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EFFECT OF DIFFERENT GROWTH SUBSTANCES AND NATURAL PRODUCTS ON PROPAGATION OF *AECHMEA FASCIATA* PLANTS FROM STEM SEGMENTS *IN VITRO* CULTURE

Azza M. S. Arafa

Ornamental Horticulture Dept., Faculty of Agric., Cairo Univ., Egypt.

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ABSTRACT: *Stem segments of Aechmea fasciata were successfully propagated in MS-basal medium with different concentration of growth substances (benzyladenine, kinetin, naphthalene acetic acid and indole butyric acid) and natural products (casein hydrolysate, yeast extract, coconut milk, banana pulp and pineapple juice) through two subcultures. Clusters (3shootlets, 1cm length and 9leaves/cluster) were taken as explants from in vitro proliferated shoots of Aechmea fasciata established in aseptic culture from stem segments of greenhouse-grown plants. Benzyladenine (BA)played a very significant role for explants multiplications than kinetin with or without adding 0.5 mg/l naphthalene acetic acid (NAA).In both seasons, the highest shoot number, leaf number ,fresh weight(gm) and growth value /cluster were obtained using MS basal medium supplemented with 5.0 mg /l BA +0.5mg/l NAA after second subculture. The use of yeast extract, casein hydrolysate and coconut milk promoted large-scale production of Aechmea fasciata. The best multiplication of cluster was obtained on control medium (MS- basal medium + 5.0 mg/l BA + 0.5mg/l NAA) containing yeast extract compared to casein hydrolysate and coconut milk.Yeast extract incorporation in the MS-basal medium + 5.0 mg/l BA + 0.5mg/l NAA(control medium) resulted in a strong stimulation of shoot and leaf numbers as well as fresh weight(gm) and growth value /cluster with an optimum at 50 or 150 mg/l. Pineapple juice and banana pulp. were not suitable for Aechmea fasciata cluster multiplication, adding 150 mg/l pineapple juice on control medium decreased all parameters recorded. The highest number of roots and good healthy shootlets / cluster (highest value of fresh weight (gm) and growth value) were registered by adding 2.0 mg/l NAA to MS- basal medium. During acclimatization stage , 100% transplanted plantlets were survived and had a higher growth through 6 months of planting into peatmoss growing medium compared with other planting media in the two seasons. After 18 months, the highest rate of survival (97.22%) of plantlets and growth were found using peatmoss: sand: clay mixture medium3:1:1 by volume.*

Key words: *In vitro –Aechmea fasciata - ornamental bromeliads -. growth substances- natural products-casein hydrolysate- yeast extract- coconut milk - banana pulp - pineapple juice*

INTRODUCTION

Aechmea fasciata–Baker (Common names: Silver-Vase Bromeliad, Urn plant) belongs to the family Bromeliaceae. *Aechmea* is one of the most beautiful of all Bromeliads used for indoor decoration. *Aechmea* is native to tropical America. 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 seed. Vegetative multiplication through suckers is too slow to be practical. Therefore, *'in vitro'* propagation of *Aechmea* has become more and more widely used. *Aechmea fasciata* is a bromeliad that is propagated by tissue culture as an ornamental plant. (Bak, 2002 and Rongyi *et al.*, 2003).

To start the *in vitro* multiplication of *Aechmea*, plantlets can be obtained from *in vitro* germination or from explanted shoot-tips or axillary buds (Proft *et al*, 1985 and Van Duck *et al*, 1988). The importance of growth substances for differentiation and regeneration of bromeliaceae were studied by Mercer and Kerbauy (1992)on *Vriesea forteriana* mature seeds, Vinterhalter and Vinterhalter (1994) on *Aechmea fasicata* leaf explants. Pickens *et al* (2003 and 2006) on *Tillandsia* seeds, and Cueva *et al* (2006) on *Tillandsia* and *Aechmea* seedlings.

Complex organics (natural products) are a group of undefined supplementes such as casein hydrolysate, coconut milk, orange juice, tomato juice, grape juice, pineapple juice, carrot juice sap from birch, banana puree, potato extract . These compounds are often used when no other combination of known defined components prouces the desired growth or development. However, the composition of these supplements is basically unknown and may also vary from lot to lot causing variable responses. Some complex organic compounds are used as organic sources of nitrogen such as casein hydrolysate, a mixture of about 20 different amino acids and ammonium, peptone, tryptone, and malt extract .These mixtures are very complex and contain vitamins as well as amino acids. Yeast extract (0.25 to 2.0g/l) is used because of the high concectration and quality of B vitamins (George and Sherrington, 1984; Butcher and Marlow, 1989; Thorpe, 1994; Beyl, 2000; kumar, 2000 and Puchooa and Rambum, 2004)

The influence of natural products on growth and development were studied by Hao *et al* (2000) they reported that, modified MS with CH at 500 mg/l was the best medium for inducing the development of axillary buds of *Ginkgo biloba*. Mahanta and Paswan (2001) showed that ,MS basal medium supplemented with coconut milk 150 ml/l was the best for multiple shoot production of anthurium The use of additives such as coconut milk and casein hydrolysate promotes large-scale production of *Plantago ovata* through *in vitro* somatic embryogenesis (Pal and Raychaudhuri, 2001). Shoot

formation of *Cymbidium* were produced when explants cultured on Nitsch medium containing 50ml/l pineapple juice by Siddique and Paswan, (2001).Banana pulp was added to Vacin and Went medium for germination of *Dendrobium hybrid* by Sobhana and Rajeevan, (2002). Lo-ShuFung *et al* (2004) found that, seedlings of *Dendrobium tobaense* were developed into healthy plantlets after transfer to MS medium supplemented with 8%banana homogenate or coconut milk for 20 weeks.

Differentiation of pigean pea explants was observed when the medium was supplemented with CH. (Vaithiyalingan and Nadarajan, 2004). Van Minh (2005) indicated that, the highest mean number of *Garcinia mangostana* shoots per explant (18-22) formed after 8 weeks, when explant were cultured on MS medium supplemented with 2g/l yeast extract and coconut milk (10%). For rooting, the buds were cultured on WPM medium supplemented with 2g/l yeast extract and coconut milk (10%).Dominic and Joseph (2006) mentioned that, the highest embryogenic response was recorded on a medium containing10%coconut milk and 100mg/lCH of *Zamia furfuracea* .Kim and Moon (2007) found that,the highest number of matured embryos of *Larix leptolepis* was obtained with 1/2 LM medium supplemented with 250mg/l CH.

The role of auxin(NAA and IBA) on root formation of bromeliad plants was studied by Vinterhalter and Vinterhalter (1994); Xu, *et al* (2000) Hong, *et al* (2002); and Guo-Min, *et al* (2005) on *Aechmea fasciata*. Mercer and Kerbauy(1992) on *Vriesea forteriana* and Hong, *et al* (2002) on*Vriesea poelmannii; Cryptanthus zonatus and Neoregelia carolinae*. While, Dolgov, *et al* (1998) and Mogollon, *et al* (2004), on *Ananas comosus.*

Studies on growing media during acclimatization stage,showed that ,the plantlets transplanted on media contining coconut-bant +sand (1:1) or coconut-bant + turf + vermiculite (1:1:1) recorded survival rates of up to 95% of *Aechmea fasciata* (Hong ,*et al* 2002). Micropropagated plants of *Tillandsia eizii*,another variegated bromeliad species, over 86% survival and rapid growth were obtained with either an all-pine-bark medium, or a mixture of 2 red wood bark:2 fir bark: 2 potting mix:1 perlite Pickens *et al* (2003) Pickens, *et al* (2006) found that, plants were successfully acclimatized and planted into the greenhouse.

This work aims to study the effect of different growth substances and natural products on propagation of *Aechmea fasciata* plants during two subcultures and in the two seasons from stem segments using *in vitro* culture.

