In vitro propagation of cactus (*Cereus peruvianus l.)*

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ABSTRACT

The effect of various treatments on the behaviour of in vitro consecutive micropropagation stages of cactus was studied. The statistical analysis of data revealed that for shootlet proliferation stage; recycling the in vitro culture of shootlets for six times at monthly intervals on MS medium amended with BAP at 5 mg/l plus NAA at 0.1 mg/l concentration significantly augmented the shootlet proliferation rates to 5.12-fold higher than on MS-free of hormons medium. Moreover, the highest amounts of chlorophyll-a &-b and carotenoids were detected in shootlet tissues grown on MS-medium provid with either NAA at 0.1 mg/l plus BAP at 3, 4 or 5 mg/l or NAA at 0.2 mg/l plus BAP at 3 mg/l concentration. For rooting stage, using full, half or quarter strength of MS medium with or without 1 g/l activated charcoal (AC) gave the highest rooting rates (100%) and longest root length, while applying full MS medium without AC resulted in the greatest root number formed per shootlet. For acclimatization stage; culturing the obtained vitroplants on either peat moss alone, sand alone or a mixture of peat moss and sand at the rate of 1:1 gave the highest survival capacity (100%); but using peat moss alone allowed the stem of the acclimatized vitroplant to grow up to the highest tall. In conclusions, it is benefitable to use successfully the in vitro culture techniques for mass micropropagation Cereus peruvianus.

Key words: *In vitro, cactus, Cereus peruvianus, BAP, NAA, micropropagation stages*

INTRODUCTION

Cereus peruvianus (family *Cactaceae*) is climbing cactus of tropical origin and has widely branched crown. They are mostly treelike or shrublike column cacti and rather atractive; they grow quickly and easily and can be used for many ornamental purposes. It is well known that many cactus species are diffecult to propagate sexually or asexually by using the conventional method comparing with the new *in vitro* culture techniques (Bustamante and Heras, 1990).

It is evident that *in vitro* micropropagation techniques have several advantages, one of them is that plant multiplication can continue in a successive short cycle throughout the year irrespective of the season and as a result a logarithmic increase in the number of plants could be obtained as found for *Nephrolepis exaltata* (Gonzalez and Serpa, 1992) and *Melaluca armillaris* (Youssef, 1996).

It was affirmed that the consecutive *in vitro* micropropagation stages of many plant species are largly controlled by numerous factors. For shootlet proliferation stage; during recycling the culture of explants the shootlet proliferation rates and pigments content of the obtained shootlets tissues were obviously

affected by cytokinins or/and auxins in the culture medium as reported by Youssef (1994) for *Acacia salicina*; Mohamed-Yasseen *et al*. (1995) for *Stapela semota*; Feng-Feng *et al*. (2000) for *Aloe barbebsis*; and Mata-Rosas *et al*. (2001) for *Turbinicapus laui*. For rooting stage, the concentration of macro-and micro elements of culture medium with or without activated charcoal remarkebly affected the rooting rate, root number and root length of the rooted shootlets as revealed by Agrawal *et al*. (1992) on *Vanilla walkeriae*, Raghuveer-Polisetty *et al*. (1996) on *Cicer arietinum*, Le *et al*. (1998) on *Arnica montana*, Sakr *et al*. (1999) on *Yucca elelphantipes*. For acclimatization stage; the type of culture substrate had a clear influence on growth behaviour of stem explants as found for *Populus nigra* (Youssef- Aboueash, 1997).

Therefore, the present work was achieved to study the behaviour of the consecutive *in vitro* micropropagation stages of *Cereus peruvianus*, i.e. shootlet proliferation stage during recycling the culture of explants as affected by different cytokinins and auxins treatments, rooting and acclimatization stage as influenced by various strengths of MS-medium; and different types of culture substrate, respectively.

MATERIALS AND METHODS

The expermintes of this study were carried out at Tissue Culture and Germplasm Conservation Research Laboratory, Horticulture Research Institute, Agricultural Research Center, in order to investigate the behaviour of the consecutive *in vitro* micropropagation stages of *Cereus peruvianus* under the effect of different concentration of cytokinins (BAP) and auxins (NAA); various strengthes of macro-and micro-elements of MS medium; and different types of culture substrates for shootlet proliferation, rooting; and acclimatization stages, respectively. The expermintes were repeated two times during the years of 2003 and 2004.

Seedlings (three-month-old) with a height of 5-7 cm originated from the Cactus International Farm (Tahanoub-Shebien El-Kanater-Qulubia) were used as a source of plant materials.

