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Synthetic Seeds of Pear (*Pyrus communis* L.) Rootstock Storage *In vitro*

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Abstract: Synthetic seeds offer several advantages, easy handling, storability, reduced size of propagules, and transportability. Germplasm can be effectively stored in the form of synthetic seeds. Shoot tips obtained from *in vitro* shoot cultures of pear (*Pyrus communis* L.) were encapsulated in 3% calcium alginate. The present work studied the role of components of culture medium on morphogenic response of pear encapsulated buds to various planting media, different types of growth regulators in different concentrations, different types of media, MS strengths and growth retardants in different concentrations for long term storage. Germination ability of the synthetic seeds was investigated. The maximum response (35%) for growth ability of encapsulated buds into plantlets was achieved on MS medium supplemented with 0.75 mg/l BA after 16weeks of culture. The frequency of growth ability of encapsulated buds into plantlets was affected by the concentration of growth regulators and storage time. Maximum growth was recorded (47.45 %) on MS medium than B_5, N_6 and WPM media. Interesting feature was the ability of the capsules to retain their viability and germinated after storage on MS and ½MS after ten weeks, almost all capsules sprouted successfully. The frequency of conversion pear encapsulated buds into plantlets was affected by MS strengths. The culture medium supplemented with sucrose at different concentrations 0, 10, 20, 40 and 80g/l affected the growth ability of capsules. In contrast, increasing the sucrose concentration enhanced and increased growth ability of pear capsules. Maximum growth ability of encapsulated buds was obtained on MS medium supplemented with 0.5 mg/l CCC (18.13) and control (15.83%) (without growth retardants) after 16 weeks of storage. Preservation method by growth retardants slow down cell metabolism and prevent somaclonal variation. The genetic stability of the pear plantlets growing out after storage in encapsulated form was confirmed via protein SDS-PAGE. Protein bands of plantlets resulting from pear encapsulated buds using the growth retardant CCC were the same as the control (shoot tips) except density.

Keywords: *In vitro,* Synthetic seeds, Pear, Growth regulators, Growth retardants, Protein fingerprinting

INTRODUCTION

Pear is one of the most important deciduous fruit trees all over the world it took the second rank after apple in production. Moreover, *Pyrus communis* L considered as the most valuable and compatible rootstock of pear under Egyptian conditions. It can grow in different kind of soils .The traditional propagation technique of pear trees by grafting on quince, seedlings or clonal selection of *Pyrus communis* is not completely satisfactory because of the lack of compatibility with some cultivars, heterogenesis of the pear seedlings and excess growth and also due to the sensitivity of the grafted plants to pear decline. Growing pear trees with their own roots is another possibility that would overcome the previous problems. The technique of commercial *in vitro* of encapsulated bud can be employed for the storage and micropropagation of pear. In recent years, nutrient-alginate encapsulation technique for producing synthetic seeds has 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 (Naik and Chand 2006). In addition, synthetic seed technology could be

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useful in conservation of clonal germplasm of elite and endangered plants in near future, with development of appropriate storage techniques (Na and Kondo1996). Synthetic seeds (synseeds) have been defined as artificially encapsulated somatic embryos, shoots, or other tissues which can be used for sowing under *in vitro* or *ex vitro* conditions, that are able to grow into a plantlet after sowing (conversion) (Bapat 1993; Standardi & Piccioni 1998). 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 programme (Gangopadhyay, *et al.,* 2005). A simple method of encapsulated microcuttings of olive (*Olea europaea* L. cv. Moraiolo) storage in plastic cuvettes was used. Interesting feature was the ability of the capsules to retain their viability and regrowth after storage at room temperature as well as at 4 °C for 15 and 30 days and both axillary buds on the nodes of almost all capsules sprouted successfully (Micheli *et al.*, 2007). The aim of the study was to evaluate the best medium of the bead among different media made of growth regulators (BA, Kin or TDZ), different types of media (MS, B_5, N_6 or WPM), sucrose concentrations (0, 10, 20, 40 or 80g/l), MS salt strengths (MS, ½ MS, ¼ MS or ⅛ MS) and growth retardants using abscsic acid (ABA), paclobutrazole (PBZ) and cycocyl (CCC) to investigate long term storage with regard to both viability and subsequent regrowth of encapsulated pear buds throughout different storage time. To assay the genetic stability of pear *in vitro* regenerated from encapsulated shoot tips using biochemical markers of total water–soluble protein to be considered an important application of propagation for producing true to type explants and as one mean of conservation of genetic resources. Also of the possibility of inserting this storage phases in micropropagation cycles of pear.

