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IN VITRO SYNTHETIC SEEDS OF *AECHMEA FASCIATA* FOR GERMPLASM CONSERVATION AND EXCHANGE.

BY

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ABSTRACT

The present paper demonstrates the potential of nutrient alginate encapsulation buds of *Aechmea fasciata* for synthetic seeds technology, which could be useful in germplasm conservation and exchange. Of the cytokinins tested, BA was more effective than either KN, 2iP or TDZ and produced maximum proliferation when used at 3.0mg/l. The frequency of proliferation shoot was lower on KN, 2iP or TDZ. Encapsulated buds exhibited shoot development on MS supplemented with some growth retardants (PBZ, ABA and CCC). The best morphogenetic response of the plantlets was when the encapsulated buds were cultured on MS medium free cytokinin and also on the medium containing 2.0mg /l BA or 1.0 mg/l KN after eight weeks of culture. *In vitro* germination of encapsulated buds ranged from 13 to 100% on different growth regulators media after eight weeks from culture. Sucrose added to the gel matrix also influenced the capsules germination. Buds were encapsulated with 3% sodium alginate where 96.67% of the encapsulated buds converted to plantlets with well elongated buds in perlite containing sucrose as a carbon source. Although the germination % of encapsulated buds was suppressed on perlite that did not contain sucrose they produced normal plants. Rooting was induced on *in vitro* proliferated shoots by culturing in MS salts supplemented with 2.0mg/l IBA as gave the highest number of roots than the other treatments. On MS media supplemented with NAA or IBA adventious roots were short. The regenerates were successfully transferred to pots in a greenhouse.

Key words: *In vitro – Synthetic seeds – Aechmea fasciata* - ornamental bromeliads - *Sodium alginate* .

INTRODUCTION

In recent years, nutrient-alginate encapsulation technique for producing synthetic seeds had become an important asset to micropropagation. In crop plants that are vegetatively propagated and have long juvenile periods e.g citrus, grapes, mango, pomegranate, ect., the planting efficiency could be considerably improved by the use of synthetic seeds instead of cuttings. In addition, synthetic seed technology could be useful in conservation of clonal germplasm of elite and

endangered plants in near future, with development of appropriate storage techniques. Another important application of synthetic seed technology could be the exchange of axenic material between laboratories (Naik and Chand, 2006). Synthetic or artificial seeds have been defined as somatic embryos engineered for use in the commercial propagation of plants (Gray and Purohit, 1991; Redenbaugh, 1993). Various forms of synthetic seeds have been envisioned over time. The first were simply hydrated somatic embryos produced from vegetative cells in plant tissue culture. These had the particular advantage of enabling rapid clonal multiplication of some plants, but the labour and therefore cost was high and the propagules were very delicate. This was partially overcome with the development of alginate capsules that encapsulated a single embryo in a protective coating enabling mechanised handling. These hydrated encapsulated embryos could only be stored using low temperatures for a few weeks (Redenbaugh *et al.*, 1986; Fujii *et al.*, 1989 and 1992). The capability of prolonged storage was achieved when the somatic embryos could be dried to moisture contents less than 20% (McKersie *et al.*, 1989). Therefore, synseed production is the unique technique used to produce commercially some *in vitro* plants with lower cost, preserve for long period, and supply materials for transgenic plants (Bornman, 1993). Artificial seed production is now an outstanding technique for plant propagation and preservation, and has been applied on many plants (Nhut *et al.*, 2004). Furthermore, synseeds can be preserved for three months at 4°C (Datta *et al.*, 1999) or for one year in sucrose-free liquid medium in growth chamber condition (Nhut *et al.*, 2005).

The family Bromeliaceae is composed of tropical American genera, many of which are herbaceous epiphytes. Some of them are important ornamentals such as several Aechmea species (Jones and Murashige, 1974). Gangopadhyay *et al.*, (2005) found that micro shoots developed from the meristematic basal portion of the innermost leaves of pineapple *Ananus comosus* (L.) Merr were encapsulated in alginate beads for short-term storage as a prelude to the transformation programmed. Among the four temperature regimes for storage, beads stored at 8°C showed maximum percentage of shoot proliferation when placed again in MS medium; the rate of 'conversion' was satisfactory even beyond 45 days of incubation.

Several possibilities exist for the multiplication of bromeliads *in vivo* as well as *in vitro*. *In vivo* propagation of Aechmea through seed has been a standard practice. The plants obtained are variable and often of poor quality, poor germination is often another problem which is associated with propagation through seeds. Vegetative multiplication through suckers is too slow to be practical. Therefore, *in vitro* propagation of *Aechmea* has become more and more widely used. To start the *in vitro* multiplication of *Aechmea*, plantlets can be obtained from *in vitro* germination or from explanted shoot-tips or axillary buds. These plantlets can be mutiplied vegetatively by induction of lateral shoot growth, on a modified Murashige and Skoog (1962) nutrient medium containing 2 mg/l BA +2 mg/l IAA and BA as was studied in relation to lateral shoot formation and ethylene production (Van Duck *et al.*, 1988). Shoots of *Aechmea fasciata* differentiated from leaf explant callus were cultured on MS-based solid media

(Vinterhalter and Vinterhalter, 1994). Synthetic seeds of pineapple stored at 4°C remained viable without sprouting for up to 45 days. Plantlets produced *in vitro* from synthetic seeds were successfully established in soil. The protocol provides an easy and novel propagation system for pineapple, an otherwise vegetatively propagated fruit crop (Soneji *et al.*, 2002). In addition to shoot formation of *Aechmea* 1 mg/l NAA and 0.1 mg/l induced callus on approx. 30% of *Tillandsia cyanea* and 8% of *Aechmea* 'Little Harv' explants. Isolated shoots formed roots after subculture in growth regulator-free media (Cueva *et al.*, 2006). Therefore, the present study aimed to find means by which formation of plantlets of *Aechmea fasciata in vitro*, encapsulation of buds to be used for storage and germplasm exchange. We also report the effect of high concentrations of sucrose in the alginate matrix and storage duration on development of encapsulated micropropagules. This study also aimed to introduce this valuble ornamental plant with nice flowers to Egyptian Agriculture, where it is the first time in Egypt to succeed in micropropagation through synthetic seeds.

