop *in vitro* micropropagation technique of thornless cactus pear and to develop successful micropropagation technique of cactus pear.

Materials and methods

Explant material of spineless cladodes were taken from the pot grown plants of Cactus pear (*Opuntia ficus indica* (L.) Mill) from the Hi-Tech nursery of Central Institute for Arid Horticulture (CIAH), Bikaner and the experiment was conducted in CRD. The significance of various treatment effects was judged with the help of 'F' value (test) at 1%. The cladodes were harvested in the morning during the month of September and October 2006. The cladodes were thoroughly washed in detergent followed by running tap water and finally washed with autoclaved distilled water. The cladodes were then surface sterilized with 0.1% solution of $HgCl_2$ (W/V) for 5-6 minutes followed by washing with autoclaved distilled water for 6-8 times.

The culture medium was poured in conical flasks and test tubes for sterilization. Autoclaving was done for 15 minutes at 121°C and 15 p.s.i. (1.1 $kg^{-1}cm^2$) pressure. After autoclaving the media was stored in dark conditions for 48 hours at 25±2°C. The cultures were incubated in culture room at 26 ± 1°C and 16/8 hours photoperiod was provided.

To determine the influence of various concentrations of BA (0, 3, 6, 9 mg l^{-1}) and NAA (0, 0.1 and 1.0 mg l^{-1}), these were used alone and in various combinations. Murashige and Skoog (1962) medium was used as basal medium. Observations with respect to number of days taken for bud break, percentage of explants with shoots, mean number of shoot per explant and mean length of shoot were recorded. After bud breaking subculturing was done on fresh medium and observations were recorded after 21 days. To determine the influence of various concentrations of NAA and IAA on the elongation of proliferated micro-shoots, the micro-shoots were subjected for elongation phase and observations with respect to average shoot length (cm) were recorded after 30 days of micro-shoots inoculation.

To evaluate *in vitro* rooting response of micro-shoots, the proliferated micro-shoots of about 2-5 cm length obtained from *in vitro* raised explants were transferred to MS medium supplemented with various concentrations of IBA and NAA and time taken for induction of roots, per cent rooting, mean maximum length of root and number of roots/shoot were recorded 21 days after inoculation of microshoots

The rooted micro-shoots were taken out from the culture vessels and tried in different potting mixtures for successful establishment of plantlets. Initially, plantlets were kept under growth chamber and later on transferred to greenhouse (at temperature range of 28-30°C and humidity of 70-85%).

Results and discussion

Effect of BA and NAA on axillary bud/areole breaking in cladode explant

The number of days required for axillary bud breaking in cladode explants are presented in Table 1. The data revealed that different levels of BA and NAA resulted in significant variation on number of days taken for axillary bud breaking in inoculated cladode explants under *in vitro* condition. Significantly less number of days for axillary bud breaking (19.47) were observed in the cultures at T_{11} (9.0 mg l^{-1} BA + 0.1 mg l^{-1} NAA) treatment followed by (19.87) days as compared to higher (35.46 days) at T_4 (3.0 mg l^{-1} BA) treatment, whereas T_1 (control), T_2 (0.1 mg l^{-1} NAA) and T_3 (1.0 mg l^{-1} NAA) treatments failed to induce axillary bud breaking in explants.

The present results are in conformity with the findings of Mauseth (1977 and 1979). Further, Escobar *et al.* (1986) also reported that BA was necessary for axillary proliferation from pre-existing buds in the cactus *Opuntia amyclaea*. Similar results have also been noticed by Dabekaussen *et al.* (1991) and Mohamed-Yassen *et al.* (1995) in cactus *Sulcorebutia alba* Rausch and *Stepelia semota*, respectively. This shows that BA suppresses apical dominance and stimulates the lateral buds.

In the treatments without BA, all auxin concentrations failed to stimulate axillary bud initiation in the cladode explant. However, some explants exhibited root induction instead of shoot bud induction. This is due to physiological action of auxin in stimulation of root initiation.