MATERIALS AND METHODS

This work was conducted in the Department of Genetics and Cytology, National Research Center. Dokky. Giza, Egypt and Tissue Culture Laboratory, Department of Plant Biotechnology, Genetic Engineering and Biotechnology Research Institute (GEBRI), Menofyia University, Sadat City, Egypt during the years of 2006 and 2007.

Plant Material and Culture Conditions:

Meristem tips culture from pear rootstocks *Pyrus communis* L from branches of field-grown trees were obtained from the farm of Genetic Engineering and Biotechnology Research Institute (GEBRI), Menofyia University, Sadat City, Egypt. Explants were disinfected and stabilized shoot cultures of clonal pear were subcultures and maintained for three months on proliferation medium Murashige and Skoog (1962) (MS) supplemented with 1mg/l 6-benzylaminopurine (BAP), 0.5 mg/l naftalene- acetic acid (NAA), 30g/l sucrose and 7 g/l agar, pH5.7 according to Bahri-Sahloul *et al.*, (2005). The Explants were grown at 25°C during a 16 h light photoperiod with a light intensity of 2000 lux provided by cool white fluorescent tubes.

Encapsulation and Preparation of Beads:

Proliferated shoots with a healthy green appearance and well defined leaves were collected, and axillary buds removed by cutting the shoot 2-3mm. Explants were encapsulated as follows: each explants was immersed with a forceps in the alginate solution 3% w/v (Sigma USA) and then was dropped in the complexing solution, made of $CaCl_2$ 100 mM, for 30 min (Jung *et al*., 2004). After hardening, the capsul was rinsed for 10 min in distilled water to wash away calcium chloride residues. Regrowth rate of alive capsules (green and without necrosis) was evaluated as percentage of sown synseeds that had shown an increase in size, with breakage of the capsule and extrusion of at least one small shoot.

Effect of Growth Regulators and Storage Time on Encapsulated Buds:

Encapsulated shoot tips were cultured on conversion MS medium supplemented with 6- benzyladenine (BA), kinetin (Kin) or thidiazuron (TDZ). Each growth regulator added at the concentration of 0.0, 0.25, 0.50, 0.75 or 1.0mg/l to conversion medium supplemented with 30g/l sucrose and 7g/l agar. Germination % of encapsulated buds was recorded after 2, 4, 6, 8, 10, 12, 14 and 16 weeks of culture.

Effect of Different Types of Media and Storage Time on Encapsulated Buds:

This experiment aimed to investigate of encapsulated buds were cultured on different types of media, Murashige and Skoog1962 (MS), Gamborge *et al.,* 1968 (B_5), Nitsch and Nitsch 1969 (N_6) and Lloyd and McCown 1980 Woody Plant Medium (WPM) supplemented with 0.25mg/l BA, 30g/l sucrose and 7g/l agar. Germination % of encapsulated buds was recorded after 2, 4, 6, 8, 10 and 12 weeks of culture.

Effect of MS Salt Strengths and Storage Time on Growth Ability of Encapsulated Buds:

This investigation was performed to study the effect of solidified conversion media which consist of different strengths (MS, ½ MS, ¼ MS or ⅛ MS) supplemented with 0.25mg/l BA, 30g/l sucrose and 7g/l agar. Germination % of encapsulated buds was recorded after 2, 4, 6, 8 and 10 weeks of culture.

Effect of Sucrose Addition to the Storage Medium:

This study was carried out to determine the effect of added sucrose concentrations, at different concentrations 10, 20, 40 or 80g/l to the conversion medium consists of MS medium supplemented with 0.25mg/l BA, and 7g/l agar. Germination % of encapsulated buds was recorded after 2, 4, 6, 8, 10 and 12 weeks of culture.

Effect of Some Growth Retardants on the Conservation of Encapsulated Buds:

Encapsulated buds were cultured on media consist 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 7 g/l agar for long term storage of encapsulated pear buds. All pear capsules experiments were kept under the same conditions and were incubated at 15°C at dark. Germination % of encapsulated buds was recorded after 2, 4, 6, 8, 10, 12, 14 and 16 weeks of culture.

