# Synthetic seed technology for encapsulation and regrowth of *in vitro*-derived *Gypsophila paniculata* L. shoot-tips

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## ABSTRACT

*Encapsulation of in vitro-derived shoot tips of Gypsophila in different concentrations of Na-alginate dissolved either in MS-salts solution or sterilized distilled water (SDW) as solvent was investigated. The encapsulated shoot-tips were stored 30 and 90 days at 4 ℃ and cultured either on MS basal medium or on MS-medium containing 0.5 mg/l each of NAA and BA. The highest frequency of shoot emergence and maximum number of shoots were recorded for beads encapsulated in 4% Na-alginate dissolved in MS- salt solution which stored 30 days and grown on medium containing BA and NAA. However, the root emergence and the percentage of root formation in shoots obtained by beads dissolved in SDW was higher than that derived from beads dissolved in MS salt solution especially with high levels of Na-alginate. Furthermore, the degree of vitrification was affected by Na-alginate concentration. The degree of vitrification was decreased from 100% to 20% by increasing the Na-alginate concentration from 2% to 4% dissolved in SDW. SDS-PAGE analysis of shoots produced from beads after 90 days storing demonstrated the similarity of produced plantlets in the protein profile. Finally, plants retrieved from the encapsulated shoot-tips coated in 4% Na-alginate were hardened off and successfully established in the growth chamber.*

***Key words:*** *Gypsophila paniculata, sodium alginate, encapsulation, shoot-tips, SDS-PAGE, synthetic seed.*

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## INTRODUCTION

*Gypsophila paniculata* L., is an ornamental plant, belonging to the family *Caryophyllaceae*. It is the major species used in commercial cut-flower production. *Gypsophila* is used traditionally as a filler for formal floral arrangements and bouquets, especially with roses. As a result of the sterile nature of *Gypsophila paniculata* plants (do not produce seeds) the real breeding of this plant is a dilemma (Shillo, 1985). Growers propagate *Gypsophila* vegetatively from tip cuttings. The low rooting frequency of cuttings likewise hinders propagation. Alternatively, *Gypsophila* can be propagated

by tissue culture (Han *et al.* 1991a, Zamorano-Mendoza and Mejia-Munoz, 1994, Song *et al.* 1996, Lee and Bae, 1999).

Synthetic seed technology is an alternative to traditional micropropagation for the production and delivery of cloned plantlets. Several aspects of the technique are still underdeveloped and hinder its commercial application (Brischia *et al.* 2002). This technology may be of value in breeding programs and allows the propagation of many elite genotype-derived plants in a short time (Nieves *et al.* 1998) and would provide an easy and novel propagation system for the elite as well as difficult to root species (Bapat *et al.* 1987). Also, encapsulation of propagules that were produced *in vitro* could reduce the cost of micropropagation of plantlets for commercialization and final delivery (Chu, 1995). This technology also has been employed for germplasm storage and exchange purposes as reported by Danso and Ford-Lloyd, (2003).

The importance of encapsulation technique as an aid to propagation in various crop plants has been discussed previously. In this respect, propagation of *Morus indica* L. (Mulberry) by encapsulated shoot and axillary buds was reported by Bapat *et al.* (1987) and Bapat and Rao, (1990). Growth and rooting of synthetic seeds of mulberry on three different growth media, vermiculite, sand and soil was studied (Machii and Yamanouchi, 1993). Propagation of some tropical forest trees through encapsulated shoot-tips or axillary buds was reported (Maruyama *et al.* 1997). Cryopreservation of embryonic axes of five almond (*Prunus amygdalus* Batsch) was tested by El-Halwagi *et al.* (2004). Conservation and propagation of four pharmaceutically important herbs, using axillary vegetative buds was reported (Mandal *et al.* 2000). A new method was described to produce encapsulatable units for synthetic seeds in *Asparagus officinalis* was reported by Kanji and Yuji, (2001). Also, shoot buds of vasaka (*Adhatoda vasica*) were encapsulated in Na-alginate (Anand and Bansal, 2002). Recently, Brischia *et al.* (2002) reported that synthetic seeds of M26 apple rootstock can be produced through organogenesis. However, encapsulation of somatic embryos produced in tissue culture to produce synthetic seeds was investigated on carrot (Timbert *et al.*, 1995), *Citrus reticulata* Blanco (Antonietta *et al.* 1998), *Carica papaya* L. (Castillo *et al.*, 1998) and *Siberian ginseng* (Choi and Jeong, 2002).

