CHAPTER 6

PLANT REGENERATION FROM SYNTHETIC SEEDS OF *Gerbera jamesonii* Bolus Ex. Hook f. *IN VITRO*

6.1 EXPERIMENTAL AIMS

Any vegetative part of plants such as shoot tips, protocorm like bodies and nodular segments can be used as propagules for encapsulation and production of synthetic seeds. Besides that, somatic embryos are also well known to be suitable as propagules and ideally coated as synthetic seeds. Through encapsulation technique (Redenbaugh *et al.*, 1986) encapsulation matrix gel, containing nutrients is coated around the somatic embryo. Similar process can be applied to other vegetative parts of plants to produce synthetic seeds. Nutrients, carbon source and hormones were added in the encapsulation matrix to aid germination process. Synthetic seeds germination rates are also influenced by the type of germination media used such as agar, garden soil and vermiculite.

The objective of this study is to produce synthetic seeds from somatic embryos (globular and cotyledonary phase) and micro shoots of *Gerbera jamesonii*. The effects of various sucrose concentrations plant hormones in the encapsulation matrix were examined. Effect of germination rate of *Gerbera* synthetic seeds, stored at low temperature and different duration were also investigated. Germination and regeneration rates of synthetic seeds of *G. jamesonii* were determined. Varieties of propagules could be chosen as source of explants to form synthetic seeds for the aim of increasing

regeneration potentials in many plant species particularly plants that encounter germination problems and seedless plants. Plantlets obtained from the germination of synthetic seeds of *G. jamesonii* were further acclimatized in the greenhouse.

Natural seeds of *G. jamesonii* are hardly found and cannot be purchased in Malaysia. They are imported and expensive. Hence, the production of synthetic seeds of *G. jamesonii* allows large supply of the plant stock. Moreover, it is valuable and worthy to plant temperate grown ornamental plants in tropical countries like Malaysia since the temperate plants are very popular here. Synthetic seeds produced could be stored at low temperatures and germinated when necessary. With the success production of synthetic seeds of *G. jamesonii*, the cost for obtaining *Gerbera* stock could be minimized since the plant stock is readily available. Nevertheless, plant production cost is in some ways minimized. Synthetic seed technology is now an important tool and alternative method to mass propagate many plant species. Synthetic seeds of *G. jamesonii* could now be produced and commercialized in large scale with low production cost. Through this technology, it is now possible to plant and cultivate the temperate grown plants in tropical climate countries like Malaysia.

6.2 MATERIALS AND METHODS

6.2.1 Preparation of Explants

Micro shoots sized ranging from 3-5 mm, globular and cotyledonary phase of somatic embryos were used as source of explants in this experiment. These two explants were encapsulated to form synthetic seeds. Regeneration of plantlets from synthetic seeds of *G. jamesonii* Bolus ex. Hook f. was investigated in this chapter.

6.2.2 Formation of Synthetic Seeds

Micro shoots and somatic embryos were encapsulated using encapsulation technique. Optimum encapsulation matrix consists of 3.0% sodium alginate added with Ca-free MS with the addition of 3.0% sucrose supplemented with 2.0 mg/l BAP and 0.5 mg/l NAA (based on experiment in chapter 5). Each explant was encapsulated using encapsulation matrix that consists of 3.0% sodium alginate solution and then treated with 100 mM $CaCl_2.2H_2O$ solution for 30 minutes. Subsequently, all synthetic seeds formed were rinsed with liquid MS basal medium for 3 times. These beads were retrieved using a nylon mesh.

6.2.3 Plant Regeneration from Synthetic Seeds

Up till now, regeneration of *G. jamesonii* Bolus ex. Hook. f from germination of synthetic seeds has not been reported. Thus, in this study, factors that influenced regeneration of *G. jamesonii* through the production of synthetic seeds were studied.

Germination of synthetic seeds in *G. jamesonii* was found to be successful and regeneration of new plantlets was achieved. The factors studied were;

6.2.3.1 Germination Media

For germination of synthetic seeds, encapsulated explants were inoculated and cultured in various germination media through *in vivo* and *in vitro* system. The germination media used were;

i. MS basal solid medium
ii. MS basal liquid medium
iii. MS medium added with 2.0 mg/l BAP and 0.5 mg/l NAA
iv. Sterile 'vermiculite'
v. Sterile garden soil

MS media were prepared with the addition of 30 g/l sucrose and 8.0 g/l agar (for solid medium). *In vitro* germination was carried out in MS liquid and solid medium and autoclaved vermiculite, whereas, *in vivo* germination was done using garden soil. From these experiments, optimum germination medium could be determined based on the rate of synthetic seeds germination.

All cultures were maintained in the culture room at 25 ± 1 ^{o}C under 16 hours light and 8 hours dark. Seeds inoculated in liquid medium were placed on a shaker at 100 rpm and incubated in the culture room. A set of unencapsulated explants were also inoculated

on all germination media and maintained under the same conditions as control. Each treatment consisted of 30 replicates.

6.2.3.2 Effects of Different Sucrose Concentrations in Culture Medium

Different sucrose concentrations ranging from 1.0%, 2.0%, 3.0%, 4.0%, 5.0% and 6.0% were added in every 1000 ml of encapsulation matrix for the preparation of synthetic seeds from micro shoots and somatic embryos. All explants were encapsulated with encapsulation matrix consists of 3.0% sodium alginate solution with the addition of MS basal medium containing sucrose at different concentrations. These synthetic seeds were cultured on optimum germination medium (refer to 5.2.3.1). Thirty replicates were used in each treatment.