Protein Fingerprinting:

Leaves samples of pear were randomly taken from the donor plant as control, selected encapsulated pear buds from medium without growth retardants (control) or with growth retardants (CCC or ABA) at 0.5 or 1.5mg/l. Sodium dodecyl sulphate polyacrylamide (SDS-PAGE) was performed according to Laemmli (1970) The gel electrophoresis of protein was scanned with Gel Dec. (Bio Rad). The software used for integrating of peek areas was the quantity one gel doc 2000 data system (Hofer Scintific instruments) scans of the one lane sequentially negative to positive. Data were scored on bases of presence or absence of bands. The data analysis was carried out with statistical software package SPSS SYSTAT for windows, version 11.5.

Data Analysis:

All experiments were arranged in completely randomized design. Each treatment was represented as sixty replicate jars with five capsules per jar. Significant differences among the various treatments were compared using LSD test at 5% Steel and Torri, (1980).

RESULTS AND DISCUSSIONS

Effect of Some Growth Regulators and Storage Time on Growth Ability of Capsules:

Shoot tips excised from *in vitro* shoot cultures of pear were encapsulated to prepare synthetic seeds. Encapsulated were cultured onto medium supplemented with 0.0, 0.25, 0.50, 0.75 or 1.0mg/l BA, Kin or TDZ exhibited shoot development from encapsulated buds as shown in Table (1). Increasing of BA from 0.25 to 0.75mg/l gradually increased growth ability of capsules than other treatments. Maximum growth ability of shoots was observed in beads after 16 weeks from cultured Figure (1). All factors, the length of storage time, growth regulators type and different concentrations affected growth ability of stored capsules. The lowest growth was found in all Kin concentrations than other treatments. The growth of donor cultures on Kin did not improve their survival after ten weeks of storage, but it did improve their survival after 16 weeks from cultured. The development of germination % increased gradually from 2 to 16 weeks. Interaction between growth regulators and storage time gave highest significant differences at 5%. Encapsulated SEs of banana v. Rasthali (AAB germonic group) cultured on MS + 4.00 mM BA exhibited multiple shoot development (five to seven shoot per embryo) whereas only single shoot emergence was noted in the case of synthetic seed cultured on medium with 2.89 µM GA_3 or 5.37 µM NAA (Ganapathi *et al.,* 2001). In both cultivars of pear (*Pyrus communis)* and Rocha, an increase in BAP concentration resulted in a higher number of shoots per explant (Freire *et al*.,2002). The maximum frequency (91%) of conversion of encapsulated beads into plantlets was achieved on Murashige and Skoog (MS) medium containing 2.5 µM 6- benzyladenine (BA) and 0.5 µM α-naphthalene acetic acid (NAA) after 6 weeks of culture (Faisal and Anis 2007). The best morphogenetic response of the plantlets was when the encapsulated buds of *Aechmea fasciata* culture on MS medium supplemented with control, 2 mg/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 of cultured (Badr-Elden *et al*., 2007).

Table 1: Effect of growth regulators and storage time on growth ability of pear capsules.

Growth regulators type and conc. (mg/l)		Growth ability of pear encapsulated buds (week) %(B)								
		2	4	6	8	10	12	14	16	Mean(A)
Control		0.00	0.00	0.00	0.00	33.33	40.00	40.00	53.33	20.83 (cd)
BA	0.25	0.00	0.00	0.00	0.00	0.00	46.67	60.00	53.33	20.00 (cd)
	0.50	0.00	0.00	0.00	0.00	6.66	26.67	80.00	100.00	26.67 (bc)
	0.75	0.00	0.00	0.00	20.00	60.00	60.00	60.00	80.00	35.00 (a)
	1.0	0.00	0.00	0.00	0.00	20.00	26.67	93.33	100.00	30.00 (ab)
Kin	0.25	0.00	0.00	0.00	0.00	0.00	0.00	6.66	20.00	3.33 (e)
	0.50	0.00	0.00	0.00	0.00	0.00	6.66	6.66	26.67	6.00 (e)
	0.75	0.00	0.00	0.00	0.00	0.00	6.66	26.67	100.00	16.67 (d)
	1.0	0.00	0.00	0.00	0.00	0.00	46.67	46.67	46.67	17.50 (d)
TDZ	0.25	0.00	0.00	0.00	13.33	26.67	40.33	53.33	40.00	21.67 (cd)
	0.50	0.00	0.00	0.00	26.66	26.66	26.66	40.00	100.00	27.50 (adc)
	0.75	0.00	0.00	0.00	33.33	33.33	33.33	46.67	73.33	27.50 (adc)
	1.0	0.00	0.00	0.00	0.00	13.33	26.67	80.00	100.00	27.50 (abc)
Mean (B)		0.00 (f)	0.00 (f)	0.00 (f)	8.18 (e)	16.92 (d)	29.74 (c)	49.23 (b)	68.72 (a)	
LSD at 5% level						A=6.09 B=5.44 AXB=17.21				

Fig. 1: Development of synthetic seeds of pear during storage on growth regulators *in vitro*.