The seedlings were surfaces sterilized with 70% (v/v) ethanol for one min and then they were treated with 15% (v/v) clorox with a few drops of tween-20 for ten min, followed by rinsing three times with a steril distilled water. After that they were immeresed in 0.1% mercuric chloride with a few drops of tween-20 for ten min. Finally they were rinsed three times with sterile distilled water.

The culture medium under trials consisted of the macro- and micro-elements and vitamins of MS-medium (Murashige and Skoog, 1962) enriched with 25 g/l sucrose and 0.7% (w/v) Anachemia agar.

In shootlet proliferation treatments, the main shoots of the previous desinfected seedlings were firstly sectioned transversally into shoot tip explants and the remained basal shoots were divided longitudinally into shoot segment explants; each type of explants consisting an axillary bud. The explants were aseptically placed on full strength MS-medium supplemented with BAP at 0, 3, 4 or 5 mg/l alon or with NAA at 0.1 or 0.2 mg/l concentration.

One month after culturing, the clusters of the formed shootlets were recultured on fresh medium of the same components. These procedures were repeated many times in which six shootlets proliferation cycles have been achieved at monthly intervals.

Therefore, in six consecutive shootlets proliferation cycles, nine components of culture media were done and twenty shootlet explants in five replicates were used.

The shootlets obtained from the sixth cycle of culture were submitted to chemical analysis for determing the endogenous chlorophyll-a and-b and carotenoids according to the procedure described by Saric *et al.* (1967).

In rooting treatments, shootlet explants (2-4 cm) resulting from the sixth shootlet proliferation cycle, were excised and planted on MS- free-hormone-medium at full, half or quarter strength of macro and micro-elements with or without 1g/l activated charcoal.

Therefore, six culture media were tested and twenty-five shootlets in five replicates were used.

At the end of the fourth week of culturing, the produced rooted shootlets (vitroplants) were submitted to the acclimatization trial.

In each of shootlet proliferation and rooting treatments, all the used culture media were adjusted to pH 5.7±0.1 with KOH/H_2PO_4 and autoclaved at 121°C and $1.2 kg/cm^2$ for 20 minutes before using. The shootlet explants were placed vertically in 200 ml capacity glass jars containing 25 ml medium. The cultures were incubated at 24±1°C under fluorescent lamps with light intensity of 3000 lux at culture level and 16-hours photoperiod.

In the acclimatization trial, the rooted shootlets (vitroplants) resulting from rooting treatments were transferred to plastic pots (0.2 liter) containing peat moss, washed sand, or peat moss plus sand at 1:1 (v/v). Each substrate used was adjusted to pH 6.2, irrigated with a solution of 0.2% Topsin-M70 fungicide and covered by trasprent polyethylene bags. The acclimatized vitroplants were kept in acclimatized glass-house for four weeks before transplanting out-of-doors.

Therefore, three types of culture substrates were examined and five vitroplants in three replicates were used.

Data recorded

During the six successive cycles of shootlet proliferation the cumulative number of the formed shootlets per explant were counted at monthly intervals. But endogenous contents of chlorophyll-a and -b and carotenoids were determined in (mg/100gfw) at the end of the sixth cycle. At the end of rooting treatment, the rooting response (%); number of roots formed per shootlet and length of roots in cm were recorded. For acclimatization trial, the survival percentage of the acclimatizated vitroplants and the length of vitroplant stem in cm were measured after four weeks and four months respectivly from acclimatization procedure.

The analysis of data for each experiment was done for a completly randomized design. Data of the two series of experiments were combined and statistically analyzed for testing differences between means using L.S.D according to Steel and Torrie, (1980).

RESULTS AND DISCUSSION

Shootlet proliferation stage

Shootlet proliferation rate

Data in Table (1) revealed that different concentrations of the cytokinin BAP and the auxin NAA tested in this trial had a significant effect on shootlets proliferation rate of explants during the successive proliferation cycles.

Recycling the culture of shootlet explants for six times at monthly intervals greatly increased the cumulative number of the formed shootlets. The highest number of shootlets (47.3) was exhibited for explants cultured on MS-medium provided with BAP (5 mg/l) plus NAA (0.1 mg/l), and this value represents 6.22-fold higher than that found for explants cultured on control medium (free-hormons). These results showed that the most adequate culture medium for obtaining the

greatest number of shootlets per explant after six proliferation cycles was MS-medium supplemeted with of BAP at 5 mg/l plus NAA at 0.1 mg/l. This means that using the cytokinin BAP at high concentration (5 mg/l) in combination with NAA at low concentation (0.1 mg/l) in this study showed the highest synergetic effects on inreasing the metabolic processes leading to shootlet formation in the greatest number. These findings are in agreement with those reported by Bustamante and Heras (1990) on Cacti (*Pelecyphora aselliformis*) and *Nealolydia lophophoroides*; Feng-Feng *et al*. (2000) on *Aloe barbebsis* and Mata-Rosas (2001) on *Turbinicapus laui*. They concluded that applying a combination of BAP and NAA in different concentrations was a limiting factor for shoot formation.