MATERIALS AND METHODS

This study was performed at the laboratory of plant tissue culture, Agricultural Development Systems Project (ADSP), Ministry of Agriculture during the study period (2004-2006).The greenhouse-grown plants of *Aechmea fasciata* were obtained from the Ornamental Horticulture Department, Faculty of Agriculture Cairo University, stem explants from

three-years old plants (Ca.2.0 cm in length) were surface sterilized under complete aseptic conditions in sterilized laminar air-flow hood. The explants after washing by water and a soap solution were immersed for 20 min in 2.0% NaOCL and a few drops 0.1% Tween 20 (polyoxyethylene sorbitan monolaurate) as a wetting agent.The explants were then rinsed three times in srerilized distilled water.Tips of stems were removed and the remainder stems were cut into 0.3cm,and used as initial explants.

Growing sterilized explants were recultured, at 4 weeks intervals, on fresh medium (MS-basal medium+2mg/lBA) until the onest of proliferation during 4recultures and 4 reculture for multiplication stage .Clusters (3shootlets,1cm length and 6leaves) were produced from multiplication stage and were used for studing experiments as follows:

Exp.1.The effect of MS-basal media supplemented with benzyladenine (BA) and kinetin (kin) at levels of 2.5 and 5.0 mg /l with or without adding 0.5 mg/l naphthalene acetic acid (NAA) and control treatment (MS-basal media only). Data on number of shoots, number of leaves , leaf length (cm), number of roots, root length (cm), fresh weight (gm) and growth value/ cluster were recorded *Growth value was estimated and presented as described by (Ziv,1992).

Exp.2.This experiment was carried out to study the influence of natural products (casein hydrolysate (CH), yeast extract (YE) and coconut milk (CM) added to MS-basal medium + 5.0 mg /l BA +0.5mg/l NAA) at different concentrations 50,100,150,200 mg/l and 0.0 mg/l, respectively on number of shoots, number of leaves, leaf length (cm), fresh weight (gm) and growth value/cluster.

Exp.3. The effect of different concentrations of banana pulp. and pineapple juice (50,100,150,200mg/l and 0.0 mg/l respectively) combind with the pervious medium MS- basal medium + 5.0 mg/l BA + 0.5 mg/l NAA was studied .Data were recorded on number of shoots, number of leaves, leaf length (cm), fresh weight (gm) and growth value/cluster.

All previous data Exp.1, 2 and 3 were recorded every two months through two subcultures.

Exp.4.The effect of indole butyric acid (IBA) and naphthalene acetic acid (NAA) added to MS-basal medium at the rates of 0.1,0.5,1.0,2.0 mg/l, respectively on number of roots and root length (cm)/cluster, number of shoots/cluster, fresh weight (gm) /cluster and growth value /cluster were obtained after two months.

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*Growth value (GV) is expressed as a value calculated by dividing the difference between final and initial fresh weight (FW) by initial FW: (FW final– FW initial / FW initial).

GVsub1= (FW final sub1- FW initial exp./ FW Initial exp.)

GVsub2= (FW final sub2- FW final sub1 / FW final sub1.)

Exp.5. This experiment was conducted e*x vitro* to evaluate the effect of growing media on plantlet growth during the acclimatization stage. On March 2005 and 2006 in two seasons respectively the plantlets (3plantlets, 3cm long and 7 leaves) produced from the pervious best treatments of *in vitro* rooting stage were transplanted into the following medium peatmoss(P), sand(S), clay(C) and P:S:C (1:1:1 , 1:1:2 , 1:1:3 , 1:2:1 , 1:3:1 , 2:1:1 and 3:1:1 v/v/v).Each treatment included 15 pots (3 replicates).After planting ,the plantlets were irrigated with water containing kristalon fertilization (N:P:K at 19:19:19) at the rate 0.25 g/l 2 times /week for 2 months and one time / week until the end of the experiment. The survival percentage, number of leaves and leaf length (cm) / plantlet were recorded after 6 months in September for the two seasons 2005 and 2006.

The survival percentage, shoot formation percentage , number of shoots / plantlets, number of leaves /plant and leaf length (cm) /plant were recorded after 18 months (September 2006) for only season (March2005).

MS-basal medium (Murashige and Skoog, 1962) was used in all experiments containing 30 g/l sucrose,100 mg/l inisitol ,0.5 mg/l pyrodoxin, 0.5 mg/l nicotinic acid,0.1 mg/l thiamine and 2mg/l glycine . and were solidified with agar (7gm/l). The media pH (5.8) was distributed in 200ml pyrex glass –jars (2.5ml/jar),autoclaved for 20 min. at 121°C and 1.2 kg /cm^2,then cooled and kept for 4 days before use. All cultures were incubated at 27°Cand 2 k-lux (16 hr/day) provided by cool white-fluorescent lamps. All experiments were repeated twice, unconditions, and conducted by using a completely randomized design in factorial arrangement with 10 replicate. all data were averaged and statistically analyzed by using one and two way analysis of variance. In case of percentages, the original data were firstly arcisine-transformed prior to statistical analysis. The least significant difference (L.S.D at 5%) was used to compare between means (Steel and Torrie, 1980).

RESULTS AND DISCUSSION

Exp.1. Data in Tables (1 and 2) and photo.(1) indicated that, highly significant differences in all studied characters under the influence of growth substances and subculture numbers. Shoot munber (51.1 and 49.2 shoot/cluster), number of leaves (204.5 and (6.71and 6.54/cluster)in two seasons respectively and growth value Fig. (1) were increased to maximum by using MS basal medium +5mg/lBA+0.5mg/lNAA after subculture two.

When BA with and without NAA were added to MS basal medium to stimulate mean number of shoots, mean number of leaves,fresh weight and growth value , adding 5.0mg/lBA+0.5mg/lNAA markedly stimulated, but mean leaf length(cm)decreased. Mean root number and length(cm)/cluster were absent by adding BA with or without 0.5mg/l NAA.

The greatest number of roots and root length (cm) were recorded by using MS basal medium free growth substances. The difference between two subcultures was observed significantly with almost parameters. These

results are in line with Almeida *et al*(1997)who reported that shoots were transferred to MS medium supplemented with BAP (0,1 or 2mg/l)in order to induce proliferation.Also,differentiation of *Aechmea fasciata* was produced on MS medium supplemented with 4mg/lBA,1mg/lNAA and 1mg/l IAA as indicated by Xu *et al* (2000).Hong *et al*(2002)showed that ,using 1mg/lBA and0.1 mg/l NAA with MS medium was optimum for micropropagation in subculture of *Aechmea fasciata* ,*Vriesea poelmannii,Cryptanthus zonatus* and *Neoregelia carolinae*.while, Mogollon *et al* (2004),found that ,eight weeks later,the number of shoots per explant was greater when using 1.0 mg/l BA+0.01mg/l NAA of *Ananas comusus*.whereas,on *Aechmea fasciata* Guo-Min *et al*(2005)observed that, the establishment of sterile material ingredients of proliferating medium were the same as those of initial medium (MS medium +2.5 BAmg/ l+0.25mg/ lNAA), and the propagation coefficient could reach 8.9 after continue culture of six months.Adventitious buds of *Tillandsia eizii* were induced in 40%of the explant placed on media with 2mg/lBA and NAA with some cultures becoming highly prolific are repeated subculture(Pickens *et al*, 2006).

Table (1): Effect of growth substances and subculture numbers on number of shoots, number of leaves and leaf length(cm) of *Aechmea fasciata* clusters(3shootlets(1cm)&9leaves/ cluster) through 2004-2005 and 2005-2006 seasons.