Gypsophila, as a vegetatively propagated plant and often cuttings proved difficult to rooting thus posing problems for breeding, is a particularly suitable candidate for synthetic seed technology, since only clonal propagation can guarantee high-yielding lines and good flower quality. The development of a synthetic seed technology from uniform clones may be a practical means of mass propagation for this valuable plant. However, only one report is available on cryopreservation of shoot tips of three species of *Gypsophila* (Fukai *et al.*, 1991). In this report we describe a method for encapsulation of *Gypsophila paniculata* shoot-tips from micropropagated shoots in order to evaluate the effect of this technique on the recovery of plantlets, and the applicability of the synthetic seed technology to *Gypsophila*. Also, the recovered plantlets were evaluated at the molecular level by SDS-PAGE analysis.

## MATERIALS AND METHODS

### Plant material and establishment of shoot cultures

Shoot tips of *Gypsophila paniculata* mature plants were surface sterilized by 70% Ethanol for 1 min, followed by 20% commercial clorox (containing 5.25% sodium

hypochlorite) for 20 min. After three successive rinses in sterile distilled water the explants (about 0.25 cm in length) were excised and placed in glass tubes containing 20 ml of MS-medium (Murashige and Skoog, 1962) supplemented with 0.5 mg/l BA (6-benzyladenine) + 0.5 mg/l NAA (∝-naphthaleneacetic acid). After one month, the proliferated *Gypsophila* shoots were established (Fig. 3-a).

**Culture media and conditions**

The MS basal medium was supplemented with 3% sucrose and 100 mg/l inositol. Media were solidified with 0.7% agar and adjusted to pH 5.8 prior to autoclaving for 20 min at 121°C and a pressure of 1.2 kg $cm^{-2}$. NAA and BA were added to the media before pH was adjusted. Cultures were incubated in a growth chamber at 25 ± 2°C under a 16 hour photoperiod (irradiance of about 40 µmol $m^{-2}$ $s^{-1}$ provided by cool white fluorescent lamps).

**Encapsulation of Gypsophila shoot tips**

After several proliferation subcultures, shoots were randomly chosen at the end of the third subculture period and used to obtain shoot tips for encapsulation. Shoot tips (0.25 cm long) were isolated and mixed with different solutions of autoclaved Na-alginate (2, 3, 4 and 5%) prepared in either MS basal salt solution or sterilized distilled water for 20 min, then the shoot tips were dropped into a solution of $CaCl_2.2H_2O$ (0.9% w/v). The drops were allowed to remain in this solution for 30 min on a gyratory shaker (80 rpm) to complete the complexation process. After the incubation period, the beads were recovered by decanting off the $CaCl_2.2H_2O$ solution and washing them 3 times with autoclaved washing liquid MS-medium. After encapsulation, beads were stored at 4°C (in darkness) in Petri dishes containing MS basal liquid medium and sealed with parafilm.

**Growth of stored beads on media**

After 30 and 90 days, the stored beads were sown on MS basal medium and MS-medium supplemented with 0.5 mg/l NAA + 0.5 mg/l BA and solidified with 7 g/l agar, then the cultures were incubated in a growth chamber. Shoot number, shoot length, rooting percentage and vitrification percentage (evaluated by visual observations) were recorded after one month of cultivation. Data obtained were subjected to statistical analysis using standard error (SE) according to the method described by Snedecor and Cochran, (1967).

**Electrophoresis (SDS-PAGE)**

Proteins were extracted from fresh leaves (0.5 g) of plantlets produced from beads stored 90 days by homogenizing in sodium phosphate buffer, pH 6.8. A 30 µg portion of protein was separated in 10% SDS-PAGE (Sodium dedocyl sulphate - Polyacrylamide gel electrophoresis) according to Laemmli, (1970). The separation was carried out using EC mini gel unit at 60 volt for four hr. Gels were stained with Coomassie brilliant blue (R-250), destained with high methanol solution (40 % methanol in 10% acetic acid), photographed and the molecular weights of polypeptide bands were calculated from a calibration curve of low molecular weight marker standards of Pharmacia.