6.2.3.3 Effects of Hormones in Encapsulation Matrix

In the preparation of encapsulation matrix 3.0% sodium alginate solution was prepared in MS basal medium, Ca-free MS basal medium and distilled water. Combination of 2.0 mg/l BAP and 0.5 mg/l NAA were also added during the preparation of encapsulation matrix. Sodium alginate solution without the addition of hormone was used as control. Based on experiments in chapter 2, 2.0 mg/l BAP and 0.5 mg/l NAA was identified as the optimum hormone combination in regeneration of shoots. Thirty replicates were used in each treatment.

6.2.4 Storage of Synthetic Seeds

Encapsulated explants were preserved at 4 ± 1 ^{o}C for 0, 30, 60, 90, 120, 150 and 180 days. After storage, the synthetic seeds were inoculated and cultured in the optimum germination medium (refer to 5.2.3.1). Thirty replicates were used in each treatment.

6.2.5 Data Analysis

Data obtained were analyzed using Duncan's Multiple Range Test (DMRT). Mean with different letters in the same column differ significantly at p=0.05

6.3 RESULTS

Micro shoots and somatic embryos of *G. jamesonii* were used as propagules for the production of synthetic seeds. Globular, heart and cotyledonary stages of *Gerbera* somatic embryos were induced. Globular and cotyledonary phase of *Gerbera* somatic embryos were encapsulated to form synthetic seeds. Explants encapsulated using 3.0% sodium alginate ($NaC_6H_7O_6$) which was the optimum concentration for the formation of encapsulation matrix. Encapsulated explants were dropped in calcium 100 mM chloride dehydrate ($CaCl_2.2H_2O$) solution and soaked for 30 minutes. Through the encapsulation process, 3 types of *Gerbera* synthetic seeds were produced, encapsulation of micro shoots, globular and cotyledonary phase of somatic embryo. All synthetic seeds showed formation of ideal texture with uniform, isodiametric shape and size.

The main aim of this study was to achieve regeneration of *G. jamesonii* Bolus ex. Hook f. through germination of synthetic seeds. Several factors in obtaining optimum germination of *Gerbera* synthetic seeds were examined. The factors were the effects of germination media on germination duration, germination and survival rates *in vitro* and *in vivo*, different sucrose concentrations in encapsulation matrix, different compositions of encapsulation matrix and the effect of storage at low temperature (4 ± 1^oC).

6.3.1 Germination of Synthetic Seeds

Synthetic seeds of *G. jamesonii* were achieved by encapsulation of micro shoots (Figure 6.1) and somatic embryos (globular and cotyledonary phases) (Figure 6.2). All

synthetic seeds derived from various propagules showed different germination and survival rates depending on the factors involved. Comparisons were made regarding the regeneration potentials with unencapsulated explants. Complete regeneration of synthetic seeds with shoots and roots formation from each propagules used were also observed.

Based on this experiment, synthetic seeds derived from the encapsulation of micro shoots (Figure 6.1) took 5 days to germinate on MS media supplemented with 2.0 mg/l BAP and 0.5 mg/l NAA while encapsulated globular and cotyledonary phases (Figure 6.2) of somatic embryos germinated after 15 and 9 days, respectively (Table 6.1). Synthetic seeds formed from encapsulation of micro shoots exhibited complete germination with the formation of shoots and roots. However, incomplete germination was observed when synthetic seeds derived from globular and cotyledonary phases of somatic embryos were used as propagules. Unencapsulated micro shoots took 7 days to germinate and unencapsulated globular and cotyledonary somatic embryos took 19 and 12 days respectively. On MS solid media, encapsulated micro shoots germinated on day 6, while encapsulated globular and cotyledonary phases germinated on day 17 and 12. On MS liquid media, encapsulated micro shoots germinated on day 6, globular and cotyledonary somatic embryos geminated on day 18 and 12, respectively. All unencapsulated propagules were observed to germinate slightly later then encapsulated propagules.

Synthetic seeds took the longest period to germinate when they were germinated on sterile garden soil. Encapsulated micro shoots took 12 days to germinate, globular

somatic embryos germinated on day 24 and cotyledonary phase somatic embryo germinated after 21 days. From the experiment it showed that encapsulated micro shoot germinated earlier and followed by cotyledonary phase somatic embryos and globular phase somatic embryos. Germination rates of the synthetic seeds were observed and recorded within 10 days.

Various germination rates were observed when synthetic seeds of *G. jamesonii* produced from micro shoots and somatic embryo (globular and cotyledonary phases) were left to germinate on 5 different sowing media or substrates. Encapsulated micro shoots produced 100.00 ± 0.0% germination when germinated on MS solid media and 96.5 ± 0.6% on MS liquid media (Table 6.2). Encapsulated globular and cotyledonary somatic embryos both showed highest germination rates when germinated on MS solid media with 78.4 ± 0.8% and 90.6 ± 0.7%, respectively. Unencapsulated explants showed lower response compared to encapsulated explants in all treatments (Table 6.2).