Treatment (mg/l)	Number of shoots /cluster			Number of Leaves/cluster			Leaf length (cm)/cluster		
	Sub1	Sub2	Mean	Sub1	Sub2	Mean	Sub1	Sub2	Mean
	2004-2005 season								
MS+2.5BA	7.50d	28.90c	18.20 c	27.80 ef	115.60 c	71.70 c	1.87 gh	1.96 f-h	1.92ef
MS+5.0 BA	7.90d	36.90b	22.40 b	23.30e-g	147.70 b	85.50 b	1.59 gh	1.40 h	1.50 f
MS+2.5BA+0.5NAA	8.90d	34.30b	21.60 b	31.70 e	137.30 b	84.50 b	4.18 a-c	1.79 gh	2.99 bc
MS+5.0BA+0.5NAA	9.00d	51.10a	30.05 a	30.30 e	204.50 a	117.4 a	4.08 bc	1.35 h	2.72 cd
MS+2.5K	2.30e	3.60e	2.95 d	10.50 h	22.70 e-g	16.60 e	2.80 e	4.08 bc	3.44 ab
MS+5.0K	2.90e	3.10e	3.00 d	11.60 gh	16.90 f-h	14.25 e	2.67 ef	4.53ab	3.60 a
MS+2.5 K+0.5NAA	3.50e	3.50e	3.50 d	11.60 gh	22.33 e-g	16.95 e	2.30 e-g	3.66 cd	2.98 bc
MS+5.0K+0.5NAA	2.80e	3.10e	2.95 d	9.10 h	16.80 f-h	12.95 e	2.02 f-h	2.85 e	2.44 de
MS	3.60e	3.90e	3.75 d	28.80 e	48.00 d	38.40 d	3.00 de	4.90 a	3.95 a
Mean	5.38 a	18.71 b		20.52 a	81.31 b		2.72 a	2.95 a	
.	2005-2006 season								
MS+2.5BA	8.10d	28.10c	18.10 c	26.80 ef	112.50 c	69.65 c	1.90gh	2.02gh	1.96 e
MS+5.0BA	8.60d	35.80b	22.20 b	24.20 ef	143.20 b	83.70 b	1.55hi	1.25i	1.40 f
MS+2.5BA+0.5NAA	9.30d	33.50b	21.40 b	33.60 de	134.00 b	83.80 b	5.00a	1.70gi	3.35bc
MS+5.0BA+0.5NAA	8.40d	49.20a	28.80 a	28.10 ef	196.00 a	112.1 a	4.63a	1.17i	2.90 cd
MS+2.5K	2.20e	3.50 e	2.85 d	11.00 gh	23.00 e-g	17.00 e	2.85de	3.89bc	3.37 b
MS+5.0K	2.60e	3.00 e	2.80 d	10.60 h	17.70 f-h	14.15 e	2.72d-f	4.42ab	3.57 ab
MS+2.5 K+0.5NAA	3.90e	4.10 e	4.00 d	12.00 gh	26.50 ef	19.25 e	2.20fg	3.33cd	2.77 d
MS+5.0K+0.5NAA	2.90e	2.70 e	2.80 d	9.40 h	17.70 f-h	13.55 e	2.31e-g	2.96de	2.64 d
MS	3.90e	4.20 e	4.05 d	26.60 ef	45.00 d	35.80 d	3.10d	4.60a	3.85 a
Mean	5.54a	18.2 b		20.2 a	79.61 b		2.92 a	2.82 a	

Means followed by different letters are significantly different at the 5%level according to L.S.D.
Sub1: (after two months) Sub2: (after four months)

Table (2): Effect of growth substances and subculture numbers on number of roots, root length(cm), fresh weight(gm) and growth value of *Aechmea fasciata* clusters(3shoots(1cm) and 9 leaves/cluster) through 2004-2005 and 2005-2006 seasons.

Treatments (mg/l)	Number of roots /cluster			Root length(cm) /cluster			Fresh weight (gm)/ cluster	Growth value /cluster
	Sub1	Sub2	Mean	Sub1	Sub2	Mean	Sub2	Sub2
	2004-2005 season							
MS+2.5BA	0.00 e	5.30 b	2.65 d	0.00 h	1.91 fg	0.96 c	6.78 b	4.42 b
MS+5.0BA	0.00 e	0.00 e	0.00 e	0.00 h	0.00 h	0.00 d	6.91 b	4.53 b
MS+2.5BA+0.5NAA	0.00 e	0.00 e	0.00 e	0.00 h	0.00 h	0.00 d	7.29 b	4.83 b
MS+5.0BA+0.5NAA	0.00 e	0.00 e	0.00 e	0.00 h	0.00 h	0.00 d	9.64 a	6.71 a
MS+2.5K	2.55 c	4.80 b	3.68 c	2.46 ef	4.34 a	3.40 a	3.33 d	1.66 e
MS+5.0 K	1.67 d	4.80 b	3.24 c	1.69 g	3.88 a-c	2.79 b	1.83 e	0.46 f
MS+2.5K+0.5NAA	5.10 b	5.25 b	5.18 b	2.86 de	4.10 ab	3.48 a	4.00 cd	2.20 d
MS+5.0K+0.5NAA	3.17 cd	4.70 b	3.94 c	2.24 e-g	3.28 b-d	2.76 b	3.01 de	1.41 e
MS	6.22 b	8.50 a	7.36 a	3.20 cd	3.80 a-c	3.50 a	4.70 c	2.76 c
Mean	2.08 a	3.71 b		1.38 a	2.37 b			
.	2005-2006 season							
MS+2.5BA	0.00 e	5.60 ac	2.80 c	0.00 g	1.98 f	0.99 d	7.10 b	4.68 b
MS+5.0BA	0.00 e	0.00 e	0.00 d	0.00 g	0.00 g	0.00 e	6.70 b	4.36 b
MS+2.5BA+0.5NAA	0.00 e	0.00 e	0.00 d	0.00 g	0.00 g	0.00 e	7.06 b	4.65 b
MS+5.0BA+0.5NAA	0.00 e	0.00 e	0.00 d	0.00 g	0.00 g	0.00 e	9.43 a	6.54 a
MS+2.5K	2.43 cd	4.25 bc	3.34 bc	2.20 ef	4.49 a	3.35 bc	3.42 cd	1.64 d
MS+5.0 K	1.80 d	5.25 ac	3.53 bc	2.31 ef	3.95 a-c	3.13 bc	1.91 e	0.42 f
MS+2.5K+0.5NAA	4.43 a-c	5.38 ac	4.89 ab	2.65 de	4.11 ab	3.38 ab	4.26 c	2.41 c
MS+5.0 K+0.5NAA	4.33 b-d	4.43 b-c	4.38 b	2.36 ef	3.24 cd	2.80 c	2.35 de	0.88 e
MS	6.45 ab	9.00 a	7.73 a	3.50 bc	4.10 ab	3.80 a	4.33 c	2.46 c
Mean	2.16 a	3.77 b		1.45 a	2.43 b			

Means followed by different letters are significantly different at the 5%level according to L.S.D.
Sub1: (after two months) Sub2: (after four months)

Exp.2. The aim of this experiment was to study the effect of different natural products (casein hydrolysate (CH), yeast extract (YE) and coconut milk (CM)) and subculture number on multiplication of *Aechmea fasciata* clusters.