Effect of Different Types of Media on Regrowth Ability of Encapsulated Pear Buds:

Results in Fig. (2) show the effect of different media (MS, B_5, N_6 and WPM) on regrowth ability of stored encapsulated pear buds. MS and N_6 media gave the highest growth ability of capsules followed by B_5 and WPM media without any significant differences. Concerning the effect of storage time of encapsulated buds, the mean value clarified that increasing the storage time gradually from 2 to 12 weeks increased the percentage of growth ability of storage capsules. Interaction between different types of medium and storage time, when encapsulated cultured on MS, B_5 and N_6 scored the highest conversion of all capsules into shoots than WPM. Shoot tips have been encapsulated pear buds to prepare synthetic seeds that offer a novel delivery system for tissue cultured plants. Encapsulated buds can be handled like a seed and could be useful in minimizing the cost of production as 1 ml of medium is sufficient for encapsulation of a single shoot tip compared to

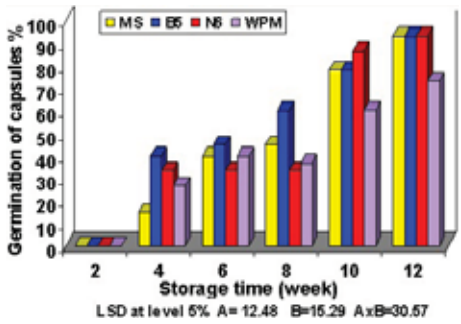

Fig. 2: Effect of different media and storage time on growth ability of stored encapsulated buds of pear *in vitro*.

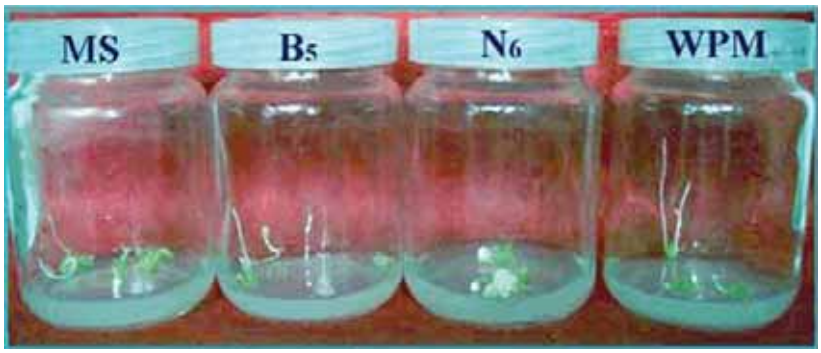

Fig. 3: Development of synthetic seeds of pear after storage on different types of media *in vitro*.

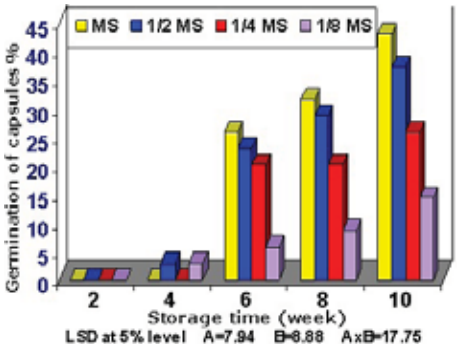

Fig. 4: Effect of different MS strengths and storage time on growth ability of encapsulated pear buds *in vitro*.

15-20 ml for conversion of shoot tips into plantlets. Niranjan and Sudarshana (2005) showed that the frequency of regeneration from the encapsulated embryos of Lagerstroemia indica L. was significantly affected by the concentration of alginate, the duration of storage and the effect of different types of media**.**

Effect of MS Salt Strengths on Growth Ability of Encapsulated Buds of Pear:

This investigation was performed to study the effect of different strength of MS medium (MS, ½ MS, ¼ MS or ⅛ MS) on growth ability of stored encapsulated pear buds. After storage, some beads appeared to be dry and did not generate while some of the others did not regenerate although they appeared to be healthy and still green. Growth of shoot was increased and observed in beads stored for ten weeks on MS and ½MS than other treatments. The growth of beads dependent on mineral concentration and storage time as shown in

Table 2: Effect of growth retardants concentration and storage time on regrowth of pear encapsulated buds *in vitro*.