Table 6.1: *In vitro* germination of synthetic seeds of *Gerbera jamesonii* derived from micro shoots, globular phase, cotyledonary phase somatic embryos. Results were observed based on germination period (days). Thirty replicates were used in each treatment

Observations (Germination Media)	**Germination period (days)**					
	Micro shoots		**Somatic Embryo (globular phase)**		**Somatic Embryo (cotyledonary phase)**	
	Encapsulated	**Unencapsulated**	**Encapsulated**	**Unencapsulated**	**Encapsulated**	**Unencapsulated**
MS Solid Media	6	8	17	19	12	14
MS Liquid Media	6	8	18	21	12	13
MS Media supplemented with 2.0 mg/l BAP + 0.5 mg/l NAA	5	7	15	19	9	12
Sterile Vermiculite	11	14	22	27	20	23
Sterile Garden Soil	12	16	24	30	21	25

Table 6.2: I*n vitro* germination of synthetic seeds of *Gerbera jamesonii* derived from micro shoots, globular phase, cotyledonary phase somatic embryos. Results were observed based on germination rate (%). Thirty replicates were used in each treatment

Observations (Germination Media)	**Germination rate (%)**					
	Micro shoots		**Somatic Embryo (globular phase)**		**Somatic Embryo (cotyledonary phase)**	
	Encapsulated	**Unencapsulated**	**Encapsulated**	**Unencapsulated**	**Encapsulated**	**Unencapsulated**
MS Solid Media	$100 \pm 0.0_{a}$	$22.2 \pm 0.5_{d}$	$78.4 \pm 0.8_{a}$	$33.0 \pm 1.0_{d}$	$90.6 \pm 0.7_{a}$	$43.6 \pm 1.0_{d}$
MS Liquid Media	$96.5 \pm 0.6_{b}$	$27.2 \pm 0.7_{d}$	$69.3 \pm 1.2_{c}$	$31.2 \pm 0.8_{d}$	$84.0 \pm 1.1_{c}$	$40.5 \pm 0.7_{d}$
MS Media supplemented with 2.0 mg/l BAP + 0.5 mg/l NAA	$98.7 \pm 1.1_{a,b}$	$89.4 \pm 1.0_{c}$	$75.2 \pm 0.7_{a,b}$	$38.6 \pm 1.2_{c,d}$	$87.4 \pm 1.3_{b}$	$52.0 \pm 0.8_{c,d}$
Sterile Vermiculite	$70.3 \pm 1.3_{c}$	0.0_{e}	$31.7 \pm 1.0_{d}$	0.0_{e}	$45.7 \pm 0.5_{c,d}$	0.0_{e}
Sterile Garden Soil	$90.6 \pm 0.7_{b,c}$	0.0_{e}	$36.6 \pm 1.3_{d}$	0.0_{e}	$53.3 \pm 1.0_{c,d}$	0.0_{e}

Mean ± SE, n=30. Mean with different letters differ significantly at p=0.05

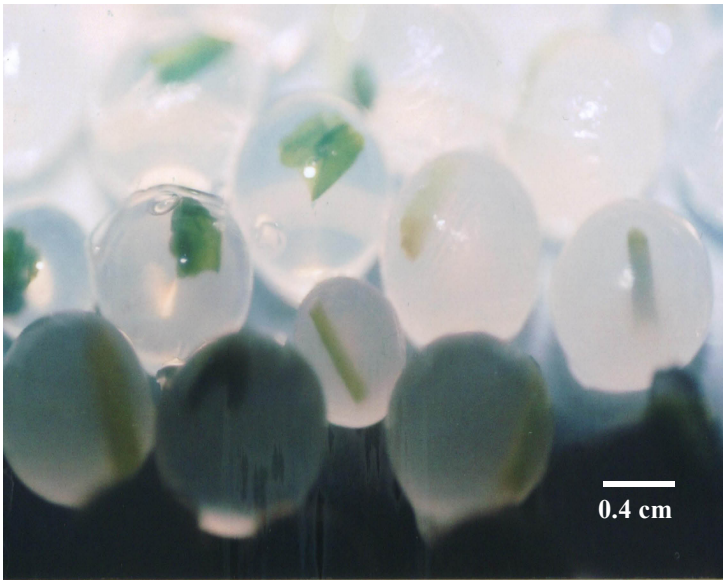

Figure 6.1: Synthetic seeds of *Gerbera jamesonii* obtained from the encapsulation of micro shoots

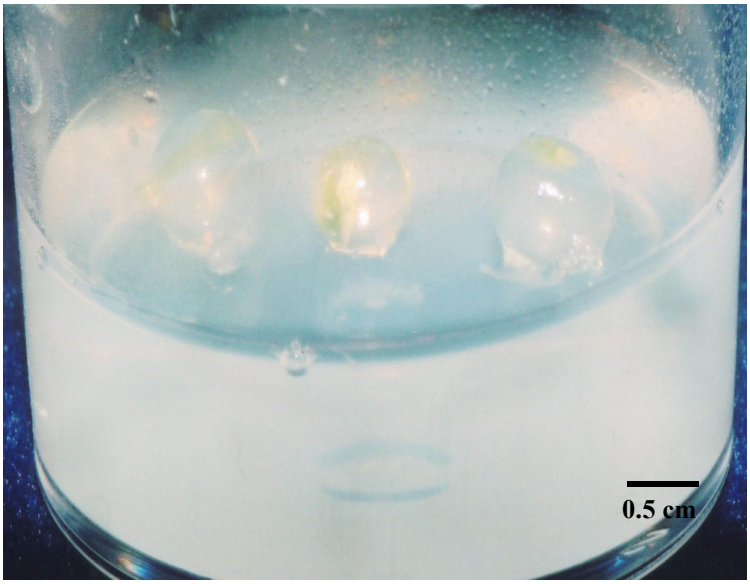

Figure 6.2: Synthetic seeds of *Gerbera jamesonii* obtained from the encapsulation of cotyledonary phase somatic embryo

Lower germination rates were observed when all encapsulated explants were germinated on sterile vermiculite, for example 70.3 ± 1.3 % for encapsulated micro shoots, 31.7 ± 1.0% for encapsulated globular somatic embryo and 45.7 ± 0.5% for encapsulated cotyledonary somatic embryo. All unencapsulated explants failed to germinate on sterile vermiculite and sterile garden soil. Encapsulated globular and cotyledonary phase somatic embryos both produced incomplete germination with formation of shoots only.