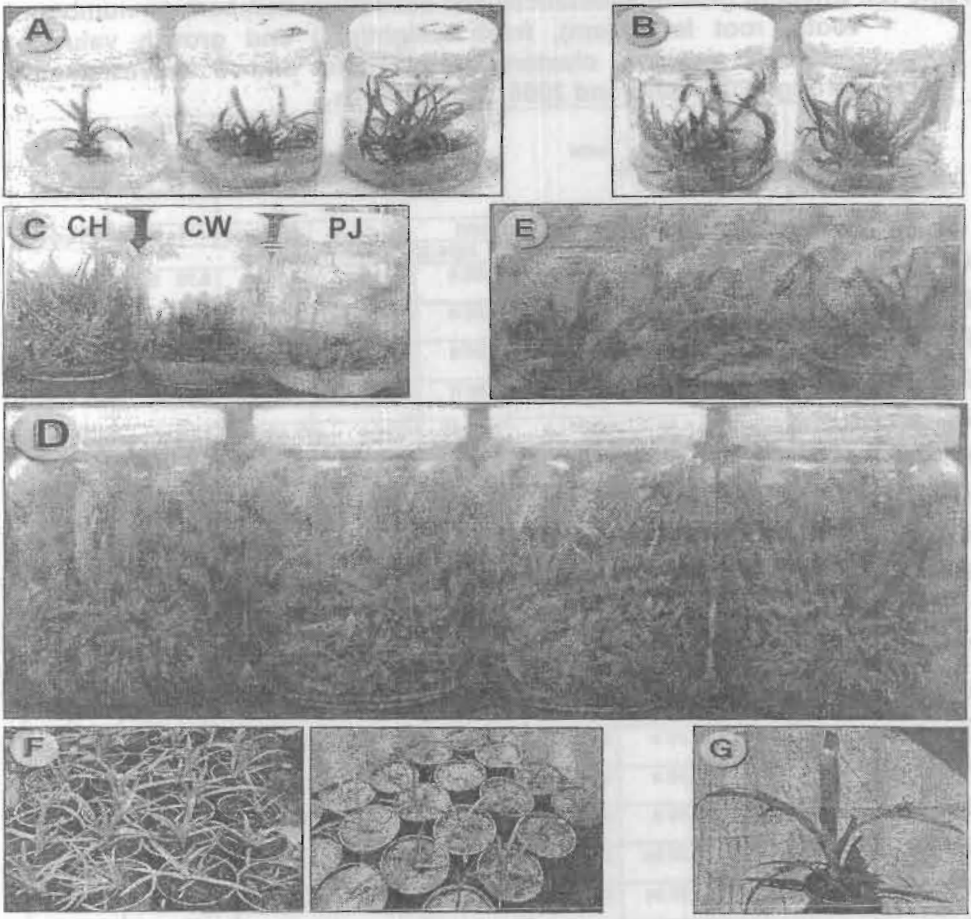

Photo (1) Successive stages of development of *Aechmea fasciata propagation in vitro* culture. These stages included (A) propagation from stem segments and stock shoots formation on MS basal medium + 2 mg/L BA (B) MS basal medium + 5 mg/L BA +0.5 mg/L NAA (control medium) was recorded the best medium for regeneration from cluster explants. (C) Different responses for shoot formation on control medium supplemented with: 1) 150 mg/L casein hydrolysate (CH). 2) 150 mg/L coconut milk (CM). 3) 150 mg/L pinapple juice (PJ). (D) Higher shoot multiplication at 150 mg/L yeast extract. (E) Clusters were cultured on the best rooting medium (MS medium + 2mg/L NAA). (F) plantlets were transplanted to 1) the suitable medium for survival and growth with peatmos medium. 2) The lowest survivial and growth with sandy medium during acclimatization stage after 6 months. (G) Plantlets after 18 months in the greenhouse.

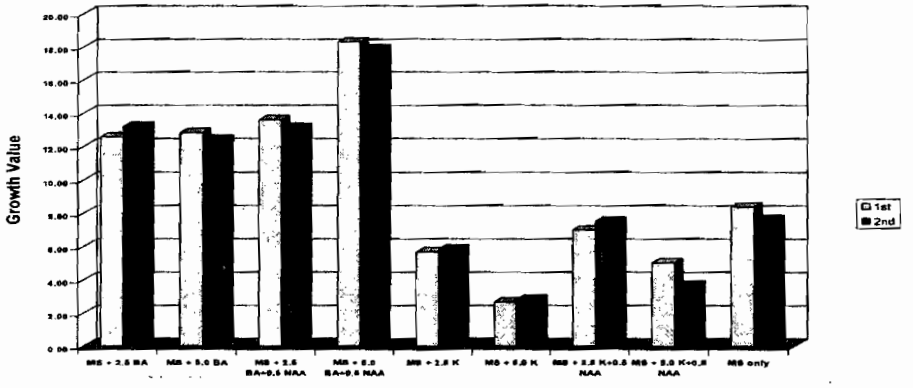

Fig.(1) : Effect of BA and kinetin combined with 0.5 mg/L NAA during two seasons on growth value of *Aechmea fasciata* clusters.

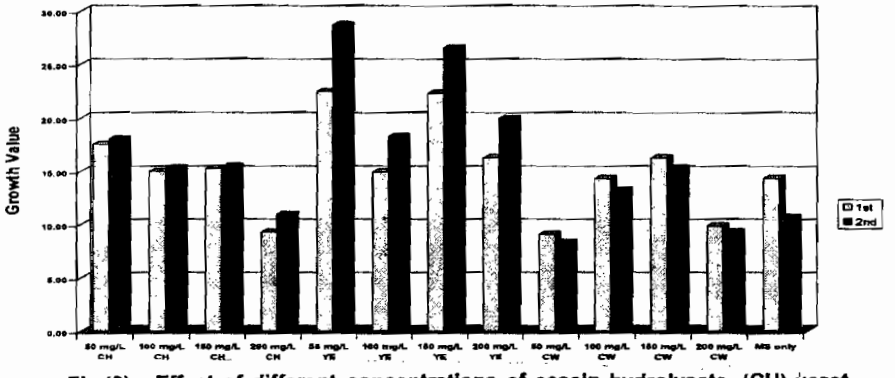

Fig.(2) : Effect of different concentrations of casein hydrolysate, (CH) yeast extract (YE) and coconut milk (CM) on growth value of *Aechmea fasciata* clusters during two seasons.

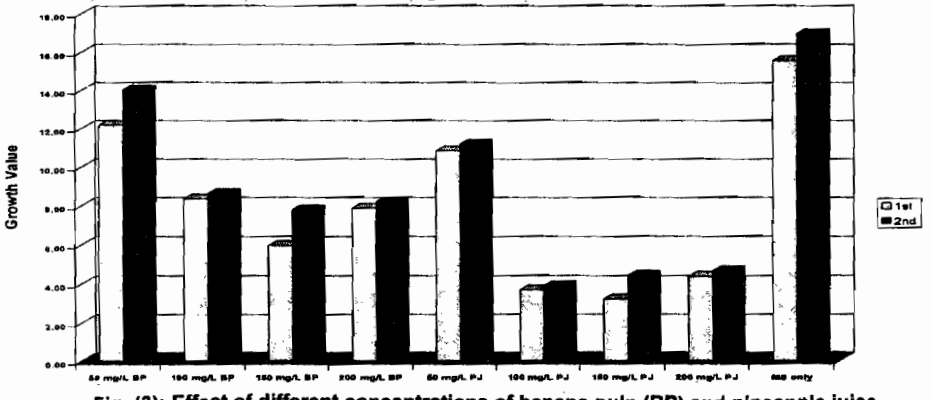

Fig. (3): Effect of different concentrations of banana pulp (BP) and pineapple juice (PJ) on growth value of *Aechmea fasciata* clusters during two seasons.

According to the results of previous experiment, MS basal medium + 5.0 mg / IBA + 0.5mg/l NAA proved to be the most suitable for multiplication, so this medium was chosen for control medium. Results in Tables (3 and 4) and photo. (1) indicated that ,in the two seasons, among the natural products at different concentratios, yeast extract resulted in the maximum multiplication of shoot number (67.50 and 69.50 shoots /cluster, respectively) at 150 mg/l, number of leaves (210 and 217 leaves / cluster, respectively) at 50 mg/l and produced the highest value of fresh weight and growth value at 50 mg/l or150 mg/l in second subculture .The mean data recorded that, adding 150 mg/l YEwas the most effective concentration for number of shoots, leaf length as well as fresh weight and growth value (Fig. 2) .