Effect of BA and NAA on percentage of explants with shoots

Data in Table 1 indicate significant effect of various levels of BA and NAA on the percentage of explants with shoots. It is clear from the data that different levels of BA and NAA produced a remarkable effect on the percentage of explant with shoots. The mean maximum percentage of explants with shoots (80%) were obtained in T_8 (6.0 mg l^{-1} BA + 0.1 mg l^{-1} NAA) and T_{11} (9.0 mg l^{-1} BA + 0.1 mg l^{-1} NAA) treatment as compared to mean minimum (26.67%) in T_6 (3.0 mg l^{-1} BA + 1.0 mg l^{-1} NAA) treatment. Whereas, T1 (control), T_2 (0.1 mg l^{-1} NAA) and T_3 (1.0 mg l^{-1} NAA) treatments failed to induce shoot formation in the explant.

These results are in agreement with the findings of Escobar *et al.* (1986), Mohamed-Yaseen *et al.* (1995), Giusti *et al.* (2002) and Garcia-Saucedo *et al.* (2005). In cactus spp, they observed better establishment of explants under *in vitro* condition with proliferation of micro-shoots. The less percentage of explant with shoots was observed with 3.0 mg l^{-1} BA + 1 mg l^{-1} NAA treatment. This is due to higher concentration of NAA with respect to BA i.e., 1.0 mg l^{-1} which might have suppressed stimulative effect of BA.

Effect of BA and NAA on number of shoot per explant

The treatment without BA failed to induce shoot in the explants (Table1) whereas, in all the treatments of BA alone and in combination with NAA produced one shoot per explant. *In vitro* propagation, subculturing process generally enhances shoot proliferation and plays an important role in increasing number of shoots per explant (Giusti *et al.*, 2002). In this experiment, cultures were not subjected to repeated subculturing hence only one micro-shoot was observed per explant. Similar results were obtained by Juarez and Passera (2002) *in vitro* propagation of *Opuntia ellisiana* Griff.

Effect of BA and NAA on length of shoot per explant

In the present study, all the BA concentrations along with NAA significantly influence the shoot length in the explants. The mean maximum shoot length (1.02 cm) was obtained in T_{11} (9.0 mg l^{-1} BA + 0.1 mg l^{-1} NAA) treatment followed by (0.95 cm) in T_8 (6.0 mg l^{-1} BA + 0.1 mg l^{-1} NAA) treatment as compared to mean minimum shoot length (0.45 cm) in T_6 (3.0 mg l^{-1} BA + 1.0 mg l^{-1} NAA) treatment (Table 1). Maximum length of shoot as observed in BA treatments at higher concentrations are due to emergence of shoot earlier with higher concentration of BA. Minimum length of the shoots recorded under the treatments with minimum BA concentration and higher concentration of auxins might be due to the suppression of BA effect by the higher concentration of NAA in shoot growth. Starling (1985) also observed variable response of BA and NAA at different

Table 1. Effect of BA and NAA on the number of days taken for axillary bud breaking in explant and others parameters of shoot proliferation