Based on this study, most of the germinated synthetic seeds survived after 30 days of germination period. On MS media, encapsulated micro shoots survived with 100 ± 0.0%, encapsulated globular somatic embryos with 83.5 ± 1.0% survival rate and encapsulated cotyledonary somatic embryos with 88.4 ± 0.8% (Table 6.3). These results are the optimum survival rates reported when all encapsulated explants were germinated on MS media. The lowest survival rates were reported when encapsulated micro shoots were germinated on sterile vermiculite with 52.5 ± 0.7%, encapsulated globular and cotyledonary somatic embryos showed the lowest survival rates on sterile garden soil with only 14.1 ± 1.3% and 23.2 ± 1.0%. Unencapsulated explants showed lower survival rates compared to encapsulated explants. All encapsulated and unencapsulated showed no response on *in vivo* germination when germinated on vermiculite and garden soil (Table 6.3). Dehydration occurred when the synthetic seeds were placed on to non-sterile vermiculite and garden soil. From these experiments, it is obvious that germination of synthetic seeds directly on vermiculite and garden soil *in vivo* was not suitable and need further experiments for improvement.

Table 6.3: Germination of synthetic seeds of *Gerbera jamesonii* derived from micro shoots, globular phase, cotyledonary phase somatic embryos. Results were observed based on survival rate (%) after 8 weeks of germination. Thirty replicates were used in each treatment.

Observations (Germination Media)	Survival rate (%)					
	Micro shoots		Somatic Embryo (globular phase)		Somatic Embryo (cotyledonary phase)	
	Encapsulated	Unencapsulated	Encapsulated	Unencapsulated	Encapsulated	Unencapsulated
MS Solid Media	$100 \pm 0.0_{a}$	$14.0 \pm 0.7_{e}$	$83.5 \pm 1.0_{a}$	$36.6 \pm 0.5_{c}$	$88.4 \pm 0.8_{a}$	$44.2 \pm 1.1_{c}$
MS Liquid Media	$98.3 \pm 1.2_{a,b}$	$19.5 \pm 1.2_{e}$	$70.2 \pm 1.2_{a,b}$	$32.5 \pm 1.0_{c}$	$73.0 \pm 1.5_{b}$	$35.9 \pm 1.0_{c,d}$
MS Media supplemented with 2.0 mg/l BAP + 0.5 mg/l NAA	$93.0 \pm 1.0_{a,b}$	$85.2 \pm 1.0_{c}$	$76.1 \pm 0.7_{a,b}$	$30.5 \pm 1.0_{c}$	$76.7 \pm 1.2_{b}$	$41.7 \pm 0.7_{c}$
Sterile Vermiculite	$52.5 \pm 0.7_{d}$	0.0_{f}	$20.6 \pm 0.5_{c,d}$	0.0_{f}	$26.8 \pm 0.5_{e}$	0.0_{f}
Sterile Garden Soil	$60.2 \pm 1.3_{d}$	0.0_{f}	$14.1 \pm 1.3_{d,e}$	0.0_{f}	$23.2 \pm 1.0_{e}$	0.0_{f}
Vermiculite	0.0_{f}	0.0_{f}	0.0_{f}	0.0_{f}	0.0_{f}	0.0_{f}
Garden Soil	0.0_{f}	0.0_{f}	0.0_{f}	0.0_{f}	0.0_{f}	0.0_{f}

Mean ± SE, n=30. Mean with different letters differ significantly at p=0.05

Overall, encapsulated micro shoots gave better germination rate when compared to encapsulated globular and cotyledonary somatic embryos. Table 6.4 showed the response of germination of synthetic seeds of *G. jamesonii* (encapsulated micro shoots, encapsulated globular and cotyledonary somatic embryos) when different concentration of sucrose were added in the encapsulation matrix. It was observed that synthetic seed produced with lower sucrose concentration in the encapsulation matrix were more transparent and clear. As the concentration of sucrose in the encapsulation matrix increased, the synthetic seeds were less transparent.

Based on the results obtained, it showed that germination rates of the synthetic seeds reduced with the increase of sucrose concentration in the encapsulation matrix after 8 weeks. Encapsulated micro shoots with no addition of sucrose yielded 37.0 ± 1.4% germination with 1.5 ± 0.7 shoots per explant (Table 6.4). Encapsulated globular somatic embryo showed 8.6 ± 0.2% germination with only 0.5 ± 1.5 shoots per explant while encapsulated cotyledonary somatic embryo showed slightly higher germination with 18.7 ± 0.5% with 1.2 ± 0.2 shoots per explant. Encapsulation matrix containing 20 g/l sucrose yielded higher germination rate compared to 10 g/l sucrose. Optimum germination was observed when 30 g/l sucrose was used. Encapsulated micro shoots yielded 75.0 ± 0.5% germination with 7.5 ± 1.5 shoots per explant (Table 6.4). Encapsulated globular and cotyledonary somatic embryos showed 61.3 ± 1.5% with 5.6 ± 0.7 shoots per explant and 63.2 ± 1.0 with 7.3 ± 0.6% shoots per explant. It was observed that the higher sucrose concentration in the encapsulation matrix, the lower germination rates obtained. In encapsulated micro shoots, germination rate of

56.3 ± 1.0% (sucrose 40 g/l) reduced to 39.5 ± 0.8% (sucrose at 60 g/l). The same was observed in encapsulated globular and cotyledonary somatic embryos. The results showed that germination rates were reduced as the concentration of sucrose increased from 40 g/l to 60 g/l.

