In vitro STORAGE OF SYNTHETIC SEEDS OF *Aechmea fasciata*

AWATEF M. BADR-ELDEN

Department of Plant Biotechnology, Genetic Engineering and Biotechnology Research Institute (GEBRI), Menofiya Univ., Sadat City

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). *In vivo* propagation of *Aechmea* through seed has been a standard practice. The plants obtained are variable and often 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 (Van Duck *et al.*, 1988). 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 (Naik and Chand, 2006). Synthetic seed refers to encapsulated explants such as shoot tips, axillary buds and somatic embryos in cryoprotectant material like hydrogel, alginate gel, ethylene glycol, dimethylsulfoxide (DMSO) and others that can be developed into a plant. The coating protects the explants, from mechanical damage during handling, and allows germination and conversion to occur without inducing undesirable variations (Harikrishna and Ong, 2002). The synthetic seeds have the possibility of being an alternative planting material meant for forestry sector in the future, especially for the highly demanded species (Nor Asmah *et al.*, 2011). Recently, molecular markers have been utilized in the detection of variation or confirmation of genetic fidelity during micropropagation (Tyagi *et al.*, 2007). Among the polymerase chain reaction (PCR)-based markers frequently used, RAPD (random amplified polymorphic DNA) is considered to be efficient and cost effective. The technique only needs a few nanograms of DNA for a fast polymorphism analysis, does not require prior knowledge of DNA sequence, and does not involve radioactivity (Williams *et al.*, 1990). RAPD markers have been employed to determine the clonal fidelity of micropropagated plants (Rani *et al*., 1995; Tang, 2001; Feuser, 2003). The present study aims to develop an efficient protocol for *in vitro* storage of *Aechmea fasciata* capsules and carry out molecular studies using RAPD technique for the re-growth plantlets after capsulation to compare the effect of three growth retardants PBZ,

ABA and CCC on the conservation of the genetic material.

MATERIALS AND METHODS

Plant material and source of explants

Healthy, mature plants from greenhouse-grown *Aechmea* plants were collected from Genetic Engineering and Biotechnology Research Institute (GEBRI), Menofyia Univ., Sadat City. leaves were washed in running tap water and washed again thoroughly by adding a few drops of Tween-20 to remove the superficial dust particles as well as fungal and bacterial spores. The washed explants were further treated with 10% solution of commercial bleach 5% (sodium hypochlorite). Followed by rinsing them five times with double distilled water inside the laminar air flow chamber. Explants about 1.0-1.5 cm were aseptically prepared and vertically implanted on (MS) medium (Murashige and Skoog, 1962) containing inorganic salts, 3% sucrose, 0.6% agar, 100 mg/l inositol, 0.2 mg/l glycine, 0.5 mg/l B_6, 0.5 mg/l nicotinic acid and 0.4mg/l B_1. MS medium supplemented with 0.25 mg/l 2,4-D (2,4 dichlorphenoxyacetic acid) was used for callus induction and using MS medium contained 2mg/l BA was used for shoot proliferation (Vinterhalter and Vinterhalter, 1994).

Shoot multiplication

Shoots from the proliferating explants were sub-cultured again on modified MS medium, at regular intervals of four weeks, for further multiple shoot induction. For mass multiplication, different types of plant growth regulators were supplemented with modified MS medium with 0.0, 1, 2, or 3 mg/l 6-bensyladenine (BA), kinetin (KN), isopentenyl adenine (2iP), thidiazuron (N-phenyl N 1,2,4 ichlorophenoxyacetic acid) (TDZ), 0.7% agar, and 3% sucrose for shoot proliferation. Shoots were cultured in 150 ml jars containing 50 ml of medium. The number of shoots, number of leaves and shoot length were recorded after six weeks.

Production of synthetic seeds

Achemea micro buds obtained from *in vitro* shoot cultures were dipped with sterilized tweezers, for few second, in 3% sodium alginate prepared by using MS liquid medium. For complexation, using 100 mM of Ca $(NO_3)_2.4H_2O$ solution, both the sodium alginate and Ca $(NO_3)_2.4H_2O$ were autoclaved at 121°C and 1.2 kg/cm^2 air pressure for 20 min after adjusting the pH to 5.7. The droplets containing the buds were held for at least 30 min to achieve polymerization of the sodium alginate. After 30 min, the alginate beads were collected and rinsed with sterile distilled water to wash away residues and there for cultured on different medium.