## RESULTS AND DISCUSSION

### Effect of alginate matrix composition and storage duration on regrowth

**(A) Conversion of synthetic seeds after 30 days of storage**

Shoot tips were coated in different concentrations (2-5%) of Na-alginate dissolved in either MS liquid media or SDW to determine the ideal concentration for encapsulation. Firm beads were sufficiently formed (Fig. 3-b), which resulted in an increased germination percentage at the first week of cultivation. Shoots proliferation from the beads occurred within 8-10 days of cultivation by breaking open the Na-alginate matrix resulting in emergence of shoots. This was followed by gradual emergence of roots after 3 weeks. Data in Table (1) demonstrate the growth of *Gypsophila* beads stored for one month and grown on MS basal medium. Beads prepared by 4% Na-alginate in MS salt solution or SDW gave the highest number of shoots than other treatments (2.8 and 2.7, respectively). On the other hand, shoot length was relatively low especially with beads prepared in MS solution. As shown in Fig. (1-A) the encapsulated shoot tips prepared with 2% Na-alginate and dissolved in either MS solution or SDW exhibited no rooting response when grown on MS basal medium. Whereas a high rate of rooting (96 and 98% respectively) was observed when shoot tips encapsulated with 4% Na-alginate (Fig. 3 c,d). Observation after 30 days from sowing showed that increasing Na-alginate to 5% reduced shoot vitrification (Fig. 1-A). It was observed that vitreous shoots did not root vigorously. This may be partly due to the poor development of the wax layer which controls excessive evaporation through the cuticle (John and Webb, 1987).

In general, it could be concluded that the encapsulated shoot-tips (beads) stored for 30 days at 4°C could be germinated and they developed shoots on MS-medium. The best results within these treatments were obtained when beads were prepared with ½ MS basal medium mixed with 4% Na-alginate. In this respect, Bapat *et al.* (1987) reported that encapsulated axillary buds of mulberry could be stored at 4°C for 45 days without loss of viability and subsequently regenerated complete plantlets on an appropriate medium. Isolated shoot buds from multiple shoot cultures of *Adhatoda vasica* Nees. were encapsulated in 3% Na-alginate with different gel matrices (Anand and Bansal, 2002).

Growth of *Gypsophila* beads stored for one month and grown on MS medium containing 0.5 mg/l NAA + 0.5 mg/l BA was investigated (Table 2). Data obtained showed that beads encapsulated in 4% Na-alginate dissolved in MS salt solution recorded the highest shoot number and the lowest shoot length among all treatments. The percentage of root formation in shoots obtained from beads dissolved in SDW was higher than that derived from beads dissolved in MS salt solution, especially with high levels of Na-alginate (Fig. 1-B). All the plantlets recovered from this treatment were robust and had good shooting and a vigorous root system. The plantlets obtained were prepared for acclimatization. In this respect, Mandal *et al.* (2000) reported that synthetic seeds were produced by encapsulating axillary buds of four *Ocimum* species in calcium alginate gel which contained MS nutrients and 1.1-4.4 µM BA. Shoots emerged from the encapsulated buds on all six planting media tested. However, the highest frequency shoot emergence and maximum number of shoots per bud were recorded on media containing BA. Maximum conversion of the encapsulated shoot buds from shoot cultures of *Adhatoda vasica* Nees. into plantlets was achieved on Gamborg's B5 medium containing 4.65 µM kinetin and 50 mg/l Phloroglucinol and the plantlets were successfully grown in soil (Anand and Bansal, 2002).

Degree of vitrification was highest (100%) in shoots derived from beads coated in 2% Na-alginate and dissolved in either MS salt solution or in SDW, but it had decreased reaching 60% with beads encapsulated in 4% Na-alginate dissolved in SDW (Fig. 1-B). High percentage of vitrification may be due to the presence of cytokinins in the medium or due to mineral salts in beads prepared with MS salt solution, as they may stimulate stress ethylene production which is regarded as a possible trigger of vitrification (Kevers *et al.*, 1984).

**(B) Conversion of synthetic seeds after 90 days of storage**

In order to test the effect of storage duration, beads stored for 90 days at 4°C were cultured in MS basal medium and MS-medium supplemented with 0.5 mg/l NAA + 0.5 mg/l BA. Data presented in Table (3) showed similar regrowth trends as beads stored for 30 days when cultured on growth media tested, but the conversion had decreased. When SDW alone was used as the solvent for 4% Na-alginate, 2.6 shoots were proliferated from a single bead, while when alternatively, the Na-alginate was mixed with MS salt solution the shoot number frequency arose to 3 shoots per bead. Rooting of the proliferated shoots was poor when the beads coated in 2 and 3% Na-alginate dissolved in MS salt solution or in SDW (Fig. 2-A). Visual Observation for proliferated shoots demonstrated that a high percentage of vitrification was observed in shoots produced from all beads reaching 100% and 90% with beads coated in 2 or 3% Na-alginate and dissolved in MS salt solution or SDW. The lowest degree of vitrification (80%) was recorded with beads coated in 4% Na-alginate and prepared in SDW (Fig. 2-A).