	Treatment combinations	Days taken for bud breaking in explant	Percentage of explant with shoots	Number of shoots per explant	Shoot length per explant (cm)
T_1	BA +NAA (0.0 mg l^{-1} + 0.0 mg l^{-1})	0.00	0.00 (0.00)*	0.00	0.00
T_2	BA +NAA (0.0 mg l^{-1} + 0.1 mg l^{-1})	0.00	0.00 (0.00)	0.00	0.00
T_3	BA +NAA (0.0 mg l^{-1} + 1.0 mg l^{-1})	0.00	0.00 (0.00)	0.00	0.00
T_4	BA +NAA (3.0 mg l^{-1} + 0.0 mg l^{-1})	35.47	46.67 (43.09)	1.00	0.49
T_5	BA +NAA (3.0 mg l^{-1} + 0.1 mg l^{-1})	31.67	73.33 (58.91)	1.00	0.75
T_6	BA +NAA (3.0 mgl^{-1} + 1.0 mg l^{-1})	32.33	26.67 (31.09)	1.00	0.45
T_7	BA +NAA (6.00 mg l^{-1} + 0.0 mg l^{-1})	29.47	66.67 (54.74)	1.00	0.62
T_8	BA +NAA (6.0 mg l^{-1} + 0.1 mg l^{-1})	28.47	80.00 (63.43)	1.00	0.95
T_9	BA +NAA (6.0 mg l^{-1} + 1.0 mg l^{-1})	23.47	53.33 (46.91)	1.00	0.65
T_{10}	BA +NAA (9.0 mg l^{-1} + 0.0 mg l^{-1})	21.60	53.33 (46.91)	1.00	0.68
T_{11}	BA +NAA (9.0 mg l^{-1} + 0.1 mg l^{-1})	19.47	80.00 (63.43)	1.00	1.02
T_{12}	BA +NAA (9.0 mg l^{-1} + 1.0 mg l^{-1})	19.87	66.67 (54.74)	1.00	0.60
	C D at 5%		0.21	9.06	0.06

0 indicates no bud break

concentration in shoot formation in a range of cacti and other succulents.

Effect of different concentration of auxin on the shoot elongation

The effects of various levels of NAA and IAA on shoot length in inoculated micro-shoots were studied. In this experiment, there were three concentration of NAA (0.5, 1.0 and 2.0 mg l^{-1}) and three concentration of IAA (0.5, 1.0 and 2.0 mg l^{-1}) along with control.

Effect of NAA and IAA on shoot length during elongation period

The data given in Table 2 show the effect of NAA and IAA on the elongation of proliferated micro-shoots. The length of shoot was significantly influenced by all the treatments of NAA and IAA as compared to control. A decrease in shoot length was noticed with increasing concentrations of auxins. It is evident from the data that the shoot length was inversely proportional to the various levels of auxins (NAA and IAA). The mean maximum elongation in shoot length (1.33 cm) was obtained in T_5 (0.5 mg l^{-1} IAA) treatment followed by (0.63 cm) in T_2 (0.5 mg l^{-1} NAA) treatment as compared to mean minimum shoot length (0.13 cm) in T_1 (control) treatment. Addition of auxin in the medium often mitigates the inhibitory effect of

Table 2. Effect of NAA and IAA on the length of micro-shoots during elongation phase

	Treatments	Shoot length per explant (cm)
T_1	Control	0.13
T_2	NAA (0.5 mg l^{-1})	0.63
T_3	NAA (1.0 mg l^{-1})	0.47
T_4	NAA (2.0 mg l^{-1})	0.30
T_5	IAA (0.5 mg l^{-1})	1.33
T_6	IAA (1.0 mg l^{-1})	0.47
T_7	IAA (2.0 mg l^{-1})	0.23
	C D at 5%	0.09

cytokinin on shoot elongation, thus increasing the number of usable shoots of sufficient length for rooting. This is due to physiological action of NAA in cell division and stimulation of apical dominance in the micro-shoots and adventitious root formation including cell elongation.

Rooting in micro-shoots

After having successfully established the culture of cactus pear single areole cladodes followed by successful production of micro-shoots, the formation of roots on the micro-shoots formed an important part of the present investigation. Proliferated micro-shoots were subjected to root initiation the *in vitro* culture.

Effect of IBA and NAA on root induction in micro-shoots

The data given in Table 3 show the effect of IBA and NAA on root induction in micro-shoots.

In the present study the number of days taken for root induction under the influence of different IBA and NAA treatments were recorded and it was observed that earliest root induction (10.44 days) was recorded with T_4 (6.0 mg IBA l^{-1}) followed by (10.82 days) with T_7 (6.0 mg NAA l^{-1}) as compared to delayed rooting with T_1 (control) treatment. These results indicate that the higher concentration of auxins have better simulative influence on root initiation whereas lower concentration exhibited delayed rooting which may be due to in-sufficient quantity for root induction. Similar observations were reported by Mata-Rosas *et al.* (2001) with Mexican cactus *Turbinicarpus laui* and Jaurez and Passera (2002) with *Opuntia ellisiana* Griff.