MATERIALS AND METHODS

This work was conducted in the Tissue Culture Laboratory, Genetic Engineering and Biotechnology Research Institute (GEBRI), Menofyia University, Sadat City, during the years of 2006 and 2007.

Explants source and surface sterilization

Healthy, mature plants were obtained from Department of Ornamental Horticulture, Faculty of Agriculture, Cairo University Giza, Egypt. Pieces of leaves were rinsed in 70% ethanol and submersed for 20 min in 10% solution of commercial bleach 4% (sodium hypochlorite) to which one drop of liquid detergent per 100 ml was added. Bleach was decanted and material thoroughly rinsed with autoclaved water (Vinterhalter and Vinterhalter 1994). The medium used in our study contained (MS) Murashige and Skoog (1962) inorganic salts, 3% sucrose, 0.6% agar, 100mg/l inositol, 0.2mg/l glycine, 0.5mg/l B6, 0.5mg/l nicotinic acid and 0.4mg/l B1.

Callus formation and proliferation

Growth regulators supplemented to the media were 0.25mg/l 2, 4-D (2, 4 dichlorphenoxyacetic acid) for callus induction, 2.0mg/l BA for shoot proliferation (Vinterhalter and Vinterhalter 1994). Primary explants consisting of 10mm X 10mm young leaf segments were grown in 150 ml jars supplemented with 50 ml of medium.

Effect of some growth regulators on shoot proliferation

During subculturing shoot explant was transferred to MS medium supplemented with, 3% sucrose, 0.6% agar, and 0.0, 1.0, 2.0, and 3.0mg/l 6-bensyladenine (BA), kinetin (KN), isopentenyl adenine (2iP) and thidiazuron (N-phenyl N 1, 2, 4 dichlorophenoxyacetic acid) (TDZ) for shoot proliferation. Shoots were cultured in 150 ml jars supplemented with 50ml of medium. Multiplication index was calculated on bases of the production of adventurous shoots. The frequency of plant formation in the shoot multiplication stage was

calculated as shoots and leaves number and shoot length. Fifty replicate jars with one explant per jar were used for each treatment. Treatments were calculated for six weeks from culturing. All cultures were maintained at 26±°C and 16-h photoperiod. The same type of shoot explants were used for rooting which was performed in 20mmX100mm tubes supplemented with 10 ml of MS medium with indolyl-3-butyric acid (IBA) or α-naphthaleneacetic acid (NAA)at 0.0, 0.5, 1.0, 1.5 or 2.0 mg/l. All cultures were maintained at 26±°C in a 16 h photoperiod with a total irradiance of 2000 lux at culture level. Fifty replicate tubes with one shoot per tube were used for each treatment. Rooted shoots (5-6 cm) were transplanted into pot containing a mixture of peat and perlite (2:1 by volume) and were grown in a greenhouse under natural conditions.

Encapsulation and preparation of beads

Small Aechmea buds were obtained from *in vitro* shoot cultures. Individual buds were excised carefully and trimmed to a small size (approximately 5mm) using a sharp dissecting needle. These micro buds were dipped for few second in 3% sodium alginate (sigma) gel (Badr Elden, 2005). The 3% gel was prepared in adistiled water and sterilized at 121 C° and 1.2 kg /cm^2 air pressure for 20 minutes. The buds were picked up with sterilized forcepes, dropped into a solution of Ca $(NO_3)_2.4H_2O$ solution for 30 min on a rotary shaker. The encapsulated buds (beads) were removed by decanting the Ca $(NO_3)_2.4H_2O$. Beads were washed 2-3 times with sterilized H_2O. Encapsulated buds were cultured on media consisting of MS basal nutrient medium supplemented with paclobutrazol (PBZ), abscisic acid (ABA) or cycocyl (CCC) at concentrations of 0.0, 0.5, 1.0 or 1.5 mg/l. The medium of each treatment was also supplemented with 30g/l sucrose and 6 g/l agar. Fifty replicate jars with nine capsules per jar were used for each treatment. The jars were incubated at 20°C under complete dark. Germination and post-germination growth % of encapsulated buds were recorded after 2, 4, 6 and 8 weeks of culturing. After encapsulation, beads were cultured on different media supplemented with BA, KN and TDZ added to storage media. Each growth regulator was added at the concentrations of 0.0, 0.5, 1.0, 1.5 or 2.0 mg/l to storage medium which consisted of MS basal nutrient medium supplemented with 30g/l sucrose and 6g/l agar.

Effect of sucrose addition to the encapsulation matrix

Bud explants of *Aechmea* were suspended in MS medium plus 3% sodium alginate supplemented with different sucrose concentrations of 0.0, 15.0, 30.0, 45.0 and 60.0 g/l as described before. Fifty replicate jars with nine capsules per jar were used for each treatment. Germination and post-germination growth % of encapsulated buds were recorded after 2, 4, 6 and 8 weeks of culturing.

Effect of sucrose addition to perlite

Encapsulated buds were cultured on sterilized perlite containing ½MS liquid medium plus 2% sucrose or without sucrose in a 150 ml culture jars. Fifteen replicate jars with nine capsules per jar were used for each treatment. Germination was recorded after 2, 4, 6 and 8 weeks of culturing. The randomized factorial design was used and data were subjected to analysis of variance. Separation of means among treatments was determined using LSD test at 5% (Steel and Torri, 1980).

RESULTS AND DISCUSSION

Effect of some growth regulators on shoot proliferation

In Table (1) data on the main effect of BA on shoot proliferation show that increasing the concentrations of BA had significant effects on enhancement of number of shoots. The highest concentration of BA (3.0mg/l) significantly presented the highest value of shoot number. The original data showed that the addition of KN, 2iP and TDZ to the medium showed lower effect on shoot proliferation compared to the high effects of the other treatments (Fig. 1).