Effect of some growth regulators and storage period on germination percentage of synthetic seeds

The encapsulated buds were planted onto culture jar containing the following culture media supplemented with BA, KN or TDZ. Each growth regulator was added at concentrations of 0.0, 0.5, 1, 1.5

or 2 mg/l to storage MS medium supplemented with 30 g/l sucrose, 6 g/l agar, then pH value was adjusted to 5.7 before being autoclaved. Cultures were maintained at 20°C under complete dark.

Slow growth treatments. The effects of growth retardants on the germination % of encapsulated buds *in vitro* cultures were studied using MS media supplemented with paclobutrazol (PBZ), abscisic acid (ABA) or cycocyl (CCC) at concentrations of 0.0, 0.5, 1 or 1.5 mg/l. The medium of each treatment was also supplemented with 30 g/l sucrose and 6 g/l agar. Cultures were maintained at 20°C under complete dark.

Effect of sucrose added 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, 30, 45 or 60 g/l as described before. Fifty replicate jars with nine capsules per jar were used for each treatment. Germination percentage of encapsulated buds were recorded after 2, 4, 6 and 8 weeks of culturing. Cultures were maintained at 20°C in dark.

Effect of sucrose added to perlite

Encapsulated buds were cultured on sterilized perlite containing ½ MS liquid medium plus 2% sucrose or without sucrose in culture jars. Fifteen replicate jars with nine capsules per jar were used for all experiments and each treatment. Germination% of encapsulated buds was recorded after 2, 4, 6 and 8 weeks of culturing.

Rooting and transplantation

Green and healthy proliferated shoots were cultured on full and half strength MS media supplemented with 0.1 mg/l indole 3-butyric acid (IBA) or α-naphthaleneacetic acid (NAA). The percentage of shoots with root induction, number of roots per shoot, and mean root length were recorded after six weeks. Rooted shoots were carefully taken out of the medium and washed thoroughly in sterilized distilled water to remove medium attached to roots. The plants were transferred to plastic pots containing sterilized mixture of perlit and peatmoss (1:1) and covered with transparent plastic bags to maintain humidity, and then grown in greenhouse.

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).

DNA Isolation

The DNA were extracted from four samples of survival plantlets, they classified as follows: control, treated encapsulated samples with PBZ, ABA or CCC at 0.5mg/l. The DNA extraction was performed according to the protocol of Genomic DNA Purification Kit from ‘‘Fermentas’’ using 0.1 g from each fresh

sample of shoot tips, then 2.0 µl from RNase ‘‘Fermentas’’ was added to digest the RNA.

Polymerase Chain Reaction (PCR)

Five random primers were used to establish the RAPD analysis. These primers and their sequences were listed in Table (1). Each primer was used in a PCR reaction, The total amount of each reaction was 25 µl and consisted of: (1.25 U) 0.25 µl of Dream Taq DNA polymerase ‘‘Fermentas’’, 2.5 µl of Dream Taq buffer including $MgCl_2$, 0.4 µl of dNTPs (40 mM, 10 mM each) ‘‘Viogene’’, 2.0 µl of random primer (25 pmol) and 50 ng of genomic DNA. The PCR program was installed and carried out as follows; initial denaturation 94°C/10 min followed by 40 cycles with 94°C/50 sec to denaturize the DNA, 37°C/30 sec to anneal the primer with the template DNA and 72°C/30 sec to extent the primer, and then the final extension at 72°C/5 min. The PCR patterns were separated on 1.5% agarose gel ‘‘Axygen’’ and were weighted with 100 bp DNA ladder ‘‘Fermentas’’, then the patterns were visualized and photographed on the UV Transilluminator of the Gel documentation analysis system ‘‘Bio-RAD’’. The analysis of each gel was done using ‘‘Gel Analyzer 3 software’’.