In this connection, different authors reported the difficulty of synthetic seed conversion. Redenbaugh *et al.* (1987) suggested that conversion frequencies of encapsulated alfalfa somatic embryos was decreased after 7 days storage. This may be due to inhibition of embryo respiration inside the alginate capsules. Our results are in accordance with those obtained by Danso and Ford-Lloyd, (2003) who found that plant regrowth by encapsulated nodal cuttings and shoot tips of cassava was significantly affected by the duration of the storage period, as shoot recovery decreased from almost 100% to 73.3% for encapsulated nodal cuttings and 94.4% to 60% for shoot tips after 28 days of storage.

Data presented in Table (4) showed that, the highest shoot number (3.6 and 3.2) was obtained when beads coated in 4% Na-alginate prepared in MS salt solution and SDW, respectively. Shoot length of the proliferated shoots were correlated inversely with shoot number as mentioned before. It was observed that all proliferated shoots failed to produce roots in the tested medium (Fig. 2-B and Fig. 3, E and F). Degree of vitrification was less in shoots derived from beads prepared in SDW than shoots derived from beads dissolved in MS salt solution. However, a slight decrease in vitrification of the produced shoots was observed with increasing Na-alginate levels used in encapsulation (Fig. 2-B).

The shoots produced from beads (coated in 4% Na-alginate) grown in MS-medium with 0.5 BA and 0.5 NAA were generally higher than those produced from growing in MS basal medium. This may be due to cultivation of beads in medium containing BA and NAA which promoted shoot growth of the cultivated beads. Also, the absence of roots in all treatments may be ascribed to the high degree of vitrification and the presence of hormones promoting shoot multiplication in the culture medium.

However, higher or lower concentrations of Na-alginate used in encapsulation reduced the conversion frequency of beads. Redenbaugh *et al.* (1987) and Redenbaugh, (1993) noted that variables related to encapsulation method, including alginate type and levels and medium used to produce synthetic seeds, were responsible for significant variations in conversion percentages for alfalfa, carrot and celery. Adriani *et al.* (2000) stated that although encapsulation is considered necessary of the 'Hayward' kiwifruit (*Actinidia deliciosa* (A. Chev.) it depressed microcuttings' vigour and vegetative activity.

**Electrophoresis (SDS-PAGE) of converted plantlets**

Total soluble proteins of eight randomly selected tissue-culture derived *Gypsophila* shoots represent all treatments used were extracted and subjected to protein electrophoresis. This is in order to compare the protein banding patterns of plantlets or shoots produced from beads after 90 days storing. Screening of different *Gypsophila* plantlets using SDS-PAGE revealed that the protein profiles of 100% of *in vitro* produced plantlets derived from beads are similar and no detectable differences were observed. The groups of protein which characterize the produced plantlets are approximately 66 and 45 kDa as shown in Figure (3- G and H), black arrows). Molecular characterization is a prerequisite for the identification of the *in vitro* produced plantlets. SDS-PAGE protein analysis in many published reports was used to proof the identity of regenerated plants *in vitro* to their intact plants and also to assess the variations which may occur. In this respect, some efforts have been made to employ molecular markers to distinguish cell lines of different regenerants produced *in vitro* (Ultrika *et al.* 1993, Hendriks and Veries, 1995). El-Kazzaz and Taha, (2002) found that SDS-PAGE protein profiles analysis confirmed the identity of the regenerants from shoot-tips cultures of Broccoli to their parents plants. Recently, El-Halwagy *et al.* (2004) did not detect any polymorphism in plantlets regenerated from frozen embryogenic axes of almond caused by cryopreservation using protein profiles, isozyme, RAPD analysis and cytological examination.

In conclusion, the results described here are promising, since this is considered the first report on encapsulation of *Gypsophila* shoot tips. The procedure used in this study can bring about rapid multiplication where the shoot and root system were formed *in vitro* within one month of cultivation from encapsulated *Gypsophila* beads, the intermediary stages of shoot elongation and rooting can be eliminated. Those beads can ensure availability of a planting material throughout the year. Moreover, this type of capsules could be useful in exchanges of sterile material between commercial laboratories due to the small size and relative ease in handling, transportation, or in conservation of elite germplasm (Accart *et al.* 1994) and would minimize the cost of production (Fabre and Dereuddre, 1990). Additionally, the storage life for encapsulated shoot-tips of *Gypsophila* was found to be quite short using the current procedure. There was a significant decrease in subsequent conversion especially of beads after 90 days storage. The reason may be attributed to difficulty in penetrating the outer surface of capsules or complexing time. Variables related to the encapsulation method, including alginate type and levels and medium used to produce synthetic seeds, were responsible for significant variations in the conversion percentages for alfalfa, carrot and celery (Redenbaugh, 1993).