Concerning number of the produced leaves, data on the main effect of BA and 2iP at the same concentration of 1.0 mg/l significantly increased number of leaves/culture (21.88 and 17.63, respectively) compared to the other concentrations. However, lower responses were significantly obtained with TDZ at all concentrations. Surpassed shoot length on MS medium free cytokinins, 3.0mg/l KN, 1.0 and 2.0mg/l 2iP than other treatments. Shoots of *Aechmea fasciata* differentiated from leaf explant callus were cultured on MS-based solid media. The frequency of aberrant plant formation was lower on media supplemented with kinetin + IAA than with BA + NAA (Vinterhalter and Vinterhalter 1994). Our results are in accordance with Roy *et al.*, (1996), they concluded that among the cytokinins tested, benzylaminopurine (BAP) was more effective than either 2-isopentenyl adenine (2iP) or kinetin (KN) for jackfruit. Hong *et al.* (2002) on *Neoregelia carolinae*; Mogollon *et al.* (2004) on *Ananas comusus*. Guo-Min *et al.*(2005)*on Aechmea fasciata* and Pickens *et al.* (2006)*on Tillandsia eizii* reached similar results where they found that, the highest number of shoots /culture were achieved on MS medium supplemented with BA and NAA.

Table (1): Effect of cytokinins concentrations on the shoot proliferation of *Aechmea fasciata in vitro.*

Cytokinins con. (mg/l) (A)		Shoot proliferation		
		No. of Shoots	No. of leaves	Shoot length(cm)
Control (0.00)		1.38	8.25	5.31
BA	1.0	6.00	21.88	2.31
	2.0	5.00	11.00	2.44
	3.0	7.63	14.50	2.75
Kin	1.0	1.50	12.25	4.18
	2.0	2.13	12.63	3.69
	3.0	2.25	11.88	5.81
2iP	1.0	4.50	17.63	4.31
	2.0	2.63	12.38	4.44
	3.0	3.38	12.13	3.56
TDZ	1.0	1.63	9.13	3.88
	2.0	1.25	7.13	3.19
	3.0	1.13	4.63	3.19
LSD at 5% level		1.15	8.46	0.62

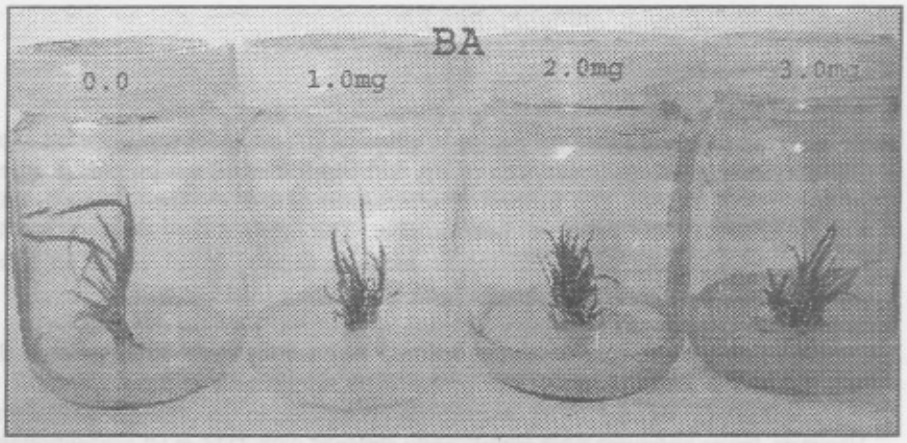

Fig. (1) : Shoot proliferation of *Aechamea fasciata in vitro* due to different BA concentrations

Effect of some growth retardants concentrations (mg/l) and different storage periods on development and germination % of encapsulated buds of *Aechmea fasciata in vitro.*

Adding different concentrations of growth retardants to storage medium, played an important role in development and germination (%) of encapsulated buds. The mean values revealed that growth retardants free medium (control) gave the highest germinated encapsulated buds and germination % as shown in Tables (2 and 3) followed by adding 0.5mg/lABA. Regarding the effect of conversion duration, the mean values clearly revealed that germination duration was significantly affected by the addition of different growth retardants concentrations to storage medium as the germination of capsules and germination (%) were increased gradually from 0.00 to 1.20, 16.67 to 46.66 % respectively when conversion frequency duration extended from 2, 4, 6 to 8 weeks. The interaction between adding different concentrations of growth retardants and storage duration, showed that MS medium free growth retardants and adding ABA at 0.5mg/l to storage medium after 8 weeks, gave the highest number of germinated encapsulated buds and germination % than the other treatments.However, the storage medium supplemented with CCC at all concentrations showed that all encapsulated buds were still without conversion to shoots after 2, 4 and 6 weeks from the culturing. Percentages of germination and conversion into plantlets were decreased gradually with increasing growth retardants concentrations in storage medium. In this respect Ara *et al.*, (1999) showed that somatic embryo of mango treated with ABA at different concentrations, and with increasing ABA concentration from 0.04 to 0.20µM, percentage of germination and conversion into plantlets were decreased gradually. Lisek and Orlikowska (2004) reported that the composition of the beads also contributed to survival, but had no effect on multiplication after storage. Growth of donor cultures on a mannitol or paclobutrazol supplemented medium did not influence multiplication of *Senga sengana* shoots after storage, but suppressed multiplication of raspberry stored for 3 months.