RESULTS AND DISCUSSION

Shoot multiplication

Effect of some growth regulators on shoot proliferation

The effect of different plant growth regulators on shoot proliferation has shown in (Table 2). In general, the higher percentage of cultures producing shoots were those placed on media with higher concentrations of BA at 3 mg/l (Fig. 1A). Maximum number of leaves obtained were BA and 2iP at the same concentration of 1 mg/l (21.88 and 17.63, respectively) compared to the other concentrations. However, lower responses were obtained with TDZ at all concentrations. The highest shoot length obtained on MS medium free cytokinins, 3 mg/l KN, 1 and 2 mg/l 2iP than other treatments. Among the cytokinins tested, benzylaminopurine (BAP) was more effective than either 2-isopentenyl adenine (2iP) or kinetin (KN). Our results are in accordance with those found by 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* they had similar results where they found that, the highest number of shoots /culture were achieved on MS medium supplemented with BA and NAA. The highest multiplication ratio of *Neoglaziovia variegata* was obtained with NAA (0, 5 µM) + BAP (4, 4 µM) treatment (Silveira *et al.*, 2009).

Effect of some growth regulators and storage duration on germination percentage of encapsulated buds

Encapsulated buds inoculated onto MS medium free cytokinin (control), 1 mg/l BA, 1 and 1.5 mg/l KN exhibited shoot development and germination% from encapsulated buds as shown in (Table 3 and Fig. 1B). The development of buds and germination % were increased

gradually from 2 to 8 weeks. Adding different concentrations of growth regulators to conversion medium with BA or KN at 1 mg/l and MS free cytokinins to conversion medium after 8 weeks, gave the highest buds germination% than the other treatments. However, the conversion medium supplemented with BA, KN and TDZ at all concentrations showed that all encapsulated buds were still without any 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, 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. Plantlet regeneration from artificial seeds was achieved in the presence of NAA, BAP and GA_3 (Tabassum *et al.*, 2010).The optimum medium for the highest artificial seeds germination rate (83.8%) was 1/4 MS (without sugar) + 1.0 mg/l BA + 0.1 mg/l NAA *(*Ma *et al.*, 2011).

Effect of growth retardants and storage duration on germination percentage of encapsulated buds

Adding different concentrations of growth retardants to storage medium, played an important role in development and germination (%) of encapsulated buds as indicated in (Table 4 and Fig. 1C). Maximum germination % was achieved in cytokinin-free medium (control) followed by adding 0.5 mg/l ABA. Germination duration was affected by the addition of different growth retardants concentrations to storage medium and increased gradually from 0.0 to 1.20 and 16.67 to 46.66%, respectively, when conversion frequency duration extended from 2, 4, 6 to 8 weeks. After 8 weeks, cytokinin-free medium supplemented with ABA at 0.5 mg/l gave the highest number of explants. 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 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 where somatic embryos of mango were treated with ABA at different concentrations, and with increasing ABA concentration from 0.04 to 0.20 µM, percentage of germination and conversion into plantlets were decreased gradually. Lisek and Orlikowska (2004) reported that the composition of the beads also affected to survival rate 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.

Effect of sucrose addition to the encapsulation matrix

Complexing 4% sodium alginate with 100 M of $Ca(NO_3)_2.4\ H_2O$ afforded

firm, clear and isodiametric beads suitable for handling. In the experiment to evaluate the effect of sucrose concentrations at 0.0, 15, 30, 45 and 60 g/l in gel matrix on development of capsules were demonstrated (Table 5 and Fig. 1D). Encapsulated buds in the alginate matrix with 60.0 g/l sucrose and storage for eight weeks lost the high amount of moisture, leading to low germination 30.63%. Almost 100% shoots recovery was 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 polymerization 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 formation through vitrification (Wolfe and Bryant, 2001). The germination rate was enhanced through the supplying of sucrose to the artificial endosperm (Ma *et al.*, 2011).