Vitrification, is a known phenomenon usually occurred in tissue culture of *Gypsophila*. In the current study, high degree of vitrification was observed in most treatments, but it can be overcome by increasing agar levels or nitrate:ammonium ratio of the medium and incubation of cultures under low temperature (Han *et al.* 1991a,b) or by using special vessels in culture (Lee and Bae, 1999). These results are a step forward in the development of economically produced synthetic seeds in cut-flower species.

***Table (1). Growth of Gypsophila beads stored for 30 days and grown on MS basal medium.***

| Na-alginate (%) | Beads prepared in MS-medium | | Beads prepared in SDW | |
|---|---|---|---|---|
| | Shoot No. | Shoot length (cm) | Shoot No. | Shoot length (cm) |
| 2 | 2.6 ± 0.24 | 3.62 ± 0.20 | 2.4 ± 0.24 | 4.68 ± 0.16 |
| 3 | 2.4 ± 0.24 | 4.44 ± 0.28 | 2.2 ± 0.20 | 4.14 ± 0.14 |
| 4 | 2.8 ± 0.37 | 4.28 ± 0.23 | 2.7 ± 0.37 | 4.60 ± 0.17 |
| 5 | 2.4 ± 0.24 | 4.72 ± 0.15 | 2.4 ± 0.24 | 4.74 ± 0.38 |

Values are means ± SE

***Table (2). Growth of Gypsophila beads stored for 30 days and grown on MS medium containing 0.5 mg/l NAA + 0.5 mg/l BA.***

| Na-alginate (%) | Beads prepared in MS-medium | | Beads prepared in SDW | |
|---|---|---|---|---|
| | Shoot No. | Shoot length (cm) | Shoot No. | Shoot length (cm) |
| 2 | 2.8 ± 0.20 | 4.2 ± 0.14 | 2.4 ± 0.24 | 4.32 ± 0.15 |
| 3 | 3.4 ± 0.25 | 4.9 ± 0.13 | 2.8 ± 0.38 | 4.10 ± 0.07 |
| 4 | 4.0 ± 0.32 | 4.0 ± 0.13 | 3.6 ± 0.25 | 3.74 ± 0.19 |
| 5 | 3.0 ± 0.31 | 4.6 ± 0.16 | 2.6 ± 0.24 | 4.14 ± 0.16 |

Values are means ± SE

***Table (3). Growth of Gypsophila beads stored for 90 days and grown on MS basal medium.***

| Na-alginate (%) | Beads prepared in MS-medium | | Beads prepared in SDW | |
|---|---|---|---|---|
| | Shoot No. | Shoot length (cm) | Shoot No. | Shoot length (cm) |
| 2 | 2.2 ± 0.37 | 3.68 ± 0.11 | 2.0 ± 0.32 | 3.58 ± 0.18 |
| 3 | 2.4 ± 0.40 | 3.66 ± 0.12 | 2.0 ± 0.32 | 3.38 ± 0.14 |
| 4 | 3.0 ± 0.32 | 3.14 ± 0.10 | 2.6 ± 0.40 | 3.04 ± 0.08 |
| 5 | 2.2 ± 0.20 | 3.65 ± 0.10 | 1.8 ± 0.20 | 3.30 ± 0.07 |

Values are means ± SE

***Table (4). Growth of Gypsophila beads stored for 90 days and grown on MS medium containing 0.5 mg/l NAA + 0.5 mg/l BA.***

| Na-alginate (%) | Beads prepared in MS-medium | | Beads prepared in SDW | |
|---|---|---|---|---|
| | Shoot No. | Shoot length (cm) | Shoot No. | Shoot length (cm) |
| 2 | 2.6 ± 0.25 | 3.96 ± 0.14 | 2.2 ± 0.20 | 3.76 ± 0.10 |
| 3 | 2.8 ± 0.37 | 3.94 ± 0.13 | 2.2 ± 0.38 | 3.30 ± 0.16 |
| 4 | 3.6 ± 0.24 | 3.48 ± 0.17 | 3.2 ± 0.32 | 3.26 ± 0.15 |
| 5 | 2.4 ± 0.24 | 4.06 ± 0.10 | 1.8 ± 0.38 | 3.92 ± 0.06 |