Growth retardants and conc. (mg/l)(A)		Growth ability of pear encapsulated buds (week) %(B)								
		2	4	6	8	10	12	14	16	Mean(A)
Control		0.00	0.00	0.00	0.00	20.00	26.67	33.33	46.67	15.83 (ab)
PBZ	0.5	0.00	0.00	0.00	0.00	0.00	0.20	6.66	6.66	1.67 (d)
	1	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00 (d)
	1.5	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00 (d)
ABA	0.5	0.00	0.00	0.00	0.00	15.00	15.00	25.00	25.00	10.00 (c)
	1	0.00	0.00	0.00	0.00	10.00	10.00	25.00	30.00	9.38 (c)
	1.5	0.00	0.00	0.00	0.00	5.00	5.00	5.00	5.00	2.50 (d)
CCC	0.5	0.00	0.00	0.00	0.00	15.00	30.00	40.00	60.00	18.13 (a)
	1	0.00	0.00	0.00	0.00	15.00	20.00	30.00	45.00	13.75(abc)
	1.5	0.00	0.00	0.00	0.00	10.00	20.00	20.00	30.00	10.00 (bc)
Mean (B)		0.000 (d)	0.000 (d)	0.000 (d)	0.000 (d)	9.00 (c)	12.67 (c)	18.50 (b)	24.83 (a)	
LSD at 5% level							A=6.09 B=5.44 AXB=17.21			

Fig. (4). Generally, our presented results concerning the effect of different MS salt strengths and storage time after ten weeks appeared good activity of encapsulated buds when cultured in MS and ½ MS which gave the highest conversion percentage compared with other treatments. In this respect Ara *et al*., (1999) reported that the germ inability of somatic embryos (ESEs) of *Mangifera indica* was increased when the medium supplemented with full-strength B_5 micro salts with full-strength MS micro salts, iron EDTA and organics, 3% sucrose in the agar- gelled medium compared with quarter or half strength B5 macro salts. Multiplication, growth rate and plant mineral statues were influenced by mineral concentration in the medium. Increasing mineral concentration resulted in increased multiplication, growth rate and total mineral uptake by pear *(Pyrus pyrifolia* (Burm) Nak.) cv. Sebri explants (Amiri 2002). Of the planting media evaluated, % sprouting (shoot development) was the highest in MS medium supplemented with 4.44 µM BA and 0.54 µM NAA and lowest in ½ MS medium (Naik and Chand 2006). The effect of different MS salt strengths and duration of germinated encapsulated buds appeared good activity of encapsulated buds of jackfruit when cultured in MS and ½ MS which gave the highest conversion percentage compared with other treatments (Badr-Elden 2005).

Effect of Sucrose Concentrations and Storage Time on Growth of Encapsulated Pear Buds In vitro:

This experiment aimed to evaluate the effect of sucrose concentrations on the development of capsules (Figs. 5 and 6). Concerning the effect of storage time, the mean values showed that when storage time increased from 2 to 12 weeks the percentage of growth ability of encapsulated buds increased significantly from 0.00 to 53.75 %, respectively. Interaction between sucrose concentrations and different storage time it was clear that, the highest significant percentage(100%) growth ability was observed on 80 g/l sucrose after 10 and 12 weeks of storage, while, 40g/l sucrose gave highest growth ability (100%) at 12 weeks. This study indicates bright prospects of commercial application of alginate nutrient capsule technology in various situations like supply of certified plant material from both official and private organizations to the plant nurseries and/or exchange of germplasm at international level without refrigerated containers just in plastic cuvettes. This agreement with Adriani *et al*., (2000) they concluded that increase the concentration of sucrose in some steps of the protocol enhanced conversion, which in some conditions reached a rate of 57.5% in kiwifruit. Ganapathi *et al.*, (2001) reported that MS medium supplemented with sucrose gave the maximum conversion of encapsulated somatic embryos banana cv. Rasthali. The encapsulated shoot tips of Strawberry and Raspberry could be stored for 9 months in beads containing sugar or a culture medium (Lisek and Orlikowska2004).