Usually, encapsulation matrix was prepared by dissolving sodium alginate in Ca-free MS basal medium with the addition of sucrose. In this experiment, 3.0% sodium alginate was added in 4 different solutions to form the encapsulation matrix. Table 6.5 showed the response of germination from synthetic seeds that were composed of various types of encapsulation matrix.

Encapsulation matrix composed of MS media gave 70.5 ± 1.2% germination rate when micro shoots were used as explant with 4.0 ± 1.3 shoots per explant. Globular somatic embryo yielded 28.7 ± 1.5% germination with 2.1 ± 1.1 shoots per explant while cotyledonary somatic embryo germinated at 48.8 ± 0.7% with 4.1 ± 1.0 shoots per explant (Table 6.5). The optimum germination (74.5 ± 2.6%) from encapsulated micro shoots were observed when encapsulation matrix used were composed of Ca-free MS + 3.0 % sucrose with the addition of 2.0 mg/l BAP and 0.5 mg/l NAA with 4.6 ± 0.8 shoots per explant (Figure 6.3). Germination rate of 30.2 ± 1.2 from encapsulated micro shoots was observed when distilled water with the addition of 2.0 mg/l BAP and 0.5 mg/l NAA was used as the encapsulation matrix with 2.3 ± 0.7 shoots per explant. Encapsulated globular somatic embryo yielded 17.5 ± 2.0% of germination with 1.2 ± 0.4 shoots per

explant while cotyledonary somatic embryo gave 26.4 ± 0.8% with 1.8 ± 0.7 shoots per explant when the same composition was used as encapsulation matrix.

Overall, the absence of $CaCl_2.2H_2O$ in MS media as encapsulation matrix helped to increase germination potentials of the synthetic seeds. It was also observed that the addition of growth hormones (BAP and NAA) in the encapsulation matrix also aided in early germination of synthetic seeds. It was also observed that encapsulation matrix composed of Ca-free MS formed firm, uniform and round shaped beads. Encapsulation matrix composed of MS media and distilled water gave soft, less uniformed sizes of synthetic seeds.

Most of the germinated synthetic seeds (formed from various types of encapsulation matrix) survived after 8 weeks of germination. The highest survival rate was observed when Ca-free MS media with 3.0 % sucrose supplemented with 2.0 mg/l BAP and 0.5 mg/l NAA were used as encapsulation matrix. Encapsulated micro shoots showed 75.0 ± 0.5% survival rate with the increase number of shoots per explant (7.5 ± 1.5) (Figure 6.4). Survival rates for encapsulated globular and cotyledonary (Figure 6.5) somatic embryos were 61.3 ± 1.5% and 63.2 ± 1.0%, respectively (Table 6.6).

Encapsulation matrix composed of distilled water supplemented with 2.0 mg/l BAP and 0.5 mg/l NAA showed the lowest survival rates for encapsulated micro shoots (42.5 ± 1.2%), globular somatic embryo (22.6 ± 1.7%) and cotyledonary somatic embryo

(38.6 ± 1.2). Synthetic seeds formed from encapsulation matrix that were composed of distilled water (absence of nutrient) did not promote the germination and survival of the seeds. Thus, distilled water is not a suitable choice to be used as encapsulation matrix.

Plantlets obtained from synthetic seeds of *G. jamesonii* were acclimatized to the green house (Figure 6.6). Detail results were discussed in chapter 8.

Table 6.4: Effect of sucrose at different concentrations on germination rate of synthetic seeds of *Gerbera jamesonii*

Sucrose concentration (g/l)	Micro shoots		Somatic Embryo (globular phase)		Somatic Embryo (cotyledonary phase)	
	Germination (%)	No. shoots per explant	Germination (%)	No. shoots per explant	Germination (%)	No. shoots per explant
0	$37.0 \pm 1.4_{c}$	1.5 ± 0.7	$8.6 \pm 0.2_{c,d}$	0.5 ± 1.5	$18.7 \pm 0.5_{d}$	1.2 ± 0.2
10	$42.8 \pm 2.1_{b,c}$	3.4 ± 1.2	$19.5 \pm 0.8_{c}$	1.6 ± 0.8	$29.0 \pm 0.8_{c}$	2.8 ± 0.8
20	$54.1 \pm 1.2_{b}$	5.4 ± 0.8	$32.6 \pm 0.8_{b}$	3.1 ± 0.9	$42.5 \pm 1.0_{b}$	4.8 ± 1.0
30 (control)	$75.0 \pm 0.5_{a}$	7.5 ± 1.5	$61.3 \pm 1.5_{a}$	5.6 ± 0.7	$63.2 \pm 1.0_{a}$	7.3 ± 0.6
40	$56.3 \pm 1.0_{b}$	4.5 ± 1.0	$34.2 \pm 1.1_{b}$	3.2 ± 1.1	$45.8 \pm 0.7_{b}$	4.3 ± 1.3
50	$47.4 \pm 1.5_{b,c}$	2.1 ± 0.7	$17.2 \pm 0.7_{c}$	1.1 ± 1.5	$27.4 \pm 0.9_{c}$	1.6 ± 1.5
60	$39.5 \pm 0.8_{c}$	0.9 ± 0.4	$7.8 \pm 1.3_{c,d}$	0.2 ± 0.1	$18.3 \pm 1.3_{d}$	0.5 ± 1.0

Mean ± SE, n=30. Mean with different letters in the same column differ significantly at p=0.05

Table 6.5: Growth response of micro shoots and somatic embryo of *Gerbera* encapsulated in different encapsulation matrix. Results were observed based on germination rate (%) after 10 days of germination. Thirty replicates were used in each treatment.