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. Bud germinated from capsules on perlite containing ½ MS medium with 2% sucrose was 56% more than those on perlite with ½ strength MS medium without sucrose was 9.17% (Table 6 and Fig. 1E). Almost 100% shoots didn't show recovery was obtained from encapsulated buds stored for 2, 4 or 6 weeks with medium free sucrose. However, a high rate of germination was 96.67 % occurred only on perlite containing ½ MS liquid medium with 2% sucrose after eight weeks of culturing than other treatments. 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 those of 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. The use of 2 mg/l Kinetin and 2 mg/l NAA applied

with S23 gave the optimal response with both perlite and compost. This study showed high growth capacity of cauliflower artificial seeds in commercial substrates which are considered a promising step for their direct use *in vivo* (Rihan Hail *et al.,* 2011).

Effect of auxin concentration on rooting in vitro

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 mg/l resulted in the tallest plants and highest, leaves and root number as shown in Table (7). Results revealed that, the greatest length of roots was obtained on MS medium supplemented with 0.5 mg/l NAA than the other treatments (Fig. 1F). In general, it could be concluded that, *Aechmea fasciata* shoots cultured on MS medium supplemented with IBA, at 2mg/l resulted in the greatest number of roots/explant while, NAA at 0.5 mg/l resulted in the longest root/plant. The plants were successfully transferred to pots in a greenhouse with 97% survival percentage and gave 100% flowering percentage after one year from adaptation (Fig. 1F, G and H). These results are in harmony with those of Badr-Elden (2003) who mentiond that using full-strength MS medium containing 2 mg/l IBA + 30 g/l sucrose increased number and length of roots *in vitro* of pineapple. The greatest value of root formation of *Aechmea fasciata* was observed with IBA (Guo-Min *et al.,* 2005). Shoot conversion to entire plantlets was most effective in response to 4.4 or 8.8 μM BA combined with 2.5 μM NAA (Carneiro *et al.,* 2006). The roots produced on IBA were longer with better quality shoots whereas NAA produced poor response with necrotic leaves and leaf abscission (Ali *et al.,* 2009).

RAPD Analysis

The present molecular study aimed to compare the effect of growth retardant (PBZ, ABA or CCC) on the conservation of the genetic material of the encapsulated buds using RAPD technique (Fig. 2). The use of RAPD primers offers the potential of acquiring more cost effective data than in the case with other technologies. The genetic analysis was performed using RAPD markers because this method does not require previous knowledge of the sequence of the DNA under study (Wolfe and Liston, 1998). The most attractive feature of RAPD analysis is that it can be used on pooled DNA samples to rapidly screen for linked DNA markers (Michelmore *et al.,* 1991). With the advent of molecular markers, it is now possible to make direct comparison of genetic diversity at the DNA level without some of the over simplifying assumptions associated with calculating genetic diversity based on pedigree history (Brar, 2002). Primer OP-A03 failed to reveal any amplified fragments. While the other four primers OP-A05, OP-B07, OP-C08 and OP-

H11 succeeded to detect 10, 9, 10 and 6 amplified fragments, respectively. Primers OP-A05, OP-C08 and OP-H11 scored the highest percentage of polymorphism 90, 90 and 83% respectively, while OP-B07 exhibited 77% polymorphism. On the other hand, treatment with PBZ was more suitable than CCC and ABA to conserve the genetic material comparing with the control (Table 8). Santos *et al.* (2008) showed that RAPD technique was effective in showing the occurrence of somaclonal variations that occur during the micropropagation process of ornamental pineapple cultivation. Also Maria *et al.* (2002) detected 11 amplified fragments when they used OP-A03.

In conclusion, we have shown that addition of carbon source 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 these capsules. Using RAPD technique for the re-growth plantlets after capsulation to compare the effect of three growth retardants PBZ, ABA and CCC on stability of the conservation of the genetic material.