Table (2): Effect of some growth retardants and storage duration on the viability of encapsulated buds of *Aechmea fasciata in vitro.*

Growth retardants conc. (mg/l)		Number of germinated encapsulated buds (week)				
		2	4	6	8	Mean(A)
Control		0. 00	7.20	7.80	9.00	**6.00**
PBZ	0.5	0. 00	1.80	2.40	3.00	**1.80**
	1.0	0. 00	0.60	1.20	1.20	**0.75**
	1.5	0. 00	0. 00	0.60	0.60	**0.30**
ABA	0.5	0. 00	1.20	1.80	7.80	**2.80**
	1.0	0. 00	0.60	0.60	6.00	**1.80**
	1.5	0. 00	0.60	0.60	4.20	**1.35**
CCC	0.5	0. 00	0.00	0.00	3.60	**0.90**
	1.0	0. 00	0.00	0.00	3.60	**0.90**
	1.5	0. 00	0.00	0.00	3.00	**0.75**
Mean (B)		**0.00**	**1.20**	**1.50**	**4.20**	
LSD at 5% level		A=0.789 B= 0.499 AXB= 1.578				

Table (3): Effect of some growth retardants and storage duration on germination % of encapsulated buds of *Aechmea fasciata in vitro*

Growth retardants conc. (mg/l)		Germination of encapsulated buds (%)				
		2	4	6	8	Mean(A)
Control		0. 00	78.80	86.67	100.00	**66.37**
PBZ	0.5	0. 00	20.00	26.66	33.33	**20.00**
	1.0	0. 00	6.66	13.33	13.33	**8.33**
	1.5	0. 00	0. 00	6.66	6.66	**3.33**
ABA	0.5	0. 00	13.33	20.00	86.67	**30.00**
	1.0	0. 00	6.66	6.66	66.66	**20.00**
	1.5	0. 00	6.66	6.66	46.66	**15.00**
CCC	0.5	0. 00	0.00	0.00	40.00	**10.00**
	1.0	0. 00	0.00	0.00	40.00	**10.00**
	1.5	0. 00	0.00	0.00	33.33	**8.33**
Mean (B)		**0.00**	**13.21**	**16.67**	**46.66**	
LSD at 5% level		A=8.79 B= 5.56 AXB= 17.57				

Effect of some growth regulators concentrations and conserving duration on germination number and germination% of encapsulated buds of *Aechmea fasciata in vitro.*

Encapsulated buds inoculated onto MS medium free cytokinin (control), 1.0 mg/l BA, 1.0 and 1.5 mg/l KN exhibited shoot development and germination % from encapsulated buds as shown in Tables (4 and 5). The development of germinated buds

and germination % were increased gradually from 2 to 8 weeks. Interaction between adding different concentrations of growth regulators to conversion medium showed that adding BA at 1.0 mg/l, 1.0mg/l KN and MS free cytokinins to conversion medium after 8 weeks, gave the highest number of germinated buds and germination % than the other treatments. Howevers, the conversion medium supplemented with BA, KN and TDZ at all concentrations showed that all encapsulated buds were still without conversion to shoots after 2 weeks from culturing. The importance of the production of synthetic seeds or encapsulated buds is a novel delivery system for the multiplication and long storage of *Aechmea fasciata*.In this concern, Jayasree *et al.*, (1997) stored synthetic seeds of *Rosa hybrid* in sterile Petri dishes for 40 days in the dark at 4 °C before germination on different MS media. Highest germination (25%) was recorded on full strength MS medium supplemented with 1.0 BAP [benzyladenine] and 0.1 NAA/l. Ganapathi *et al.*, (2001) recorded that encapsulated SEs of banana cv. Rasthali (AAB germonic group) cultured on MS + 4.00 mM BA exhibited multiple shoot development (five to seven shoots per embryo) whereas only single shoot emergence was noted in the case of synthetic seeds cultured on medium with 2.89 µM GA_3 or 5.37 µM NAA. Saiprasad and Polisetty (2003) showed that the best encapsulation buds response was observed with 3% sodium alginate upon complexation with 75 mM CaCl2.2H2O. An encapsulation matrix prepared with MS medium (three-quarter strength) supplemented with 0.44 mM BA and 0.54 mM alpha -naphthaleneacetic acid gave 100% conversion of encapsulated buds of *Dendrobium* to plants after storage.

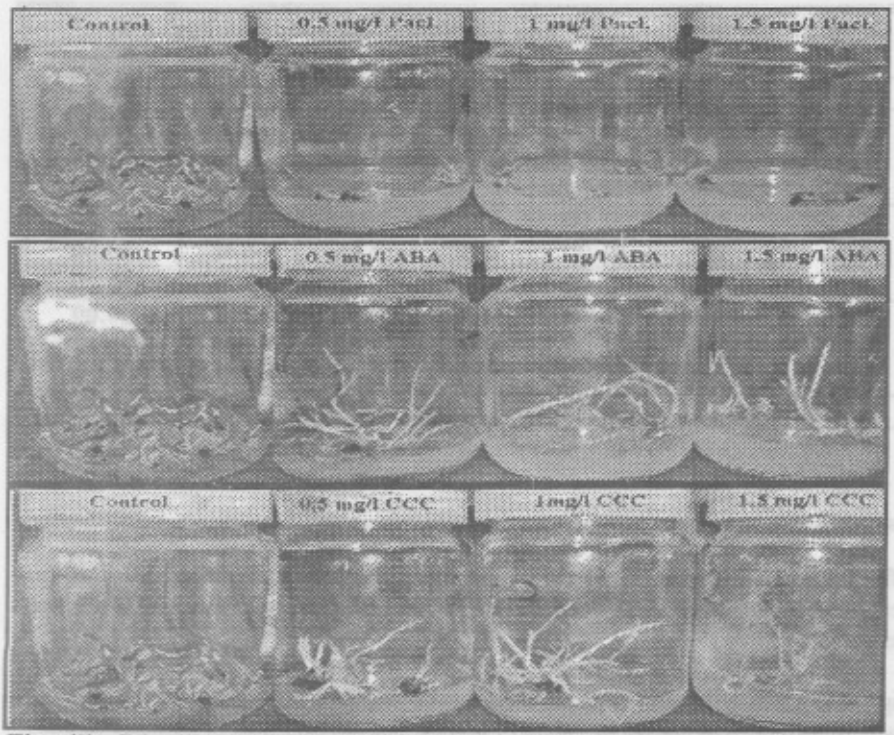

Fig. (2): Development of synthetic seeds of *Aechmea fasciata* after eight weeks of storage on growth retardants *in vitro*.