Encapsulation matrix	**Germination Rate (%) (10 Days)**					
	Micro shoots		**Somatic Embryo (globular phase)**		**Somatic Embryo (cotyledonary phase)**	
	Germination (%)	**No. shoots per explant**	**Germination (%)**	**No. shoots per explant**	**Germination (%)**	**No. shoots per explant**
MS	$70.5 \pm 1.2_{b}$	4.0 ± 1.3	$28.7 \pm 1.5_{b,c}$	2.1 ± 1.1	$48.8 \pm 0.7_{b,c}$	4.1 ± 1.0
Ca-free MS + distilled water	$56.0 \pm 1.1_{b,c}$	3.2 ± 0.7	$24.0 \pm 0.8_{c}$	1.6 ± 1.5	$41.4 \pm 1.0_{c}$	3.4 ± 1.0
Ca-free MS + 3.0% sucrose	$72.1 \pm 0.8_{b}$	3.6 ± 1.2	$29.2 \pm 0.5_{b,c}$	1.6 ± 0.8	$50.6 \pm 1.5_{b}$	3.6 ± 0.8
MS + 3.0% sucrose + 2.0 mg/l BAP + 0.5 mg/l NAA	$73.4 \pm 1.0_{b}$	4.4 ± 1.0	$32.5 \pm 1.2_{b}$	2.4 ± 1.0	$52.0 \pm 1.3_{b}$	4.3 ± 1.2
Ca-free MS + 3.0% sucrose + 2.0 mg/l BAP + 0.5 mg/l NAA	$74.5 \pm 2.6_{a}$	4.6 ± 0.8	$34.8 \pm 1.2_{a}$	2.5 ± 0.7	$54.2 \pm 1.3_{a}$	4.4 ± 1.5
Distilled water + 2.0 mg/l BAP + 0.5 mg/l NAA	$30.2 \pm 1.2_{c}$	2.3 ± 0.7	$17.5 \pm 2.0_{d}$	1.2 ± 0.4	$26.4 \pm 0.8_{d}$	1.8 ± 0.7

Mean ± SE, n=30. Mean with different letters differ significantly at p=0.05

Table 6.6: Growth response of micro shoots and somatic embryos of *Gerbera jamesonii* encapsulated in different encapsulation matrix. Results were observed based on survival rates (%) after 8 weeks of germination. Thirty replicates were used in each treatment

Encapsulation matrix	**Survival Rate (%) (30 Days)**					
	Micro shoots		**Somatic Embryo (globular phase)**		**Somatic Embryo (cotyledonary phase)**	
	Survival (%)	**No. shoots per explant**	**Survival (%)**	**No. shoots per explant**	**Survival (%)**	**No. shoots per explant**
MS	$69.5 \pm 0.8_{b,c}$	6.8 ± 1.0	$41.6 \pm 1.0_{c}$	4.9 ± 0.4	$52.2 \pm 0.8_{b,c}$	6.6 ± 2.1
Ca-free MS + distilled water	$64.8 \pm 0.7_{b,c}$	5.9 ± 0.5	$36.2 \pm 1.2_{c,d}$	4.3 ± 1.0	$46.1 \pm 0.5_{c}$	5.9 ± 0.8
Ca-free MS + 3.0% sucrose	$73.6 \pm 1.2_{b}$	6.3 ± 0.9	$45.8 \pm 0.8_{b,c}$	4.4 ± 1.5	$54.5 \pm 1.1_{b}$	6.1 ± 1.4
MS + 3.0% sucrose + 2.0 mg/l BAP + 0.5 mg/l NAA	$72.9 \pm 2.2_{b}$	7.2 ± 1.1	$50.5 \pm 0.4_{b}$	5.2 ± 1.0	$59.0 \pm 0.9_{b}$	7.0 ± 1.3
Ca-free MS + 3.0% sucrose + 2.0 mg/l BAP + 0.5 mg/l NAA	$75.0 \pm 0.5_{a}$	7.5 ± 1.5	$61.3 \pm 1.5_{a}$	5.6 ± 0.7	$63.2 \pm 1.0_{a}$	7.3 ± 0.6
Distilled water + 2.0 mg/l BAP + 0.5 mg/l NAA	$42.5 \pm 1.2_{d}$	5.1 ± 0.7	$22.6 \pm 1.7_{d}$	3.9 ± 1.2	$38.6 \pm 1.2_{c,d}$	4.5 ± 1.0

Mean ± SE, n=30. Mean with different letters in the same column differ significantly at p=0.05

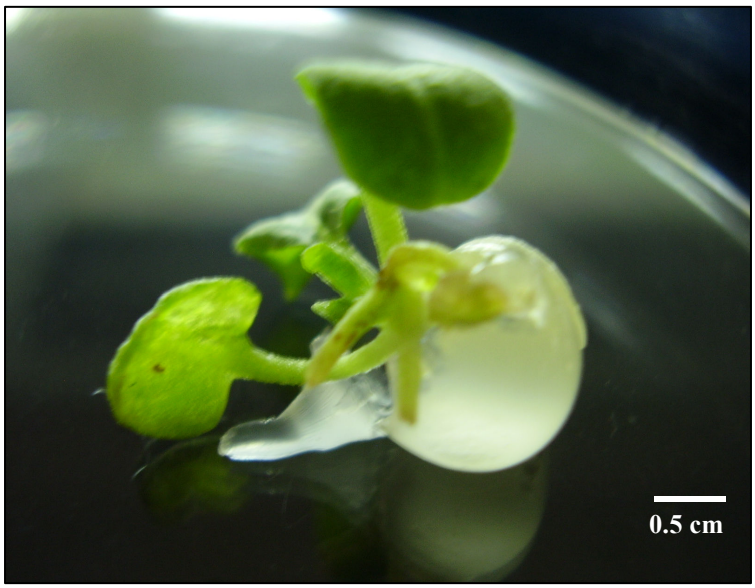

Figure 6.3: Germination of synthetic seed of *Gerbera jamesonii* from encapsulated micro shoots