The previous data showed that, a positive effect of yeast extract, casein hydrolysate, fllowed by coconut milk on shoots multiplication and growth. The same results were recorded by Echeverrigaray *et al.* (2000), useful effect of 0.1% YE on shoots multiplication and growth was detected of *Anthemis nobilis* L. and Zayed (2000), found that, YE at 100 mg/l gave the highest number of shoots and leaves during multiplication stage of *Spathiphyllum wallisii*. While, the induction of direct somatic embryogenesis of *Malva silvestris* increased with CH at 500mg/l Kintzios *et al*, (2002) and Gao *et al* (2004) on *Amorpha fruticosa*,showed that, the addition of CH 200mg/l enhanced the number of shoots up to 8.77 per subculture, coconut milk was found to promote the shoot elongation and make them grow more vigorously. Addition of 100mg/l CH to medicinal plant shoot induction medium enhancement these was recorded by Surya-Prakash *et al* (2003), CH proved inhibitory for shoot differentiation in male and female jojoba plants. Liu *et al* (2004) found that increasing the concentrations of casein hydrolysate did not improve overall plant regenerative ability of *Lolium perenne*.

Coconut milk (CM) 20% concentration was used as a source of nutrients. Nearly 50% of the explants produced multiple promot protocorm-like bodies (PLBs) of *Vanda coerulea* (Kanika and Vij, 2004). Dominic and Joseph, (2006), found that,somatic embryos of *Cycas circinalis* induction from megagametophyte explant was optained on MS medium supplemented with 10% CM.

Table (3): Effect of different concentrations of natural products (casein hydrolysate, yeast extract and coconut milk)and subculture numbers on number of shoots , number of leaves and leaf length (cm) of *Aechmea fasciata* clusters(3shootlets(1cm) &9leaves / cluster)through 2004-2005 and 2005-2006 seasons

Treatment (MS+5BA+0.5NAA) (mg/l)	Number of shoots /cluster			Number of leaves /cluster			Leaf length (cm)/cluster		
	Sub1	Sub2	Mean	Sub1	Sub2	Mean	Sub1	Sub2	Mean
	2004-2005 season								
50 Casein hydrolysate	7.75 no	44.00e	25.88 ef	38.25i-k	137.50 bc	87.88 cd	1.34 e-h	1.18 g-j	1.26 c-f
100 Casein hydrolysate	8.50m-o	47.50d	28.00 d	40.00i-k	130.00 c	85.00 de	1.74 ab	1.12 h-k	1.43 a-c
150 Casein hydrolysate	8.75m-o	60.25b	34.50 c	44.50i	143.00 b	93.75 c	1.50 b-f	0.96 jk	1.23 d-f
200 Casein hydrolysate	9.00mn	26.13h	17.56 hl	35.50 jk	70.25 f	52.88 h	1.84 a	1.27 f-i	1.55 a
50 Yeast extract	17.25jk	56.75c	37.00 b	70.25 f	210.00 a	140.1 a	1.38 d-h	1.17 h-k	1.27 c-f
100 Yeast extract	10.50lm	34.13g	22.31 g	42.50 ij	115.50 d	79.00 ef	1.63 a-d	1.17 h-k	1.40 a-d
150 Yeast extract	15.75k	67.50a	41.63 a	64.00 fg	142.50 b	103.3 b	1.55 b-e	1.18 g-j	1.37 b-d
200 Yeast extract	12.00l	39.13f	25.56 f	56.00 gh	118.00 d	87.00 d	1.82 a	1.28 f-i	1.55 ab
50 Coconut milk	6.25op	23.13i	14.69 j	33.00 k	56.50 gh	44.75 i	1.37 d-h	0.93 jk	1.15 ef
100 Coconut milk	6.75n-p	26.25h	16.50 i	21.00 l	71.00 f	46.00 i	1.15 h-k	1.03 i-k	1.09 f
150 Coconut milk	8.75m-o	46.13de	27.44 de	34.25 jk	119.50 d	76.88 f	1.66 a-c	0.91 k	1.28 c-e
200 Coconut milk	4.50p	19.75j	12.13 k	19.75 l	54.50 h	37.13 j	1.44 c-g	1.06 i-k	1.25 c-f
MS	9.25mn	27.75h	18.50 h	40.50 i-k	88.50 e	64.50 g	1.58 a-e	0.99 jk	1.29 c-e
Mean	9.62a	39.88b		41.50a	112.06b		1.54a	1.10b	
	2005-2006 season								
50 Casein hydrolysate	8.50 o	45.50 f	27.00 d	39.00 h	135.0 c	87.00 c	1.50 c-f	1.20 j-l	1.35 b-d
100 Casein hydrolysate	8.75 o	52.50 d	30.63 c	36.00 h	137.0 c	86.50 c	1.54 b-e	1.43 d-h	1.49 ab
150 Casein hydrolysate	8.50 o	63.25 b	35.88 b	39.75 h	144.0 c	91.88 c	1.25 h-k	0.88 n	1.07 f
200 Casein hydrolysate	8.50 o	25.25 jk	16.88 f	35.50 h	69.50 f	52.50 e	1.77 a	1.30 g-j	1.54 a
50 Yeast extract	13.25m	60.00 c	36.63 b	72.25 ef	217.0 a	144.60 a	1.45 c-g	1.25 h-k	1.35 cd
100 Yeast extract	8.25 o	32.13 h	20.19 e	33.25 h	118.5 d	75.88 d	1.62 a-d	1.20 j-l	1.41 a-c
150 Yeast extract	13.00m	69.50 a	41.25 a	57.25 g	157.0 b	107.10 b	1.71 ab	1.34 f-j	1.53 a
200 Yeast extract	12.00mn	40.00 g	26.00 d	55.75 g	120.0 d	87.88 c	1.63 a-c	1.35 e-j	1.49 a
50 Coconut milk	5.75 p	23.50 k	14.63 g	33.50 h	58.0 g	45.75 f	1.23 i-k	1.00 mn	1.11 ef
100 Coconut milk	6.00 p	26.13 j	16.06 fg	22.75 i	69.0 f	45.88 f	1.03 l-n	1.00k-m	1.06 f
150 Coconut milk	10.25 no	50.13 e	30.19 c	39.25 h	118.0 d	78.63 d	1.37 e-j	0.85 n	1.11 f
200 Coconut milk	4.50 p	20.50 l	12.50 h	20.75 i	52.50 g	36.63 g	1.40 e-i	1.10k-m	1.25 de
MS	8.75 o	29.25 i	19.00 e	34.25 h	80.25 e	57.25 e	1.31 g-j	1.00 mn	1.15 ef
Mean	8.92a	41.36b		39.94a	113.52b		1.45a	1.15b	

Means followed by different letters are significantly different at the 5%level according to L.S.D.
Sub1: (after two months) Sub2:(after four months)

Table (4): Effect of different concentrations of natural products (casein hydrolysate, yeast extract and coconut milk) and subculture numbers on fresh weight (gm) and growth value of *Aechmea fasciata* clusters(3shootlets(1cm) & 9 leaves/ cluster) through 2004-2005 and 2005-2006 seasons.