Table (4): Effect of growth regulators concentrations (mg/l) and conserving duration on germination of encapsulated buds of *Aechmea fasciata in vitro.*

Growth regulators con. (mg/l)(A)		Number of germinated encapsulated buds(week)				
		2	4	6	8	Mean(A)
Control		0.00	6.60	7.20	9.00	**5.70**
BA	0.5	0.00	1.20	3.60	8.40	**3.30**
	1.0	0.00	5.40	6.60	9.00	**5.25**
	1.5	0.00	1.80	4.20	7.80	**3.45**
	2.0	0.00	3.00	4.80	7.96	**3.915**
KN	0.5	0.00	4.8	6.00	9.00	**4.95**
	1.0	0.00	6.00	6.60	8.40	**5.25**
	1.5	0.00	6.00	7.20	8.40	**5.40**
	2.0	0.00	3.60	7.20	7.80	**4.65**
TDZ	0.5	0.00	4.20	4.80	7.20	**4.02**
	1.0	0.00	4.80	5.40	7.20	**4.35**
	1.5	0.00	3.60	4.80	6.00	**3.60**
	2.0	0.00	4.20	6.00	6.00	**4.05**
Mean (B)		**0.00**	**4.25**	**5.72**	**7.85**	
LSD at 5% level		A=0.749 B= 0.425 AXB= 1.497				

Table (5): Effect of growth regulators concentrations (mg/l) and conserving duration on germination% of encapsulated buds of *Aechmea fasciata in vitro.*

Cytokinin con. (mg/l)(A)		Germination of encapsulated buds(week) (%)				
		2	4	6	8	Mean(A)
Control		0.00	73.33	80.00	100.00	**63.33**
BA	0.5	0.00	13.33	40.00	93.26	**36.65**
	1.0	0.00	60.00	73.33	100.00	**58.33**
	1.5	0.00	20.00	46.66	86.66	**38.33**
	2.0	0.00	33.33	53.33	86.66	**43.33**
KN	0.5	0.00	53.33	66.66	100.00	**55.00**
	1.0	0.00	66.66	73.33	93.33	**58.33**
	1.5	0.00	66.66	80.00	93.33	**60.00**
	2.0	0.00	40.00	80.00	86.66	**51.66**
TDZ	0.5	0.00	46.66	53.33	80.00	**45.00**
	1.0	0.00	53.33	60.00	80.00	**48.33**
	1.5	0.00	40.00	53.33	66.66	**40.00**
	2.0	0.00	46.66	66.66	66.66	**45.00**
Mean (B)		**0.00**	**47.18**	**63.59**	**87.17**	
LSD at 5% level		A=8.34 B= 4.62 AXB= 16.67				

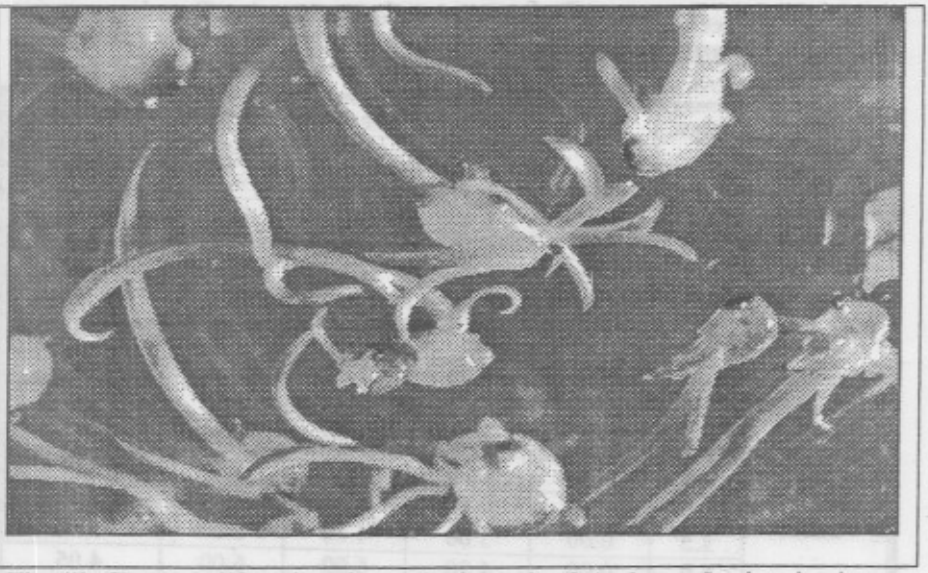

Fig. (3): Shoot proliferation via synthetic seeds of *Aechmea fasciata in vitro.*

Effect of sucrose addition to the encapsulation matrix of encapsulated buds of *Aechmea fasciata in vitro*

Complexing 3 % sodium alginate with 100M of Ca $(NO_3)_2.4H_2O$ afforded firm, clear and isodiametric beads suitable for handling beads(Fig., 4). In the experiment to evaluate the effect of gel matrix sucrose concentrations at 0.0, 15.0, 30.0, 45.0 and 60.0 g/l on development of capsules were used. Encapsulated buds in the alginate matrix with 60.0g/l sucrose and storage for eight weeks lost the high amount of moisture, leading to low germination number and germination % of 2.45 or 30.63% respectively. However, germination number and germination % of encapsulated buds were increased as the period of storage were increased as shown in Tables (6 and 7). Almost 100% shoots recovery were obtained from encapsulated buds stored for eight weeks with gel matrix free sucrose (control). Encapsulated buds of the alginate matrix of *Aechmea fasciata* responded with high frequency of plant recovery after culturing thus could be used for germplasm exchange, storage and micropropagation. The presence of nutrients affected shoot development. The presence of nutrients in gel matrix, which served as a nutrient bed around the encapsulated buds of *Aechmea fasciata* affected growth and survival. Our results are similar to those of Panis (1995) who showed that sucrose is known to provide a carbon source for *in vitro* propagules, and its inclusion in the alginate matrix enhanced plant recovery. However, relatively higher concentrations of sucrose in the alginate matrix and polymerisation medium significally decreased plant development, especially root formation. High levels of sucrose have been found to have adverse effects on shoot and root morphogenesis. In addition, an alginate matrix that contains high level of sucrose is necessary for cryogenic storage and using encapsulation dehydration, as source is known to prevent ice nucleation through vitrification (Wolfe and Bryant 2001).