Effect of Different Growth Retardants and Storage Time on Growth of Encapsulated Pear Buds In vitro:

Shoot tips excised from the shoot cultures of pear were encapsulated to prepare synthetic seeds, in 3% sodium alginate solution prepared in distilled water. Growth retardants play an important role on growth ability (%) of encapsulated buds as shown in Table (2). Synthetic seeds stored at 0.50 mg/l CCC remained viable without sprouting for up to 8 weeks and gave highest ability to growth after 16 weeks than other treatments, while 1 or 1.5mg/l PBZ blocked all capsules to grow. Meanwhile, increasing concentration of growth retardants cause a sharp decrease in the conversion %. Regarding the effect of storage time, the mean values cleared that germination duration was significantly affected by adding different concentrations of growth retardants to storage medium as the germination (%). Growth ability increased gradually from 0.0, 9.0, 12.67, and 18.50 to 24.83 % after 8, 10, 12, 14 and 16 weeks respectively. Regarding the interaction, results demonstrated that encapsulated pear buds cultured on MS medium supplemented with 0.5mg/l CCC resulted in highest

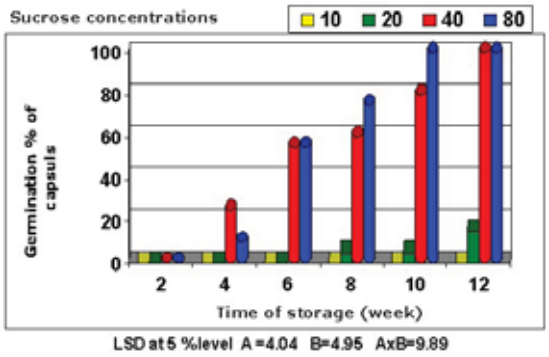

Fig. 5: Development of encapsulated buds of pear after different storage time (week) at different sucrose concentrations *in vitro*.

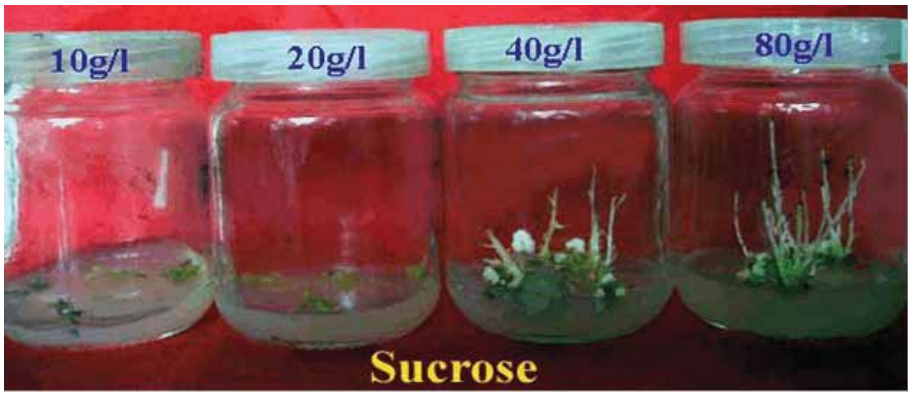

Fig. 6: Development of pear synthetic seeds after storage on different sucrose concentrations *in vitro*.

conversion % after 16 weeks of storage. Generally the encapsulated shoot tips can be handled like a seed and could be useful in minimizing the cost of production as 1 ml of medium is sufficient for encapsulation of a single shoot tip compared to 15-20 ml for conversion of shoot tips into plantlets. These results are in parallel with Rao *et al.,* (1993) they found that as compared to suckers, encapsulated shoot tips present as inexpensive, easier and safer material for germplasm exchange, maintenance and transportation**.** Somatic embryo of mango treated with ABA at different concentrations, with increasing ABA concentration from 0.04 to 0.20µM, percentage of germination and conversion into plantlets decreased gradually. The composition of the beads also contributed to survival, but had no effect on multiplication after storage (Ara *et al.,* 1999). Growth of donor cultures on a mannitol or pacloputrazol supplemented medium did not influence multiplication of Senga Sengana shoots after storage, but suppressed multiplication of raspberry stored for 3 months (Lisek and Orlikowska 2004). Encapsulated buds of *Aechmea fasciata* exhibited shoot development on MS supplemented with some growth retardants, PBZ, ABA and CCC (Badr-Elden *et al.* 2007).