Treatment (MS+5BA+0.5NAA) (mg/l)	Fresh weight (gm) /cluster			Growth value /cluster		
	Sub1	Sub2	Mean	Sub1	Sub2	Mean
	2004-2005 season					
50 Casein hydrolysate	0.76 h	9.26 b	5.01 d	0.60 no	6.62 d	3.61 e
100 Casein hydrolysate	0.92 h	7.99 d	4.45 ef	1.04 lm	5.52 e	3.28 fg
150 Casein hydrolysate	1.08 h	8.12 cd	4.60 d-f	1.32 l	5.58 e	3.45 ef
200 Casein hydrolysate	1.01 h	5.13 e	3.07 g	0.16 p	4.28 g	2.22 i
50 Yeast extract	2.01 g	11.73 a	6.87 b	3.14 i	10.89 a	7.02 b
100 Yeast extract	1.84 g	7.96 d	4.90 d	1.96 k	6.71 d	4.34 d
150 Yeast extract	3.62 f	11.65 a	7.64 a	4.66 f	10.02 b	7.34 a
200 Yeast extract	2.35 g	8.63 c	5.49 c	3.38 hi	7.38 c	5.38 c
50 Coconut milk	0.51 h	5.04 e	2.78 g	0.10 p	2.73 j	1.42 j
100 Coconut milk	0.91 h	7.66 d	4.28 f	0.40 op	4.69 f	2.55 h
150 Coconut milk	1.04 h	8.63 c	4.84 de	0.80 mn	5.53 e	3.17 g
200 Coconut milk	0.63 h	5.44 e	3.04 g	0.10 p	3.14 i	1.62 j
MS	0.83 h	7.67 d	4.25 f	0.88 mn	3.67 h	2.28 i
Mean	1.35 a	8.07 b		1.43 a	5.91 b	
	2005-2006 season					
50 Casein hydrolysate	0.80 m-o	9.52 d	5.16 d	0.52 o	6.41 b	3.47 e
100 Casein hydrolysate	1.02 mn	8.15 e	4.59 e	0.84 m	5.39 e	3.12 g
150 Casein hydrolysate	1.16 lm	8.23 e	4.70 e	1.16 k	5.50 e	3.33 f
200 Casein hydrolysate	0.85 m-o	5.98 g	3.42 g	1.02 l	3.10 i	2.06 i
50 Yeast extract	2.07 k	14.86 a	8.47 a	3.02 i	8.38 a	5.70 b
100 Yeast extract	1.48 l	9.64 d	5.56 c	2.68 j	5..37 e	4.03 d
150 Yeast extract	2.83 j	13.78 b	8.31 a	6.24 c	8.45 a	7.34 a
200 Yeast extract	2.19 k	10.47 c	6.33 b	3.70 g	5.90 d	4.80 c
50 Coconut milk	0.55 o	4.66 i	2.16 h	0.02 q	3.03 i	1.53 k
100 Coconut milk	0.70 no	7.11 f	3.90 f	0.82 m	5.13 f	2.98 h
150 Coconut milk	0.90 m-o	8.16 e	4.53 e	1.08 kl	5.90 d	3.49 e
200 Coconut milk	0.55 o	5.17 h	2.86 h	0.20 p	3.35 h	1.78 j
MS	0.94 m-o	5.84 g	3.39 g	0.66 n	5.14 f	2.90 h
Mean	1.23a	8.58b		1.69 a	5.47 b	

Means followed by different letters are significantly different at the 5%level according to L.S.D.
Sub1: (after two months) Sub2: (after four months)

Exp.3. Concerning the effect of different natural products,pineapple juice and banana pulp, the data in Tables (5 and 6) indicated that, adding juice and banana pulp at different concentrations on MS control medium (MS-basal medium+ 5.0mg/lBA+0.5mg/lNAA) inhibited growth and development/cluster compared with MS control medium. The lowest number of shoots and leaves /cluster .As well as fresh weight (gm) and growth value/cluster (Fig. 3) were obtaind when clusters were grown into MS control medium supplemented with pineapple juice at 150mg/l. In contrast to this results, banana extract produced a high rate of protocorm proliferation, while pineapple juice resulted in shoot formation of *Cymbidium* by Siddique and Paswan, 2001. Kalpana- Sachdev and Sathyanarayana (2002) found that, medium supplemented with a combination of 3% BP (w/v) and 10% CW (v/v) was more effective and enhanced the production of protocorm-like bodies and shoot formation of *Dendrobium* Lo-ShuFung, *et al.* (2004) observed that, active growth in the germinated seedlings was achieved by re-culturing on MS basal medium containing 8% banana homogenate.

Table (5): Effect of different concentrations of natural products (banana pulp and pineapple juice)and subculture number on number of shoots, number of leaves and leaf length(cm) of *Aechmea fasciata* clusters (3shootlets(1cm) &9 leaves /cluster) through 2004 -2005 and 2005-2006 seasons.

Treatment (MS+5BA+0.5NAA) (mg/l)	Number of shoots /cluster			Number of leaves /cluster			Leaf length(cm) /cluster		
	Sub1	Sub2	Mean	Sub1	Sub2	Mean	Sub1	Sub2	Mean
	2004-2005 season								
50 Banana pulp	9.50 fg	28.25 b	18.88 b	28.00 g	52.00 b	40.00 b	1.12 hi	1.27 gh	1.20 ef
100 Banana pulp	8.00 f-h	20.50 c	14.25 c	31.00 fg	52.00 b	41.50 b	1.32 f-h	1.47 e-g	1.40 de
150 Banana pulp	7.50 gh	16.50 d	12.00 d	28.00 g	40.75de	34.38 cd	1.54 d-g	1.62 b-f	1.58 cd
200 Banana pulp	7.00 gh	17.00 d	12.00 d	30.00 fg	37.50 e	33..75 cd	1.60 c-g	1.49 e-g	1.54 cd
50 Pineapple juice	6.25 h	17.00 d	11.63 de	27.50 g	44.25cd	35.88 c	1.70 a-e	1.68 a-e	1.69 bc
100 Pineapple juice	5.75 h	15.00 de	10.38 de	22.50 h	42.25d	32.38 d	1.80 a-e	1.87 a-d	1.84 ab
150 Pineapple juice	6.13 h	10.50 f	8.31 f	13.00 i	33.00 f	23.00 e	1.90 a-c	1.97 ab	1.93 ab
200 Pineapple juice	6.25 h	13.75 e	10.00 ef	22.00 h	41.00de	31.50 d	2.00 a	1.98 a	1.99 a
MS	10.50 f	32.00 a	21.25 a	48.00bc	83.75a	65.88 a	1.32 f-h	0.87 i	1.10 f
Mean	7.43 a	18.94 b		27.78a	47.39b		1.59 a	1.58 a	
	2005-2006 season								
50 Banana pulp	9.00 gh	25.50 b	17.25 b	30.00 fg	49.00 b	39.50 b	1.20 g-i	1.42 e-h	1.31 e
100 Banana pulp	7.75 gh	18.00 c	12.88 c	32.00 f	45.00 c	38.50 b	1.35 f-h	1.39 e-h	1.37 e
150 Banana pulp	8.00 gh	15.50 cd	11.75 c	29.00f-h	42.00cd	35.50 c	1.66 c-f	1.61 c-f	1.64 cd
200 Banana pulp	7.13 h	17.00 c	12.06 c	28.00gh	39.00de	33.75 cd	1.70 c-e	1.50 d-g	1.60 d
50 Pineapple juice	7.49 gh	16.50 cd	11.99 c	26.00hi	38.00 e	32.00 de	1.75 b-d	1.75 b-d	1.75 b-d
100 Pineapple juice	6..50 h	14.00 de	10.25 d	23.00 i	39.00de	31.00 e	1.80 a-d	1.86 a-c	1.83 a-c
150 Pineapple juice	6.75 h	12.50 e	9.63 d	16.00 j	38.00 e	27.00 f	2.05 ab	1.89 a-c	1.97 ab
200 Pineapple juice	7.50 gh	12.00 ef	9.75 d	18.00 j	36.00 e	27.00 f	2.10 a	1.92 a-c	2.01 a
MS	9.75 fg	30.50 a	20.13 a	42.00 cd	87.50 a	65.00 a	1.15 hi	0.90 i	1.03 f
Mean	7.76a	17.95b		27.17 a	46.00 b		1.64 a	1.58 a	

Means followed by different letters are significantly different at the 5%level according to L.S.D.
Sub1: (after two months) Sub2: (after four months)

Table (6): Effect of different concentrations of natural products (banana pulp and pineapple juice) and subculture number on fresh weight (gm) and growth value of *Aechmea fasciata* clusters (3shootlets (1cm)& 9 leaves/ cluster) through 2004-2005 and 2005-2006 seasons..