Table (6): Effect of sucrose in alginate matrix of encapsulated buds and storage duration on number of germinated buds of *Aechmea fasciata in vitro.*

Sucrose conc. g/l (A)	Number of germinated encapsulated buds(week)				
	2	4	6	8	Mean(A)
Control	0.00	4.10	5.60	8.00	**4.40**
15.0	0.00	3.00	3.80	5.80	**3.15**
30.0	0.00	4.00	4.80	6.40	**3.80**
45.0	0.00	3.00	4.00	4.60	**2.90**
60.0	0.00	2.40	3.40	4.00	**2.45**
Mean(B)	**0.00**	**3.28**	**4.32**	**5.76**	
LSD at 5% level	A=0.327 B= 0.292 AXB= 0.653				

Table (7): Effect of sucrose in alginate matrix of encapsulated buds and storage duration on germinated buds (%) of *Aechmea fasciata in vitro.*

Sucrose conc. g/l (A)	Germination of encapsulated buds (%)				
	2	4	6	8	Mean(A)
Control	0.00	51.00	70.00	100.00	**55.00**
15.0	0.00	37.5	50.00	72.5	**40.00**
30.0	0.00	50.00	60.00	80.00	**47.50**
45.0	0.00	37.5	50.00	37.50	**36.25**
60.0	0.00	30.00	42.50	50.00	**30.63**
Mean(B)	**0.00**	**41.00**	**54.50**	**72.00**	
LSD at 5% level	A=4.33 B=3.87 AXB=8.65				

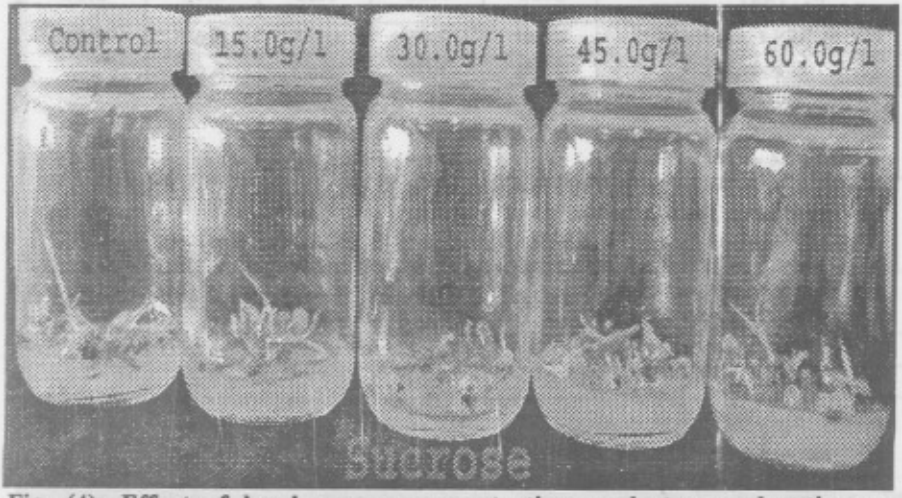

Fig. (4): Effect of bead sucrose concentrations and storage duration on viability of synthetic seeds of *Aechmea fasciata in vitro.*

Effect of sucrose addition to perlite

Encapsulated buds were cultured on perlite containing ½ MS liquid medium with or without 2% sucrose. Germination of encapsulated buds occurred regardless of sucrose addition on perlite within eight weeks of culturing. Plantlets germinated from encapsulated buds on perlite containing ½ MS medium with 2% sucrose were 6.50 number of germinated buds and 56.00% germination more than those of 0.92 or 9.17% respectively on perlite with ½ strength MS medium without sucrose. Almost 100% shoots didn't show recovery were obtained from encapsulated buds stored for 2, 4 or 6 weeks with medium free sucrose. However, a high rate of shoot and germination % (9.67 or 96.67 % respectively) to form plantlets occurred only on perlite containing ½ MS liquid medium with 2% sucrose after eight weeks of culturing than other treatments as shown in Table (8) and Fig. (5).This result indicates that sucrose was utilized as an energy source for germination and further post germinative growth of encapsulated buds, also carbon source addition to the medium enhances the conversion of encapsulated buds of *Aechmea fasciata*. Our results were in agreement with **Jung *et al.*, (2004)** who found that cotyledonary somatic embryos of *Siberian ginseng* were encapsulated with 3% sodium alginate; 96% of encapsulated embryos were converted to plantlets with well elongated epicotyls in perlite containing sucrose as a carbon source.

Table (8): Effect of sucrose addition to sterilized perlite and storage duration on germination number and germination % of *Aechmea fasciata* from synthetic seeds.