Table (1): Effect of different concentrations of the cytokinin BAP alone or in combinations with the auxin NAA on the number of shootlets proliferated from each Cereus peruvianus explant in vitro during six successive proliferation cycles.

Treatment and concentration (mg/l)	**Proliferation cycles**					
	1st cycle	**2ndcycle**	**3rd cycle**	**4thcycle**	**5thcycle**	**6thcycle**
Control	1.05	2.15	3.30	4.50	5.95	7.60
BAP (3)	1.15	5.65	10.85	18.20	29.60	43.85
BAP (4)	1.15	5.55	10.70	17.35	30.35	45.60
BAP (5)	1.50	5.35	10.30	16.90	28.15	43.45
BAP (3)+NAA (0.1)	1.35	4.15	7.70	13.70	26.60	43.05
BAP (4)+NAA (0.1)	1.50	5.60	9.70	16.50	29.80	42.7
BAP (5)+NAA (0.1)	2.10	6.00	11.90	19.35	32.95	47.30
BAP (3)+NAA (0.2)	1.65	5.75	10.90	18.75	31.45	45.40
BAP (4)+NAA (0.2)	1.60	5.75	11.50	18.65	41.95	41.95
BAP (5)+NAA (0.2)	2.15	6.05	11.30	19.95	43.65	43.65
L.S.D at 5%	**0.356**	**1.312**	**1.998**	**2.281**	**4.375**	**4.856**

Pigments contents

The various treatments of the cytokinin BAP and the auxin NAA caused a significant effect on endogenous pigments such as chlorophyll-a &-b and carotenoids in the shootlet tissues at the end of the sixth proliferation cycle (Table 2).

In case of chlorophyll-a; using MS-medium amended with BAP (3 mg/l) plus NAA (0.1 mg/l); BAP (5 mg/l) plus NAA (0.1 mg/l) and BAP (3 mg/l) plus NAA (0.2 mg/l) produced shootlet tissues having the highest amounts of chlorophyll-a (63.51, 64.48 and 69.93 mg/100g fw, respectively), while the lowest amounts (29.36, 30.96, 30.14 and 34.83 mg/100g fw) were determined in shootlet tissues growing on MS-medium of control without hormons, provided with BAP at 3 and 4 mg/l alone or BAP at 4 mg/l plus NAA at 0.2 mg/l, respectively. The other tested BAP and NAA treatments induced chlorophyll-a in amounts ranged from 44.53 to 56.43 mg/100g fw with no significant differences between these values.

Concerning the chlorophyll-b; the shootlets resulting from MS-medium supplemented with BAP at 4 mg/l puls NAA at 0.1 mg/l contained the greatest levels of chlorophyll-b (46.60 mg/100g fw), whereas the minute amount (21.77 mg/100g fw) was detected in shootlet tissues growing on MS-medium provided with BAP at 4 mg/l plus NAA at 0.2 mg/l. The other tested BAP and NAA treatments induced chlorophyll-b in amounts ranged from 26.35 to 45.01 mg/100g

fw with no significant differences between these values.

As for carotenoids, the highest value (46.03 mg/100g fw) was detected in shootlet tissues resulting from MS-medium enriched with BAP at 3 mg/l plus NAA at 0.2 mg/l, which represented about two times higher them that recorded for control MS-medium.

A wide survey of the obtained data clearly showed that MS-medium provided with BAP at 3 mg/l plus NAA at 0.2 mg/l can increase the biosynthesis activities of chlorophyll-a and carotenoids in higher amounts. While the highest chlorophyll-b content could be resulted in case of using MS-medium enriched with BAP at 4 mg/l plus NAA at 0.1 mg/l. These results coincided with the earlier data found by Tung Ming Sung (1997) on *Cucumis sativus* and Gao *et al.* (2000) on *Carthamus tinctorius.* They revealed that different concentrations of cytokinins and auxins had a significant effect on the chlorophylls and caroteniods formation capacity.

Table (2): Effect of different concentrations of the cytikinin BAP alone or in combinations with the auxin NAA on pigment contents (mg/100g fw) of Cereus peruvianus shootlet tissues in vitro.