Treatment (MS+5BA+0.5NAAmg/l)	Fresh weight(gm)/cluster			Growth value /cluster		
	Sub1	Sub2	Mean	Sub1	Sub2	Mean
	2004-2005 season					
50 Banana pulp	0.90 f	6.59 b	3.74 b	0.80 h-j	4.27 b	2.54 b
100 Banana pulp	0.85 f	4.67 c	2.76 c	0.70 j-l	2.74 d	1.72 d
150 Banana pulp	0.86 f	3.48 d	2.17 de	0.72 i-k	1.78 f	1.25 e
200 Banana pulp	0.91 f	4.44 c	2.67 cd	0.82 hi	2.55 e	1.69 d
50 Pineapple juice	0.92 f	5.92 b	3.42 b	0.84 hi	3.74 c	2.29 c
100 Pineapple juice	0.86 f	2.35 e	1.60 f	0.72 i-k	0.88 h	0.80 f
150 Pineapple juice	0.84 f	2.11 e	1.48 f	0.68 kl	0.74 i-k	0.71 g
200 Pineapple juice	0.80 f	2.69 e	1.75 ef	0.60 l	1.15 g	0.88 f
MS	0.95 f	8.28 a	4.61 a	0.90 h	5.62 a	3.26 a
Mean	0.88 a	4.50 b		0.75 a	2.61 b	
	2005-2006 season					
50 Banana pulp	0.91 f	7.52 b	4.22 b	0.82 i	5.02 b	2.92 b
100 Banana pulp	0.89 f	4.82 d	2.86 d	0.78 ij	2.86 d	1.82 d
150 Banana pulp	0.87 f	4.40 d	2.64 d	0.74 i-k	2.52 f	1.63 e
200 Banana pulp	0.85 f	4.60 d	2.73 d	0.70 jk	2.68 e	1.69 e
50 Pineapple juice	0.82 f	6.10 c	3.46 c	0.64 kl	3.88 c	2.26 c
100 Pineapple juice	0.79 f	2.45 e	1.62 e	0.58 lm	0.96 h	0.77 f
150 Pineapple juice	0.75 f	2.72 e	1.74 e	0.50 m	1.18 g	0.84 f
200 Pineapple juice	0.69 f	2.83 e	1.76 e	0.38 n	1.26 g	0.82 f
MS	0.89 f	9.00 a	4.95 a	0.78 ij	6.20 a	3.49 a
Mean	0.83 a	4.94 b		0.66 a	2.95 b	

Means followed by different letters are significantly different at the 5%level according to L.S.D.
Sub1: (after two months) Sub2:(after four months)

Exp.4. Data in Table (7) and photo.(1) registered that, NAA was superior for rooting as compared to IBA at the same concentration .the maximum number of roots was obtained on MS basal medium containing 1.0mg/lNAA, but non significant by different between this medium and medium containing 2.0mg/l NAA. The longest roots was found on MS medium +0.5 mg/l IBA. The same results for number of shoots and leaf length as well as fresh weight and growth value was observed on medium containing 2.0 mg/l NAA. Consequenthly, MS basal medium supplemented with 2.0 mg/l NAA was recorded suitable medium for rooting cluster and give rise to healthy shootlets. These results are in line with Mercer and Kerbauy (1992) and Vinterhalter and Vinterhalter (1994) They found that, the medium containing NAA was the optimal medium for rooting of *Vriesea forteriana* and *Aechmea fasciata* respectively. Hong, *et al.* (2002) mentioned that, the optimum concentration of NAA for rooting was 0.5mg/l of some bromeliad plants. Whereas, the largest number of roots was registered when using 2.0 mg/l NAA Mogollon *et al.* (2004), on *Ananas comosus* L' Queen Australia',.The reverse of these results was recorded by Xu, *et al.* (2000).and Guo-Min,*et al.* (2005) using MS medium supplemented with IBA for the induction of rooting *Aechmea fasciata*.

Table (7): Effect of different concentrations of IBA and NAA on growth and development of *Aechmea fasciata* clusters (3 shootlets (1cm) & 9leave/ cluster) after two months through 2004-2005 and 2005-2006 seasons.

Tretments (mg/l)	Number of roots/cluster	Root length (cm) /cluster	Number of shoots/cluster	Number of leaves/cluster	Leaf length (cm)/cluster	Fresh weight (gm)/ cluster	Growth value/ cluster
	Season (2004-2005)						
MS+0.1IBA	5.75 c	3.46 ab	3.00 d	25.33 c	4.12 a	1.91 c	0.53 d
MS+0.5 IBA	5.25 c	4.15 a	3.75 b-d	24.17 c	2.94 c	1.77 c	0.42 de
MS+1.0 IBA	6.00 c	4.04 a	3.08 cd	28.08 bc	4.19 a	1.80 c	0.44 de
MS+2.0 IBA	10.00 b	2.95 bc	3.58 b-d	31.75 ab	4.05 ab	1.68 c	0.34 e
MS+0.1 NAA	9.58 b	3.23 bc	3.50 b-d	25.67 c	4.11 a	1.91 c	0.53 d
MS+0.5 NAA	10.92 ab	2.47 c	3.42 b-d	25.33 c	3.81 ab	1.91 c	0.53 d
MS+1.0 NAA	12.75 a	2.77 bc	4.17 b	32.17 ab	3.28 bc	2.74 b	1.19 c
MS+2.0 NAA	11.08 ab	2.78 bc	5.92 a	37.25 a	2.84 c	6.45 a	4.16 a
MS	6.75 c	3.42 ab	4.00 bc	31.50 ab	2.93 c	3.33 b	1.66 b
	Season (2005-2006)						
MS+0.1 IBA	6.13 c	3.41 cd	2.88 c	24.88 d	4.01 ab	1.90 cd	0.52 e
MS+0.5 IBA	5.50 c	4.56 a	3.63 bc	24.63 d	2.95 cd	1.88 cd	0.50 e
MS+1.0 IBA	6.38 c	4.39 ab	3.13 bc	29.75 b	4.56 a	2.14 b-d	0.71 d
MS+2.0 IBA	7.88 bc	3.62 b-d	3.25 bc	28.63 c	4.45 a	1.44 d	0.15 f
MS+0.1 NAA	11.63 a	3.17 c-e	3.75 bc	27.88 c	3.87 a-c	2.19 b-d	0.75 d
MS+5.0 NAA	10.63 a	2.31 e	3.38 bc	24.13 d	3.86 a-c	1.86 cd	0.49 e
MS+1.0 NAA	11.88 a	2.85 c-e	4.13 ab	29.75 b	3.41 b-d	2.53 bc	1.02 c
MS+2.0 NAA	10.13 ab	2.72 de	5.00 a	35.63 a	2.95 cd	6.22 a	3.98 a
MS	6.25 c	3.66 a-c	3.63 bc	28.75 c	2.81 d	2.88 b	1.30 b

Means followed by different letters are significantly different at the 5%level according to L.S.D.

Exp.5. Data in Table (8a) and photo. (1) showed that, in two seasons, the highest survival rate (100%) was recorded when the shootlets were transplanted onto soil mixture composed of peatmoss: sand: clay (3:1:1) by volume or peatmoss medium. Whereas, the largest number of leaves and leaf length (cm) were observed only with peatmoss medium after 6 months from transferred to soil. Survival rate 20.83% and 9.72% were recorded in two seasons respectively when the plantlets were transplanted into sandy soil. After 18 months from transplanting , in only first season ,data in Table (8b), demonstrated that, healthy plants and the obtimum survival rate (97.22%)as well as a maximum number of shoots, number of leaves and longest leaf length were obtained when plantlets growing in peatmoss :sand: clay (3:1:1) by volume whereas , D-Andrea and Dematte (2000) found that,the best growth was observed on media containing tree fern fibre, although good growth was exhibited by plants grown in a medium comprising 45% coconut bark+45% pine bark+10% humus. In addition, Guo-Min, *et al.* (2005) found that, the cultured plantlets of *Aechmea fasciata* were easy survivable, with a survival rat over 95%.

Table (8a): Effect of the growing media on *Aechmea fasciata* plantlets growth during the acclimaization stage after 6 months in greenhouse through two seasons (2005 and 2006).