Values are means ± SE

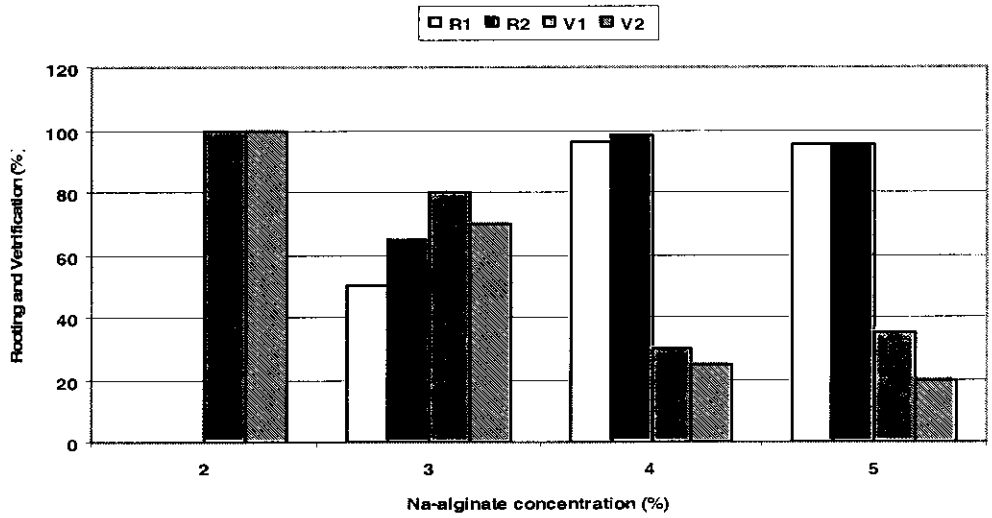


*Fig. (1-A ) Effect of different concentrations of Na-alginate used in encapsulationof beads stored 30 days and grown on MS- basal medium on rooting and vitrification .*

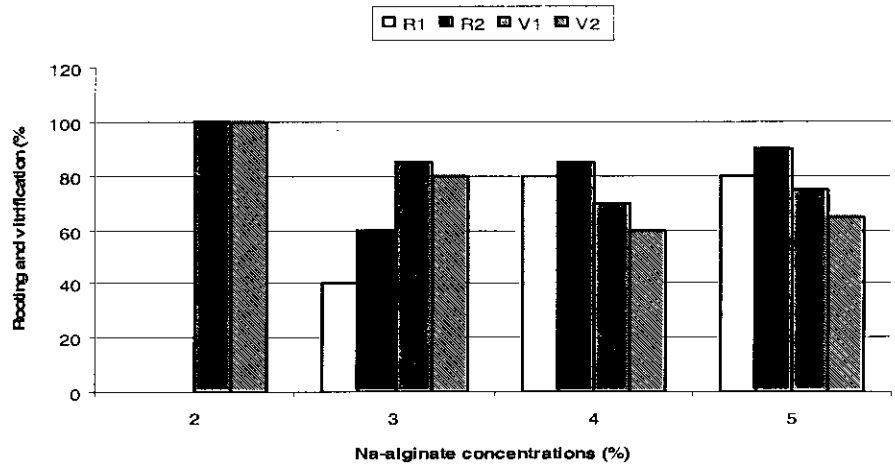


*Fig.(1-B) Effect of different concentrations of Na-alginate used in encapsulation of beads stored for 30 days and grown on MS+0.5 mg/l BA+0.5 mg/l NAA on rooting and vitrification*

*R1= Rooting of beads prepared in MS solution.*
*R2= Rooting of beads prepared in SDW.*
*V1= Vitrification of shoots produced from beads prepared in MS solution.*
*V2= Vitrification of shoots produced from beads prepared in SDW.*

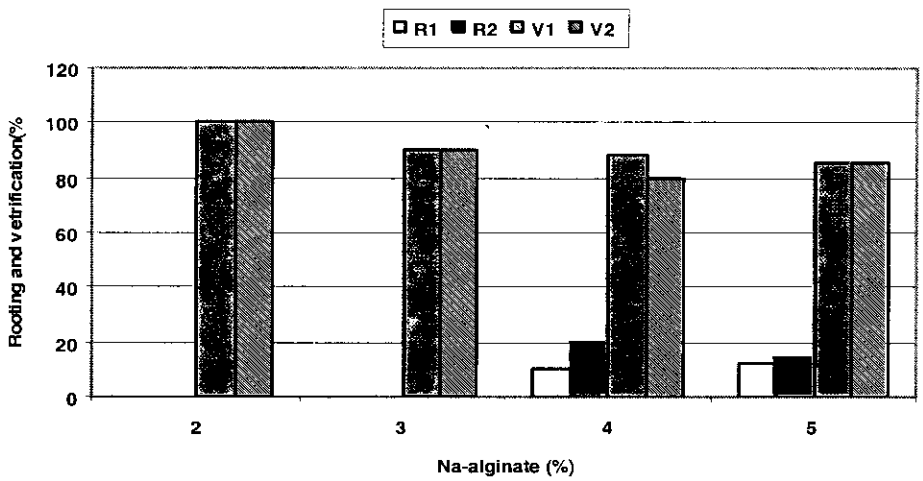


*Fig. (2-A ) Effect of different concentrations of Na-alginate used in encapsulation of beads stored for 90 days and grown on MS basal medium on rooting and vetrification*