Protein Fingerprinting:

The protein profiles of the control and plantlets obtained after encapsulation storage of pear rootstock are presented in Figure (7).Total numbers of polymorphic bands were 224, ranged from the minimum bands 49 (lan3) to maximum polymorphic bands 68 (lane 11) with an average 44.8 bands/ treatment. The highest percentage of polymorphic bands was 29.57 followed by 27.2, 24.1, 23.6 and 20.9 for bands number 11, 9, 3, 7 and 5 respectively. There isn't any monomorphic bands (present or absent) found for any lane. There is difference in the protein banding pattern between general control (lane 1) and control (encapsulated buds cultured on free growth retardants) (lan 2) , germination of capsules after storage without growth retardant (lane 3), this can be referred to the germination process. But it is important to notice that plantlets resulting from growth after germination of capsules with the growth retardant CCC 0.5 and 1.5mg/l (lanes 5 and 9) are the same of the general control (lane1) except intensities of protien bands. On the contrary, plantlets

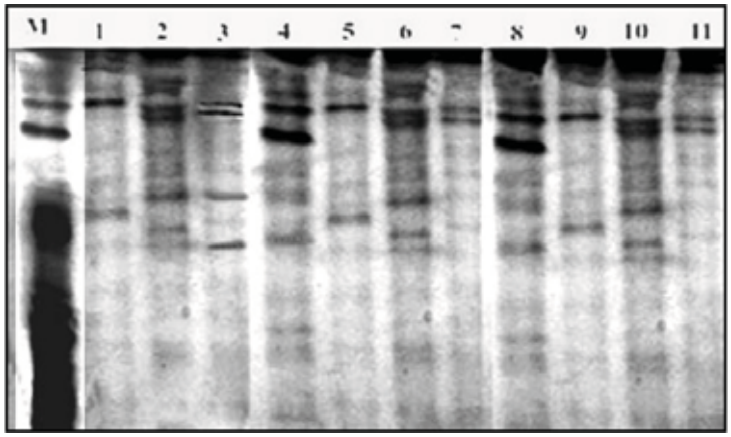

Fig. 7: Gel electrophoresis pattern for eleven treatments of pear explants after storage.
M-Marker
1 - Control (shoot tips)
2 - Encapsulated buds cultured on free growth retardants.
3 - Germination of capsules after cultured without growth retardants
4 - Encapsulated buds cultured on 0.5mg/l CCC
5 - Germination of capsules after cultured on 0.5mg/l CCC
6 - Encapsulated buds cultured on 0.5mg/l ABA
7 - Germination of capsules after cultured on 0.5mg/l ABA
8 - Encapsulated buds cultured on 1.5mg/l CCC
9 - Germination of capsules after cultured on 1.5mg/l CCC
10 - Encapsulated buds cultured on 1.5mg/l ABA
11 - Germination of capsules after cultured on 1.5mg/l ABA

resulting from germination of encapsulation and storage on growth retardant ABA 0.5 and 1.5mg/l (lanes 7 and 11) differ from the general control (lane 1). These results explain the better growth percentage when using CCC than using ABA as shown in Table (2), and emphasize that CCC is a good growth retardant for encapsulation storage and preserve genotype with genetic stability of pear. Preservation methods slow down cell metabolism and prevent somaclonal variation this is in agreement with (Yoshimatsu *et al.,* 1996 and El-Halwagy *et al.,* 2004).

REFERENCES

Adriani, M., E. Piccioni and A. Standard, 2000. Effect of different treatments on the conversion of 'Hayward' kiwifruit synthetic seeds to whole plants following encapsulation of *in vitro*-derived buds. New Zealand Journal of Crop and Horticultural Science, 28: 59-67.

Amiri, M.E., 2002. Mass propagation of a unique variety of pear (*Pyrus pyrifolia* (burm.) NAK. CV. Sebri) by shoot tip culture *in vitro*. Acta Horticulturae, 587.,2(92): 555-561.

Ara, H., U. Jaiswal and V.S. Jaiswal, 1999. Germination and plantlet regeneration from encapsulated somatic embryo of mango (*Mangifera indica L.*). Plant Cell Report, 19: 166-170.

Badr-Elden, A.M., 2005. Artificial seeds production, Germplasm conservation and Gene transfere of Jacfruit *(Artocarpus heterophyllus* L.) Plants by biotechnological Techniques. Ph.D., Thesis, Fac. Of Science, Cairo, Univ., Egypt.

Badr-Elden, A.M. , A.A. Nower and A.M.S. Arafa, 2007. *In vitro* synthetic seeds of *Aechmea fasciata* for germplasm conservation and exchange. Annals Of Agric.Sc.,Moshtohor, 45(1): 307-324.

Bahri-Sahloul, R, S. Ammar, A. Msallem and R. Mtar, 2005. Micropropagation of three Pyrus rootstocks. Advances-in-Horticultural-Science, 19(1): 21-28.