Growing media	Survival(%)		Number of leaves /plantlets		Leaf length(cm)/ plantlets	
	1st	2nd	1st	2nd	1st	2nd
Peat(P)	100.00 a	100.00 a	12.93 a	12.65 a	10.15 a	10.20 a
Sand(S)	20.83 g	9.72 h	9.10 b	8.50 b	5.42 b-d	5.32 bc
Clay(C)	38.89 f	50.00 e	8.60 bc	7.40 d-f	5.72 bc	5.66 b
P:S:C(1:1:1)	70.83 c	80.55 c	8.6 bc	8.60 b	5.90 b	5.54 bc
P:S:C(1:1:2)	59.72 d	69.44 d	7.70 d	7.50 c-e	5.00 d-f	5.02 b-d
P:S:C(1:1:3)	61.11 d	70.83 d	7.30 d	6.65 f	5.22 de	3.90 e
P:S:C(1:2:1)	50.00 e	40.28 f	8..35 c	8.15 b-d	4.90 ef	5.21 bc
P:S:C(1:3:1)	18.06 g	20.83 g	7.25 d	7.20 ef	5.38 c-e	4.97 cd
P:S:C(2:1:1)	80.56 b	90.27 b	8.60 bc	8.30 bc	4.71 fg	5.29 bc
P:S:C(3:1:1)	100.00 a	100.00 a	8.60 bc	8.45 b	4.25 g	4.41 de

Means followed by different letters are significantly different at the 5%level according to L.S.D
1st :(first season) 2nd:(secand season)

Table (8b): Effect of the growing media on growth and development of *Aechmea fasciata* plantlets after 18 months in the greenhouse.

Growing media	Survival(%)	Shoot formation(%)	Number of shoots/plant	Number of leaves/plant	Leaf length (cm)/Plant
Peat(P)	91.67 b	90.28 b	2.00 b	7.50 b	28.00 c
Sand(S)	40.28 e	15.28 f	1.11 e	4.76 e	18.50 e
Clay(C)	59.72 d	50.00 e	1.20 de	6.21 d	19.50 e
P:S:C(1:1:1)	59.72 d	59.72 d	1.50 cd	5.00 e	23.75 d
P:S:C(1:1:2)	75.00 c	61.11 d	1.80 bc	6.75 c	33.00 b
P:S:C(1:1:3)	81.94 c	58.33 d	2.00 b	6.00 d	17.50 e
P:S:C(1:2:1)	80.56 c	50.00 e	1.50 cd	6.78 c	27.50 c
P:S:C(1:3:1)	59.72 d	19.44 f	1.21 de	7.00 c	24.00 d
P:S:C(2:1:1)	65.28 d	79.17 c	3.00 a	7.81 b	31.00 b
P:S:C(3:1:1)	97.22 a	100.00 a	3.11 a	8.71 a	40.00 a

Means followed by different letters are significantly different at the 5%level according to L.S.D.

CONCLUSION

Aechmea fasciata stem segments can be successfully propagated when 1) sterilized by soaking in NaOCL (2.0%)for 20 min. 2) culture at 4 weeks intervals on fresh medium , on MS-basal medium supplemented with 2mg/l BA until the formation of the physiological base containing microshoots through 8 recultures.3)Clusters containing (3shoots(1m)&9 leaves)were used as explants for experiments .4) MS medium supplemented with BA not only increased shoot number/ cluster, but also considerably increased leaves number, fresh weight (gm)and growth value but kinetin inhibited multiplication .The greatly shoots number (51.10 shoots/cluster) production from clusters when 5mg/l BA +0.5 mg/l NAA was added to the MS-basal medium.after subculture two.5) There was a significant increase in shoot number with adding 150mg/lyeast extract and 150mg/l casein hydrosate to MS basal medium +5.0 mg/lBA+0.5mg/lNAA(control medium). 67.50 and 60.25 shoots/cluster respectively after subculture two.6) The incorporation of banana pulp. and pineapple juice at different concentration to MS control medium decrease in shoot numbr and other parameters. 7) In rooting stage: MS basal medium supplemented with 2mg/lNAA appeared to be the best for rooting formation .8) In acclimatization stage: High survival rate (100%) and healthy vigorous plantlets were recorded in carriers with peatmoss growing medium, after 6 months, while peat: sand: clay (1:1:1)by volume mixture medium helped in better growth and development after 18 months from transplanting.

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تأثير مواد النمو والمركبات الطبيعية المختلفة على إكثار نباتات الأكميا فاسيكاتا من أجزاء الساق فى مزارع الأنسجة

عزة محمد سعيد عرفة

بساتين الزينة – كلية الزراعة – جامعه القاهرة

الملخص العربى

تم بنجاح إكثار أجزاء سيقان نباتات الأكميا فاسيكاتا فى بيئة مورشيجى وسكوج مع تركيزات مختلفة من مواد النمو (بنزيل ادنين وكينتين ونفتالين حامض الخليك واندول حامض البيوتريك) والمركبات الطبيعية (كازين اللبن ومستخلص الخميرة ولبن جوز الهند وكذلك لب الموز وعصير الأناناس) وذلك خلال مرتين تجديد الزراعة.

أخذت الأجزاء النباتية للتجربة من مجاميع مكونة من (٣ أفرع بطول اسم وعدد ٩ أوراق لكل مجموعة نباتية) وذلك من نباتات الأكميا فاسيكاتا النامية فى الصوبة. إضافة البنزيل ادنين الى البيئة يلعب دورا فعالا فى تضاعف الأجزاء النباتية مقارنة بإضافة الكينتين وذلك مع إضافة ٥ملجم لكل لتر نفتالين حامض الخليك أو عدم إضافته. خلال موسمى زراعة وجد ان أعلى عدد للأفرع و عدد للأوراق ووزن طازج وكذلك قوة نمو لكل مجموعة نباتية بزراعتها على بيئة مورشيجى وسكوج مضاف إليها ٥ملجم لكل لتر بنزيل ادنين + ٥ملجم لكل لتر نفتالين حامض الخليك بعد مرتين تجديد زراعة. يستخدم مستخلص الخميرة وكازين اللبن ولبن جوز الهند كمشجع لإنتاج اعداد كبيرة من نباتات الأكميا فاسيكاتا.

أحسن تضاعف للمجموعة النباتية وجد علي البيئة الكنترول بيئة مورشيجى وسكوج + ٥ ملجم لكل لتر بنزيل ادنين + ٠,٥ ملجم لكل لتر نفتالين حامض الخليك التي تحتوى علي مستخلص الخميرة عن التي تحتوى علي كازين اللبن أو لبن جوز الهند. نتيجة إضافة مستخلص الخميرة ظهرت إستجابة قوية فى زيادة اعداد الأفرع واعداد الأوراق وكذلك فى الوزن الطازج وقوة النمو لكل مجموعة نباتية وكان التركيز الأمثل هو ٥٠ أو ١٥٠ملجم لكل لتر. وإضافة عصير الأناناس ولب الموز غير مناسبين لتضاعف المجموعات النباتية للأكميا

فاسيكاتا. وإضافة ١٥٠ملجم لكل لتر عصير الأناناس للبيئة الكنترول ادى لنقص في كل القياسات التي سجلت.

بيئة مورشيجى وسكوج المضاف إليها ٢ملجم لكل لتر نفتالين حامض الخليك سجلت أكبر عدد من الجذورواعطت فريعات قوية (أعلي قيمة للوزن الطازج (بالجرام) وقوة النمو). أثناء مرحلة الأقلمة ١٠٠% من النباتات المنزرعة نجت واستحث نموها خلال ٦ شهور من الزراعة فى بيئة بيتموس مقارنة بالبيئات الأخرى التى درست فى موسمى الزراعة.

بعد ١٨ شهر وجدت أعلى نسبة نجاة(٩٧،٢٢%) وكذلك لنموها بزراعتها فى مخلوط البيئة البيت: الرمل: الطين (١:١:٣) بالحجم.