Effect of IBA and NAA on rooting percentage of micro-shoots

The mean maximum rooting (100%) was obtained in T_3 (4.0 mg l^{-1} IBA) and T_6 (4.0 mg l^{-1} NAA) treatments,

Table 3. Effect of IBA and NAA on the various rooting parameters

	Treatments	Root induction (Days)	Rooting per cent	Number of roots / micro-shoots	Length of the Root (cm)
T_1	Control	14.69	36.30 (37.05)*	1.69	0.52
T_2	NAA (0.5 mg l^{-1})	12.15	80.00 (63.43)	2.13	2.00
T_3	NAA (1.0 mg l^{-1})	11.13	100.00 (90.00)	4.33	3.33
T_4	NAA (2.0 mg l^{-1})	10.44	66.67 (54.74)	4.47	3.80
T_5	IAA (0.5 mg l^{-1})	12.42	93.33 (75.04)	2.20	2.20
T_6	IAA (1.0 mg l^{-1})	11.68	100.00 (90.00)	4.20	2.60
T_7	IAA (2.0 mg l^{-1})	10.82	93.33 (75.04)	3.73	3.20
	C D at 5%	0.31	15.21	0.38	0.31

*Figures in parenthesis are angular transformed values

respectively and the mean minimum rooting (36.30%) was obtained in control (Table 3). Rooting percentage in micro-shoots was better with 4.0 mg l^{-1} IBA and 4.0 mg l^{-1} NAA. *In vitro* studies of Parez-Molphe-Balch *et al.* (2002) also support the present findings.

Effect of IBA and NAA on number of roots per shoot

The mean maximum numbers of roots (4.47) were recorded in T_4 (6.0 mg l^{-1} IBA) treatment closely followed by T_3 (4.33) (4.0 mg l^{-1} IBA) treatment as compared to mean minimum number of roots (1.69) in control (Table 3). The maximum numbers of roots per micro-shoot were recorded with higher concentration of IBA as compared to all treatments of NAA and control. These results are in confirmation of with Escobar *et al.* (1986), who also observed variable response of IBA and/or NAA at different concentration on root formation in a range of cacti and other succulents.

Effect of IBA and NAA concentration on root length

The data presented in the Table 3 show the effect of different concentration of auxin (IBA and NAA) fortified to ½ strength MS media. The mean maximum length of roots (3.80 cm) observed in T_4 (6.0 mg l^{-1} IBA) treatment closely followed by (3.33 cm) in T_3 (4.0 mg l^{-1} IBA) treatment as compared to mean minimum root length (0.52 cm) in control. The effect of higher concentration was at par to the IBA concentration of 4.0 mg l^{-1}. The results for longer root with higher concentration of both auxins may probably be explained by the effect that higher concentration of auxins took less time in root induction so the emerged roots get more period for their growth and development as compared to the roots which emerged later in lower concentration of auxins. The present findings are supported by the results obtained by Mausath (1979) who obtained better quality roots with NAA.

Acclimatization of the plantlets

In vitro propagated plantlets were successfully acclimatized by transferring them into small pots of plastic, containing potting mixture of different medium. The rate of survival of the plantlets in the pots was 91.67% with vermiculite + cocopeat mixture of 3:1 (v/v) which was followed by another mixture i.e. vermiculite + vermicompost (83.33%). The lowest survival of plantlets (58.33%) observed with soil. The higher percentage of survival of plantlets with vermiculite along with either cocopeat or vermicompost may be due to the properties of higher porosity, cation exchange capacity (CEC) and water holding capacity. Cent per cent survival under the same medium has also been reported by (Johnson and Emino, 1979; Vyaskot and Jara, 1984; Ault and Blackmon, 1987).

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