Medium composition (A)	Germination number of encapsulated buds				
	2	4	6	8	Mean (A)
½ MS+ perlite+2%sucrose	3.00	6.00	7.33	9.67	6.50
½ MS+ perlite	0.00	0.00	0.00	3.67	0.92
Mean(B)	1.90	3.00	3.67	6.67	
LSD at 5% level A	0.556				
B	0.787				
AxB	1.113				
Medium composition (A)	Germination of encapsulated buds(%)				
	2	4	6	8	Mean (A)
½ MS++ perlite 2% sucrose	30.00	60.00	73.33	96.67	59.00
½ MS+ perlite	0.00	0.00	0.00	36.67	9.17
Mean(B)	16.00	31.00	37.67	67.67	
LSD at 5% level A	5.567				
B	7.873				
AxB	11.13				

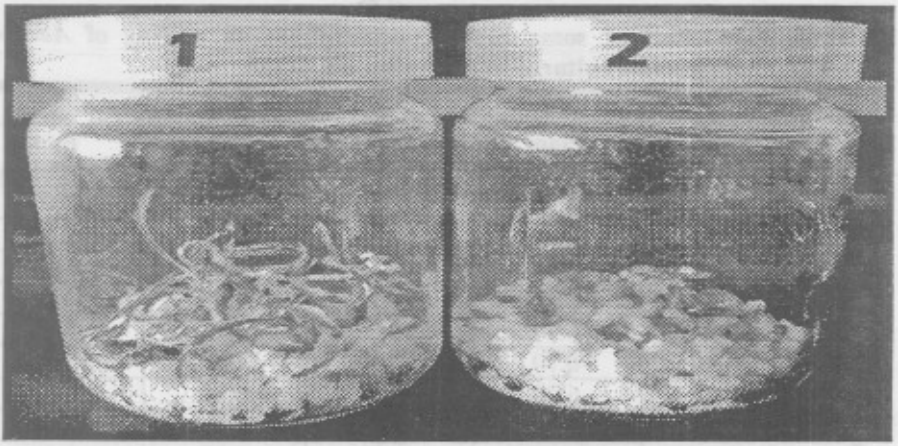

Fig. (5): Conversion of synthetic seeds of *Aechmea fasciata* on perlite *in vitro*.
1-Perlite + ½MS + 2% sucrose
2-Perlite + ½MS

Effect of some auxin concentrations on rooting of *Aechmea fasciata* cultured *in vitro* after six weeks.

Auxins (IBA and NAA) efficiently stopped shoot multiplication and supported nearly 100% rooting. Auxins increased the mean number of roots per shoot but at the same time decreased root length. The effect of auxin concentrations, was observed where, IBA at 1.5 or 2.0 mg/l resulted in the tallest plants and highest, leaves and root number as shown in Table (9). Results revealed that, the greatest length of roots was obtained on MS medium supplemented with 0.5 mg/l NAA than the other treatments.

In general, it could be concluded that, *Aechmea fasciata* shoots cultured on MS medium supplemented with IBA, at 2.0mg/l resulted in the greatest number of roots/explant while, NAA at 0.5 mg/l resulted in longest root/ shoot as shown in Fig. (6). These results are in harmony with those of Mercer and Kerbauy (1992)who showed that addition of 0.54 µM NAA was necessary to stop adventitious proliferation as well as to reestablish apical growth of the shoots of *Vriesea fosteriana* when shoots attained ca. 2cm in high (3months of culture), rooting was easily induced, and the shoots were transferred to medium supplemented with 1.1 µM NAA. After 2 months of culture every shoot formed 3-4 roots. Hong *et al.* (2002) demonstrated that at rooting stage of bromeliad plants, the highest value of rooting percentage was obtained with the medium which contained NAA at concentration of 0.5 mg/l. Mogollon *et al.*, (2004) found that, the highest number of roots of *Ananas comosus* was recorded when the medium contained 2.0mg/l NAA. Whereas, Badr-Elden (2003) mentiond that using full MS medium containing 2mg/l IBA + 30g/l sucrose increased number and length of roots *in vitro* of pineapple.And, the greatest value of rooting formation of *Aechmea fasciata* was observed with IBA(Guo-Min, *et al.*, 2005).

Table (9): Effect of some auxin concentrations on rooting of *Aechmea fasciata* cultured *in vitro* after six weeks.

Auxin conc. (mg/l)	Root formation			
	Plant length(cm)	No of leaves	No of roots	Root length(cm)
Control	4.70	9.40	1.70	2.00
0.5 NAA	7.60	11.20	2.80	2.50
1.0 NAA	6.80	10.80	2.60	1.40
1.5 NAA	6.60	9.20	3.20	1.60
2.0 NAA	6.40	12.00	4.60	1.50
0.5 IBA	6.70	12.40	4.60	1.80
1.0 IBA	6.20	10.80	4.00	1.70
1.50 IBA	8.90	14.80	5.40	2.10
2.0 IBA	10.00	14.20	6.20	1.90
LSDat5%level	1.957	1.663	1.021	0.723

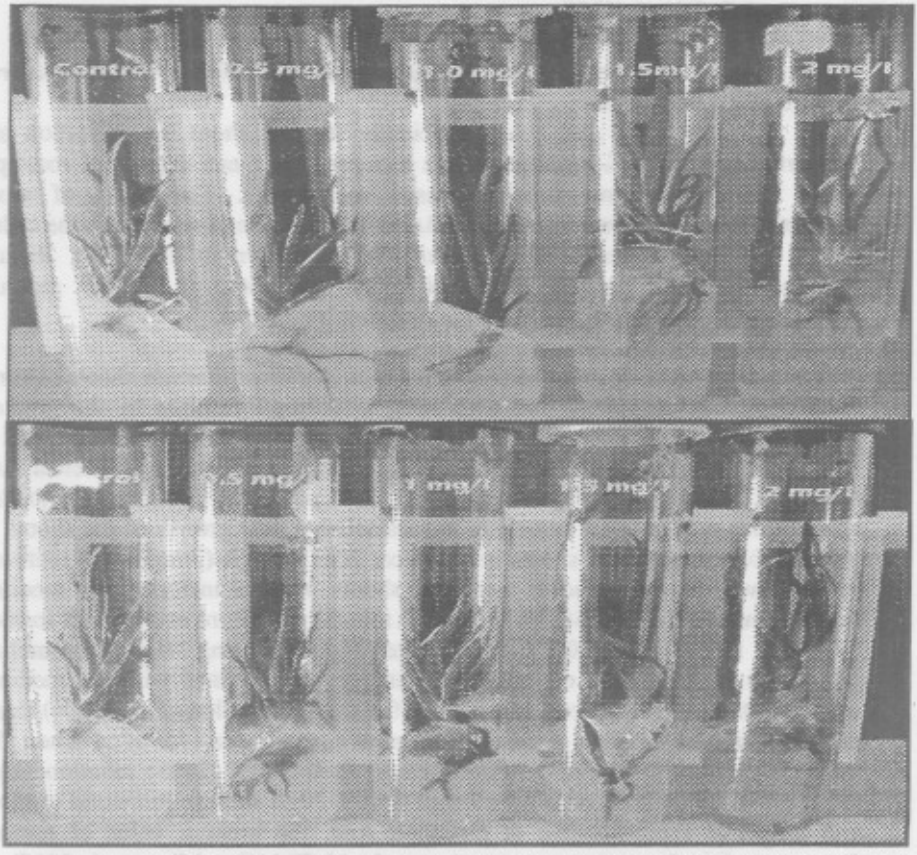

Fig. (6 A): Development of roots of *Aechmea fasciata* after six weeks of culture *in vitro*.