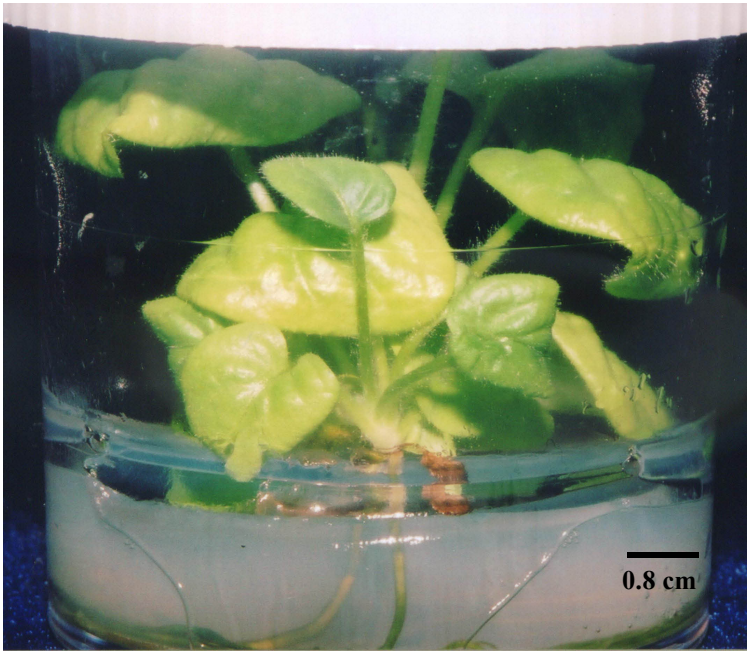

Figure 6.4: Plantlet obtained from germination of *Gerbera* synthetic seed derived from encapsulation of micro shoots

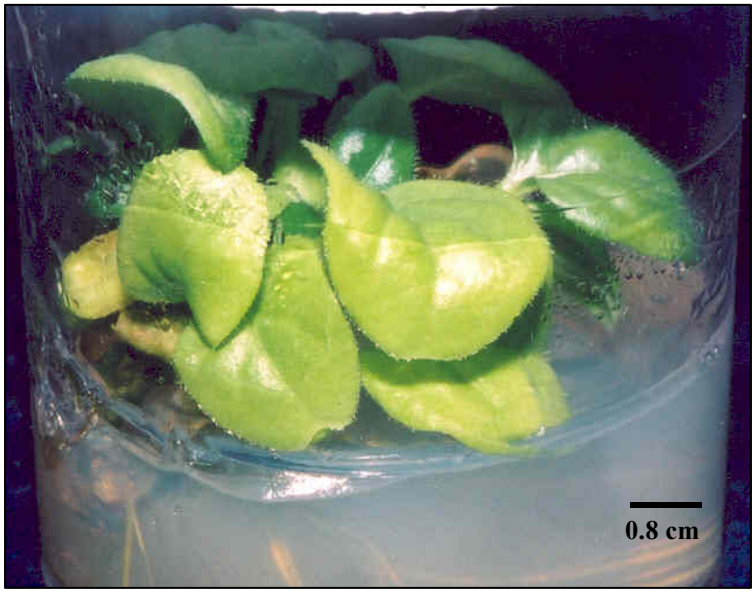

Figure 6.5: Plantlet formed from germination of *Gerbera jamesonii* synthetic seed derived from encapsulation of cotyledonary phase somatic embryos

Figure 6.6: Three-month-old plantlet obtained from germination of *Gerbera jamesonii* synthetic seed being acclimatized in the green house

6.3.2 Effects of Storage Period on Germination of Synthetic Seeds

Table 6.7 illustrates the effects of various storage periods on germination of synthetic seeds (encapsulated micro shoots, globular and cotyledonary somatic embryos). All synthetic seeds were stored under low temperature at 4 ± 1 ^{o}C for 0, 30, 60, 90, 120, 150 and 180 days. Synthetic seeds which were not incubated (0 day) were used as control. MS basal medium was chosen as the optimum germination medium based on previous experiment. The germination was observed after 8 weeks.

Storage of synthetic seeds were also done at 25 ± 1 ^{o}C , however all synthetic seeds stored at this temperature failed to germinate. Only synthetic seeds which were not stored manage to germinate. After 30 days of storage at 25 ± 1 ^{o}C, all synthetic seeds were desiccated, dehydrated and lost their viability to germinate. The results from Table 5.8 demonstrated that germination of synthetic seeds occurred at long term storage, however longer storage period (up to 180 days) reduced the quality and quantity of germination. Synthetic seeds which were not stored showed higher germination compared to stored seeds. Non-stored synthetic seeds from encapsulated micro shoots germinated at 93.3 ± 0.5% while non-stored encapsulated globular and cotyledonary somatic embryo germinated at 49.0 ± 0.8% and 67.3 ± 0.7%, respectively (Table 6.7).

Germination rates for encapsulated micro shoots reduced from 91.7 ± 0.6% (30 days storage) to 14.3 ± 0.7% (180 days storage). Germination of encapsulated globular somatic embryo decreased from 44.0 ± 1.2% (30 days storage) to 21.7 ± 1.0%

(60 days storage). Encapsulated somatic embryos (cotyledonary phase) yielded 61.7 ± 1.0% rate of germination after 30 days of storage and the germination rate was reduced to 32.3 ± 0.5% after 90 days of storage. Synthetic seeds formed from encapsulated globular and cotyledonary somatic embryos failed to germinate after 120 days of storage. Throughout the experiments, it was observed that encapsulated explants which failed to germinate showed changes in the quality which caused the propagules (micro shoots, globular and cotyledonary somatic embryos) to face necrosis and turned brownish and later failed to response.