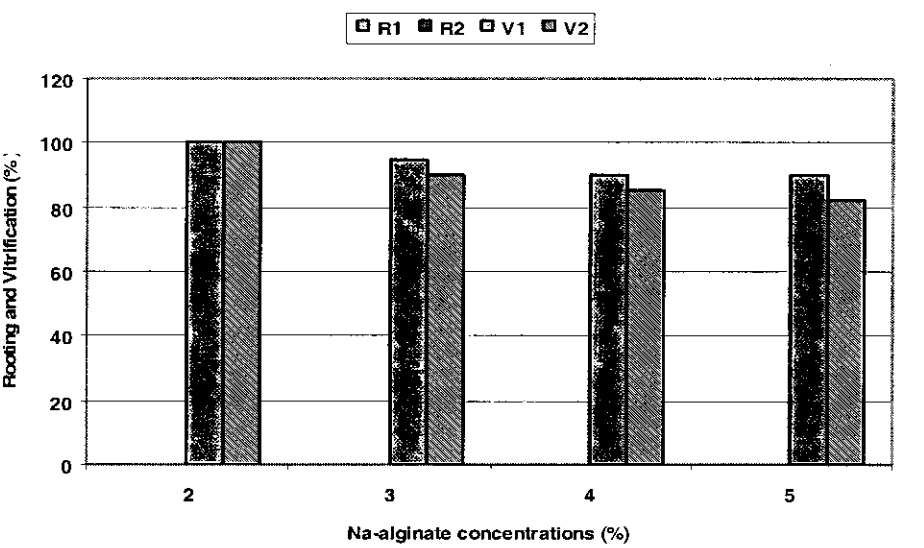


*Fig .(2-B) Effect of different concentrations of Na-alginate used in encapsulation of beads stored for 90 days and grown on MS medium + 0.5 mg/l each of BA and NAA on rooting and vitrification*

*R1= Rooting of beads prepared in MS solution.*
*R2= Rooting of beads prepared in SDW.*
*V1= Vitrification of shoots produced from beads prepared in MS solution.*
*V2= Vitrification of shoots produced from beads prepared in SDW.*