Bapat, V.A., 1993. Studies on synthetic seeds of sandalwood *(Santalum album* L.) and mulberry *(Morus indica* L.) *In:* Redenbaugh, K. *ed.* Synseeds: applications of synthetic seeds to crop improvement. Boca Raton, CA, United States, CRC Press Inc., 381-407.

El-Halwagi, A.A., S. Youssef, A. Abou-Zaied and M.H. Hussein, 2004. Molecular and chromosomal stability of cryopreservated almond tissues. Proceed. Int. Conf. Eng. And Appl., 1: 279-297.

Faisal, M. and M. Anis, 2007. Regeneration of plants from alginate-encapsulated shoots of *Tylophora indica* (Burm. f.) Merrill, an endangered medicinal plant. The Journal of Horticultural Science and Biotechnology, 82(3): 351-354.

Freire, I.C.G., C.P.S. Coelho and M.T.F. Barros, 2002. Improved culture media for the *in vitro* establishment of pear from nodal cuttings. Acta Horticulturae., 596: 2:457-461.

Gamborg, O.L., R.A. Miller and K. Ojima, 1968. Nutrient requirements of suspension cultures of soyabean root cells. Exp. Cell. Rers., 50: 151-158.

Ganapthi, T.R., L. Srinivas, P. Supranna and V.A. Bapat, 2001. Regeneration of plants from alginate – encapsulated somatic embryos of banana cv. Rasthali (*Musa* spp. AAB group). *In Vitro* Cell. Dev. Biol., 37: 178-181.

Gangopadhyay, G., T. Bandyopadhyay, R. Poddar1, S.B. Gangopadhyay and K.K. Mukherjee, 2005. Encapsulation of pineapple micro shoots in alginate beads for temporary storage. Current Science, 88(6): 972-977.

Jung, S.J., E.S. Yoon, J.H. Jeong and Y.E. Choi, 2004. Enhanced post germinative growth of encapsulated somatic embryos of Siberian ginseng by carbohydrate addition to the encapsulation matrix. Plant Cell Report., 23: 365-370.

Laemmli, U.K., 1970. Cleavage of structural proteins during the assembly of the head of bacteriophage. Nature, 227: 680-685.

Lisek A. and T. Orlikowska, 2004. *In vitro* storage of strawberry and raspberry in calcium-alginate beads at 4°C. Planl Cell, Tissue and Organ Culture., 78: 167-172.

Lloyd, G. and B. McCown, 1980. Commercially feasible micropropagation of mountain laurel *Kalmia latifolia* by use of shoot tip culture. Comb. Proc. Int. Plant Prop. Soc., 30: 431-427.

Micheli, M., I.A. Hafiz and A. Standardi, 2007. Encapsulation of *in vitro*-derived explants of olive (*Olea europaea* L. cv. Moraiolo) II. Effects of storage on capsule and derived shoots performance. Scientia Horticulturae, 113: 286-292.

Murashige, T. and F. Skoog, 1962. A resived medium for rapid growth and bioassays with tobacco tissue cultures. Physiol. Plant, (15): 473-497.

Na, H.Y. and K. Kondo, 1996. Cryopreservation of tissue cultured shoot primordial from shoot apices of cultured protocorms in Vanda pumila following ABA preculture and desiccation. Plany Science, 118: 195-201.

Naik, S.K. and P.K. Chand, 2006. Nutrient-alginate encapsulation of *in vitro* nodal segments of pomegranate (*Punica granatum* L.) for germplasm distribution and exchange. Scientia Horticulturae, 108: 247-252.

Niranjan, M.H. and M.S. Sudarshana, 2005. *In vitro* response of encapsulated somatic embryos of Lagerstroemia indica L. Indian J. Exp. Biol. 43(6): 552-554.

Nitsch, J.P. and C. Nitsch, 1969. Haploid plants from pollen grain. Science, 163: 85-87.

Rao, P.S., T.R. Ganapathi, P. Suprasanna and V.A. Bapat, 1993. Encapsulated shoot tips of banana: a new propagation and delivery system. InfoMusa, 2(2): 4-5.

Standardi, A. and E. Piccioni, 1998. Recent perspectives on synthetic seed technology using nonembryogenic in vitro derived explants. International Journal Plant science, 159(6): 968-978.

Steel, R.G.D. and Torrie 1980. Principals and procedure of statistics (2^{nd} ed.). Mc.

Yoshimatsu, K., H. Yamaguchi and K. Shimomura, 1996. Traits of Panax ginseng hairy roots after cold storage and Cryopreservation. Plant Cell Rep., 15: 555-560.