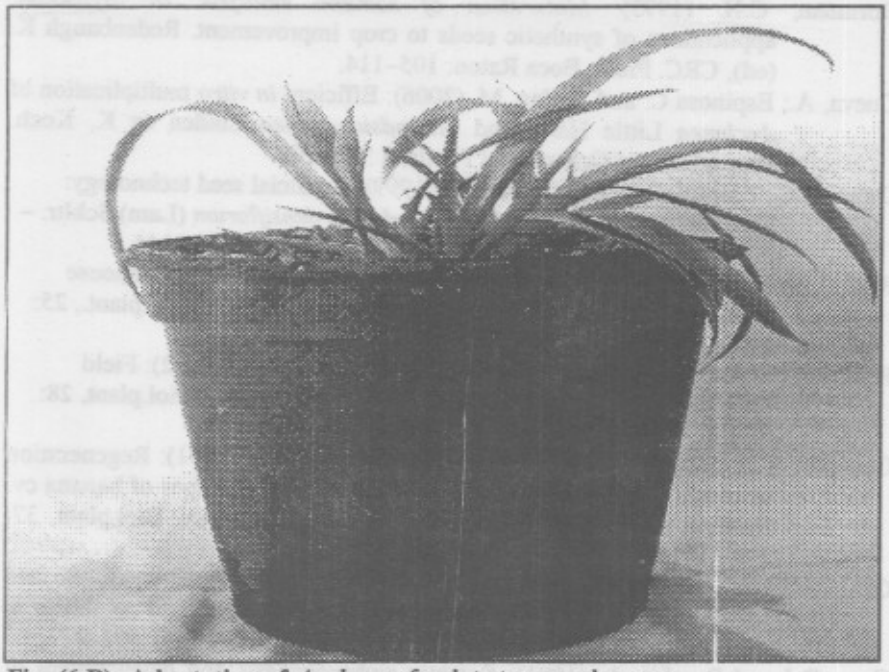

Fig. (6 B): Adaptation of *Aechmea fasciata* to greenhouses.

CONCLUSION

In conclusion, we have shown that carbon source addition to the encapsulation matrix or soil substrates markedly enhanced the conversion of encapsulated buds. This indicates that carbohydrate addition plays an important role in the further growth of germinated buds. Although encapsulation buds of *Aechmea* can be stored for shorter periods of time, the encapsulation techniques described in this work allow new possibilities for handling, transportation and delivery of *in vitro* tissue cultures of this plant.

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البذور المخلقة معمليا لنبات الأكميا كوسيلة لحفظ الأصول الوراثية وتداولها

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توضح الدراسة الحالية كفاءة التغليف لبراعم**الأكميا** بـ**ألجينات** الصوديوم المحتوية على مغذيات من خلال تقنية البذور الصناعية التي تعتبر مفيدة في حفظ وتداول الأصول الوراثية. و من السيتوكينين المختبرة كان البنزايل ادينين أكثر فاعلية عن كل من الكينيتين والايزوبنتينيل ادينين والثيوديازول يوريا وأعطى أقصى تضاعف عددى عندما استخدم بتركيز ٣ ملليجرام/لتر بينما كان معدل التضاعف العددى للأفرع اقل على البيئات المحتوية على من الكينيتين والايزوبنتينيل ادينين والثيوديازول يوريا . وقد أظهرت البذور الصناعية نموا على بيئة موراشيج وسكوج المحتوية على مثبطات النمو الباكلوبيترازول وحمض الابسيسيك والسيكوسيل. وكانت أفضل استجابة للإنبات للكبسولات عندما زرعت على بيئة موراشيج وسكوج الخالية من السيتوكينين و٢ ملليجرام/لتر بنزايل ادينين و ١,٠ ملليجرام/لتر كينيتين بعد ثمانية أسابيع من الزراعة. وتراوحت نسبة الإنبات للبراعم المغلفة من ١٣ إلى ١٠٠ % على تركيزات السيتوكينيين المختلفة بعد ثمانية أسابيع من التخزين وأثرت أيضا إضافة السكروز إلى مادة التغليف على كفاءة الكبسولات على الإنبات. وبتغليف البراعم بمادة التغليف بتركيز٣ % صوديوم ألجينات وزراعتها مع البيرليت في وجود السكر كمصدر كربون أعطت أعلى نسبة إنبات للكبسولات إلى البراعم بشكل واضح.وعلى الرغم من إعاقة نمو البراعم على بيئة البيرليت الغير محتوية على سكروز الا ان البذور الصناعية قد نمت بصورة طبيعية. و قد استحدث التجذير على الأفرع الناتجة من مرحلة التضاعف العددى بزراعتها على بيئة موراشيج وسكوج المحتوية على ٢ مليجرام/لتر اندول حامض البيوتريك حيث أعطت أكبر معدل للجذور عن باقى المعاملات. كما أعطت جذورا عرضية قصيرة فى بيئة موراشيج وسكوج المحتوية على نفتالين حامض الخليك و اندول حامض البيوتريك. وقد نقلت النباتات الناتجة بنجاح الى أصص فى الصوبة الزجاجية.