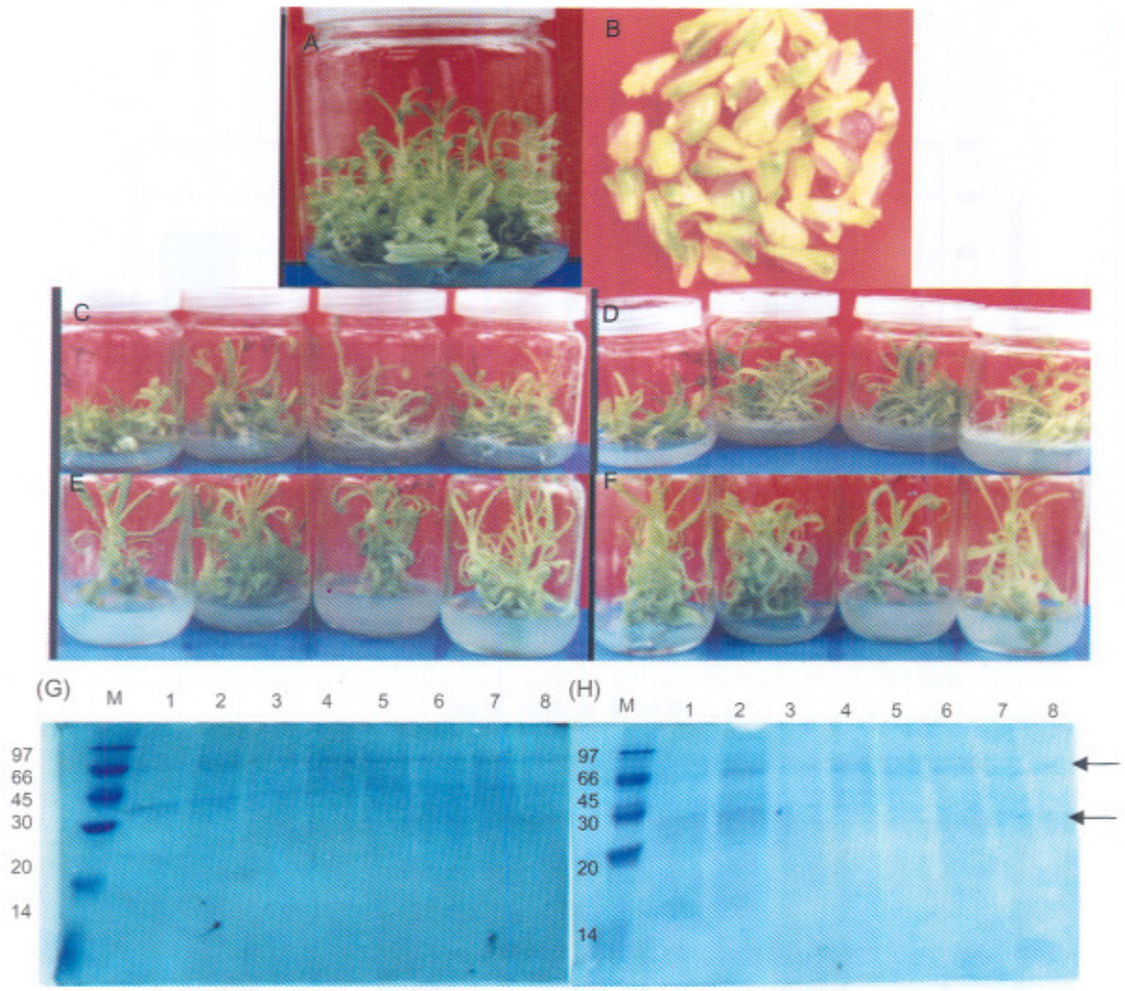


*Fig. (3) Encapsulation and regrowth of in vitro derived Gypsophila shoot tips.*
*A = Shoot cultures*
*B = Encapsulation of Gypsophila shoot-tips in 4% Na-alginate*
*C,D = Complete plantlets produced from beads stored 30 days and grown on growth media.*
*E,F = Shoots produced from beads stored 90 days and grown on growth media.*
*G,H = SDS-PAGE protein profiles of different Gypsophila shoots produced from beads stored 90 days and grown on MS basal medium (lanes 1-8-G) and MS-medium + 0.5 mg/l each of BA and NAA (lanes 1-8-H). Low molecular weight marker (M) are given in kDa at the left of the figure.*

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## الملخص العربي

### أستخدام تكنولوجيا البذور الأصطناعية فى تغليف وأعادة نمو القمم الخضرية الناتجة معمليا لنبات الجبسوفيلا

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فى هذا البحث تم تغليف القمم الخضرية الناتجة من الزراعة النسيجية لنبات الجبسوفيلا بواسطة عدة تركيزات متنوعة من ألجينات الصوديوم المذابة أما فى محلول أملاح بيئة MS موراشيج وسكوج (1962) أو فى ماء مقطر معقم وذلك بهدف دراسة أمكانية أنتاج بذور أصطناعية للأنتاج التجارى لهذا النبات. وقد خزنت الكبسولات المعدة لمدة 30 و 90 يوما تحت درجة حرارة 4 °م ثم زرعت على بيئتين الأولى هى بيئة MS بدون هرمونات والثانية تحتوى على نفثالين حمض الخليك NAA وبنزيل الأدنين BA بتركيز 0.5 ملليجرام/لتر لكل منهما. أوضحت النتائج أن أفضل نمو خضرى كان للكبسولات المخزنة 30 يوما (التى أعدت فى تركيز 4% ألجينات الصوديوم والمذابة فى محلول أملاح MS) والنامية على بيئة MS المحتوية على NAA و BA . كما كانت أفضل نسبة لتكوين الجذور فى النموات الخضرية الناتجة من الكبسولات المخزنة 30 يوما (التى أعدت فى تركيز 4% ألجينات الصوديوم والمذابة فى ماء مقطر معقم) والنامية على بيئة MS بدون هرمونات. كذلك أظهرت النتائج أيضا أن ظاهرة التزجيج Vitrification للنموات الخضرية الناتجة من الكبسولات قد قلت من 100% فى الكبسولات المعدة فى 2% ألجينات صوديوم الى 20% فى الكبسولات المعدة فى 4% ألجينات صوديوم. وبصفة عامة فأن تخزين الكبسولات لمدة 90 يوما كان لة تأثير سلبى واضح على نموها على البيئات المختبرة وفشلها فى تكوين الجذور. كما أوضحت نتائج تحليل أنماط التفريد الكهربى للبروتين عدم وجود أى أختلافات بين النموات الخضرية الناتجة من الكبسولات المعدة فى تركيزات متنوعة من ألجينات الصوديوم والتى خزنت لمدة 90 يوما. وقد تمت عملية الأقلمة المعملية للنباتات الناتجة من الكبسولات المعدة فى تركيز 4% ألجينات الصوديوم والمذابة فى محلول أملاح MS بنجاح.