Treatment and concentration (mg/l)	Chlorophyll(A) (mg/100g)	Chlorophyll(B) (mg/100g)	Carotenoids (mg/100g)
Control	29.36	28.18	23.18
BAP (3)	30.96	36.86	27.97
BAP (4)	30.14	26.35	22.43
BAP (5)	55.60	31.08	34.17
BAP (3)+NAA (0.1)	63.51	45.01	38.32
BAP (4)+NAA (0.1)	56.43	46.60	37.04
BAP (5)+NAA (0.1)	64.48	40.26	43.11
BAP (3)+NAA (0.2)	69.93	35.16	46.03
BAP (4)+NAA (0.2)	34.83	21.77	25.99
BAP (5)+NAA (0.2)	44.53	43.12	34.77
L.S.D at 5%	**18.710**	**20.000**	**10.760**

Rooting stage

Rooting response

Data presented in Table (3) revealed that all the different concentrations (full, half and quarter strength) of macro- and micro-elements of MS-medium with or without addition 1 g/l activated charcoal induced the shootlets explants to form roots in an equal rate (100%). This means that *Cereus peruvianus* shootlets had a capacity to produce roots spontanously irrespective of the strength of MS-medium. This led to a conclusion that it is possible to minimize the elements concentrations of MS-medium to quarter strength for obtaining successfully the highest rooting response at the rate of 100%. In this regard, Raghuveer-Polisetty *et al.* (1996) found that maximum rooting of *Cicer arietnum* was observed when cultured in quarter strength MS-medium. Also George and Ravishankar (1997) pointed out that the best rooting of *Vanilla planifolia* shootlets was observed in half-strength MS-medium containing activated charcoal.

Number of roots

The number of roots formed per shootlet explant was significantly affected by the different strengths of MS-medium used in this investigation (Table 3). The greatest number

of roots/explant (2.73) was exhibited for shootlet explants cultured on full strength MS-medium, while the minute one (1.07 roots/explant) was recorded for shootlet explants grew on quarter strength MS-medium provided with 1 g/l activated charcoal. These resultes affirmed that using full concentration of macro- and micro- elements of MS-medium alone allowed shootlet explants to form roots at greatest number. In this respect Sakr (1999) on *Yucca elephantipes,* reported that more roots developed per explant was related to the strength of MS-medium**.**

Acclimatization stage

Survival capacity

Data in Table (4) revealed that the survival capacity of vitoplants was not affected by the different culture substrates examined in the current experiment. All the vitroplants showed a higher survival capacity (100%) when they cultured on peat mosse, sand or a combination between them at the proportion of 1:1 (v/v). These data led to recommendation that the cheaper culture substrate such sand can be used for producing 100% of survival vitroplants during the acclimatization procedure.

Length of roots

The data in Table (3) indicated that all the tested MS-medium strengthes in this investigation allowed the root to grow up in the length ranged between 2.06 to 2.89 cm with no significant differences between these values. Thus, the root length formed was not affected by the culture medium strength, which was tested. Oppositly, Sakr *et al*. (1999) noted that when shoots of *Yucca elephantipes* were transferred to MS rooting medium, longer roots were developed on full-strength than on half-strength MS-medium.

Length of stem

As shown in Table (4) the stem length of acclimatized vitroplants was significantly influenced by different types of culture substrates under investigation. Using peat moss alone and in combination with sand at 1:1 (v:v) allowed the stem of the acclimatized vitroplants to grow up in highest values (10.6 and 9.0 cm, respectively). On the contrary, using sand alone produced the shortest stem length (6.1 cm). This may be attributed to the high nutrition value of peat moss which can permit the stem of vitroplant to grow up in higher length comparing to the poor nutrition value of sand. Accordingly, Youssef-Aboueash (1977) on *Populus nigra* cuttings found that using nile silt alone or in combination with sand at the rate of 1:1 resulted in the greatest shoot length, while using sand alone gave the smallest shoot length.

Table (3): Effect of macro- and micro- elements strength of MS- medium with or ithout activated charcoal (AC) on rooting behaviour of Cereus peruvianus shootlet explants in vitro.

Strength of MS-medium	Rooting response (%)	Number of roots/shootlet	Length of root (cm)
Full MS alone	100	2.73	2.06
Full MS plus 1g/l AC	100	2.67	2.89
Half MS alone	100	2.00	2.68
Half MS plus 1g/l AC	100	1.67	3.11
Quarter MS alone	100	1.80	2.87
Quarter MS plus 1g/l AC	100	1.07	2.43
L.S.D at 5%	**N.S**	**0.906**	**N.S**

Table (4): Effect of different culture substrates on acclimatization behaviour of *Cereus peruvianus* vitroplants.

Culture substrates	Survival capacity	Stem length (cm)
Peat moss	100	10.6
Sand	100	6.1
Peat moss : Sand (1:1)	100	9.0
L.S.D at 5%	**NS**	**2.686**

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