SUMMARY

This study has focused on the synthetic seed technology of *Aechmea fasciata* with a particular focus on the carbon source in seed bead. BA was the most effective cytokinin comparing with KN, 2iP or TDZ. It induced the maximum proliferation when used at 3 mg/l. The best morphogenetic response of the plantlets was achieved by culturing the encapsulated buds – for 8 weeks - on cytokinin-free MS medium, on media containing 2 mg/l BA or 1.0 mg/l KN. Encapsulated buds exhibited shoot development on MS medium supplemented with some growth retardants PBZ, ABA and CCC. Adding sucrose to the gel matrix influenced the capsule germination. Buds were encapsulated with 3% sodium alginate where 96.67% of the encapsulated buds were converted to plantlets. The plantlets developed elongated buds in perlite containing sucrose as a carbon source. The highest number of roots was obtained by culturing the *in vitro* proliferating shoots in MS salts supplemented with 2 mg/l IBA. The plants were successfully transferred to pots in a greenhouse with 97% survival percentage and gave 100% flowering percentage after one year from adaptation grown under controlled conditions. The molecular studies using RAPD technique with five primers proved that, the same dose of conservative material PBZ, ABA and CCC that were used to conserve the encapsulated explants. After the re-growth from plantlets it showed that adding PBZ, ABA and CCC was interaction between

the conservative materials and plays an important role in the conservation.

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Table (1): List of primers and their sequences.

Primer number	Primer code	Sequence 5'–3'
1	OP-A05	AGGGGTCTTG
2	OP-B07	GGTGACGCAG
3	OP-C08	TGGACCGGTG
4	OP-H11	CTTCCGCAGT
5	OP-A03	AGTCAGCCAC

Table (2): Effect of cytokinin concentrations on shoot development of *Aechmea fasciata* cultured *in vitro*.

Cytokinins con. (mg/l)		Shoot proliferation		
		Shoots number	Leaves umber	Shoot length (cm)
BA	0.0	1.38	8.25	5.31
	1.0	6.00	21.88	2.31
	2.0	5.00	11.00	2.44
	3.0	7.63	14.50	2.75
KN	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

Table (3): Effect of growth regulators and storage duration on germination percentage of encapsulated buds.

Cytokinin con. (mg /l)		Germination (%) of encapsulated buds (week)				
		2	4	6	8	Mean (A)
BA	0.0	0.00	73.33	80.00	100.00	63.33
	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				

Mean values followed by the same letter are not significantly different at $p<0.05$ according to LSD test − F-test significant at 0.05 level.

Table (4): Effect of some growth retardants and storage duration on germination percentage of encapsulated buds.

Growth retardants conc. (mg/l)		Germination (%) of encapsulated buds (week)				
		2	4	6	8	Mean (A)
PBZ	0.0	0. 00	78.80	86.67	100.00	66.37
	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	1757				

Mean values followed by the same letter are not significantly different at $p<0.05$ according to LSD test − F-test significant at 0.05 level

Table (5): Effect of sucrose in alginate matrix of encapsulated buds and storage duration on germination percentage of encapsulated buds.

Sucrose conc. (g/l) (A)	Germination (%) of encapsulated buds (week)				
	2	4	6	8	Mean(A)
Control	0.00	51.00	70.00	100.00	55.00
15	0.00	37.50	50.00	72.50	40.00
30	0.00	50.00	60.00	80.00	47.50
45	0.00	37.50	50.00	37.50	36.25
60	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		

Mean values followed by the same letter are not significantly different at $p<0.05$ according to LSD test − F-test significant at 0.05 level

Table (6): Effect of sucrose addition to sterilized perlite and storage duration on germination percentage of encapsulated buds of *Aechmea fasciata* cultured *in vitro*

Medium composition		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		

Mean values followed by the same letter are not significantly different at $p<0.05$ according to LSD test – F test significant at 0.05 level

Table (7): Effect of auxin concentration on rooting of *Aechmea fasciata* cultured *in vitro* for six weeks.

Auxin conc. (mg/l)		Root formation			
		Plant Length (cm)	Leaves number	Roots number	Root length (cm)
NAA	0.0	4.70	9.40	1.70	2.00
	0.5	7.60	11.20	2.80	2.50
	1.0	6.80	10.80	2.60	1.40
	1.5	6.60	9.20	3.20	1.60
	2.0	6.40	12.00	4.60	1.50
IBA	0.5	6.70	12.40	4.60	1.80
	1.0	6.20	10.80	4.00	1.70
	1.5	8.90	14.80	5.40	2.10
	2.0	10.00	14.20	6.20	1.90
LSD at 5% level		1.957	1.663	1.021	0.723

Table (8): Numbers of detected monomorphic and unique bands, total number of bands (mono. +poly. +unique) and the % of polymorphism (polymorphic + unique/total no. of bands x 100) for all primers.

Primer	Monomorphic bands	Unique bands			Total no. of bands	Polymorphism %
		No	Sample	MW		
OPA05	1	1	C	*846	10	90
OPB07	2	2	1	*1880 *1495	9	77.77
OPC08	1	6	2	*1850 *1470 *1220 *630 *360 *320	10	90
OPH11	1	4	3	*860 *660 *340 *260	6	83.33

C = Control 1 = 0.5 mg/l PBZ 2 = 0.5 mg/l ABA 3 = 0.5 mg/l CCC

Fig. (1):A- Development of shoot proliferation on BA. B- Germination of synthetic seeds. C- Effect of growth retardants on germination of synthetic seeds. D- Germination of synthetic seeds on perlite containing sucrose after 6 weeks from cultured. E- Effect of sucrose addition to perlite on germination percentage of synthetic seeds. F- *In vitro* rooting development G- Adaptation of plants in greenhouse. H- Flowering of *Aechmea fasciata* after one year.

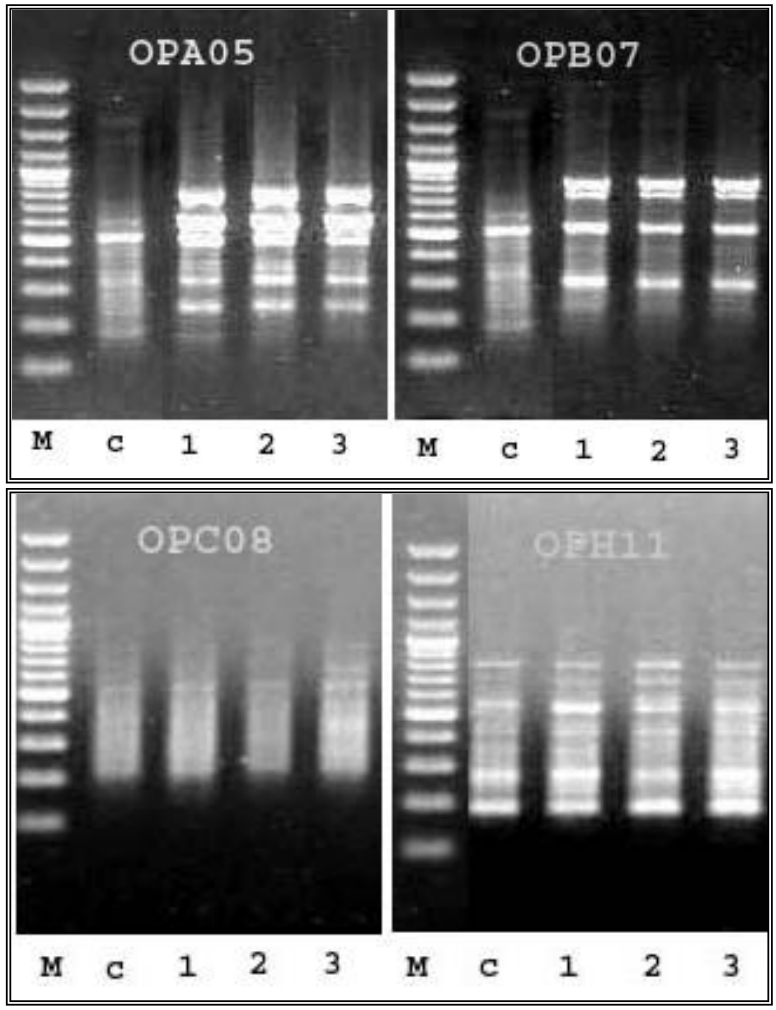

Fig. (2): RAPD profile of capsules after storage on 1-PBZ, 2- ABA and 3-CCC.