Table 6.7: Effect of storage period (days) at 4 ± 1 ^{o}C on germination of synthetic seeds of *Gerbera jamesonii,* germinated on MS basal medium. Thirty replicates were used in each experiment.

Period of Storage (Days)	Micro shoots		Somatic Embryo (globular phase)		Somatic Embryo (cotyledonary phase)	
	Germination Rate (%)	No. of Germinated Seed	Germination Rate (%)	No. of Germinated Seed	Germination Rate (%)	No. of Germinated Seed
0 (control)	$93.3 \pm 0.5_{a}$	28.0 ± 1.2	$49.0 \pm 0.8_{a}$	14.7 ± 0.9	$67.3 \pm 0.7_{a}$	20.2 ± 1.2
30	$91.7 \pm 0.6_{a}$	27.5 ± 1.1	$44.0 \pm 1.2_{a,b}$	13.2 ± 0.5	$61.7 \pm 1.0_{a,b}$	18.5 ± 0.8
60	$91.0 \pm 0.8_{a}$	27.3 ± 0.8	$35.0 \pm 0.5_{b,c}$	10.5 ± 1.2	$48.7 \pm 1.2_{b,c}$	14.6 ± 1.2
90	$77.0 \pm 1.0_{b}$	23.1 ± 1.3	$21.7 \pm 1.0_{d}$	6.5 ± 1.0	$32.3 \pm 0.5_{c}$	9.7 ± 0.7
120	$53.3 \pm 1.2_{b,c}$	16.0 ± 0.9	0.0_{e}	No seed germination	0.0	No seed germination
150	$23.3 \pm 0.8_{c}$	7.0 ± 1.5	0.0_{e}	No seed germination	0.0	No seed germination
180	$14.3 \pm 0.7_{c}$	4.3 ± 0.7	0.0_{e}	No seed germination	0.0	No seed germination

Mean ± SE, n=30. Mean with different letters in the same column differ significantly at p=0.05

6.4 SUMMARY OF RESULTS

1. Synthetic seeds of different propagules (micro shoots, globular and cotyledonary somatic embryos) were successfully formed through encapsulation process. Generally, encapsulation matrix consists of 3.0% sodium alginate dissolved in Ca-free MS media added with 30 g/l sucrose.

2. Encapsulation process was accomplished when each propagule (micro shoots, globular and cotyledonary somatic embryos) were drawn up with some of the encapsulation matrix and then dropped into CaCl2.2H2O solution for 30 minutes.

3. Encapsulated micro shoots showed the highest germination and shoots formation. On MS solid media, synthetic seeds from encapsulated micro shoots gave 100 ± 0.0% germination after 6 days. The percentage of germination for encapsulated globular somatic embryo was 78.4 ± 0.8% after 17 days while encapsulated cotyledonary somatic embryo gave 90.6 ± 0.7% germination after 12 days. Germination rates of unencapsulated propagules were lower than encapsulated propagules with 22.2 ± 0.5% germination of unencapsulated micro shoots after 6 days, 78.4 ± 0.8% germination of unencapsulated globular phase somatic embryo after 17 days and 43.6 ± 1.0% germination of unencapsulated cotyledonary somatic embyo after 12 days.

4. All synthetic seeds germinated the earliest on MS media supplemented with 2.0 mg/l BAP and 0.5 mg/l NAA. However, germination rates for all synthetic seeds (encapsulated micro shoots, globular and cotyledonary somatic embryos) were optimum on MS solid media, thus, this medium was identified as optimum germination media (sowing media) for synthetic seeds of *G. jamesonii*.

5. All synthetic seeds survived after 8 weeks of germination. Encapsulated micro shoots showed the highest survival on MS media with 100.00 ± 0.0% and lowest survival on sterile vermiculite with 52.5 ± 0.7%. Encapsulated globular somatic embryo survived with 83.5 ± 1.0% on MS solid media and the survival rate was reduced to 14.1 ± 1.3% when germinated on sterile garden soil. Encapsulated cotyledonary stage showed 88.4 ± 0.8% survival on MS solid media and the survival decreased to 23.2 ± 1.0% when germinated on sterile garden soil.

6. Synthetic seeds of *G. jamesonii* failed to germinate *in vivo* on vermiculite and garden soil. Thus, *in vivo* germination is not suitable for germination of synthetic seeds. However, the synthetic seeds managed to germinate on sterile vermiculite and sterile garden soil.

7. Encapsulation matrix containing 30.0 g/l sucrose gave optimum germination rates for all 3 types of encapsulated propagules, 75.0 ± 0.5% germination for encapsulated micro shoots, 61.3 ± 1.5% for encapsulated globular somatic embryo and 63.2 ± 1.0% for encapsulated cotyledonary somatic embryos.

8. Encapsulation matrix composed of Ca-free MS media with 3.0 % sodium alginate and 3.0 % sucrose supplemented with 2.0 mg/l BAP and 0.5 mg/l NAA were found to be the most suitable encapsulation matrix. Encapsulated micro shoots germinated at 74.5 ± 2.6%, encapsulated globular and cotyledonary somatic embryos germinated at 34.8 ± 1.2% and 54.2 ± 1.3%, respectively. All 3 types of encapsulated propagules prepared in Ca-free MS solution yielded higher germination rates compared to encapsulation matrix that was composed of MS media (with calcium macronutrient). Encapsulation matrix that was composed of distilled water produced the lowest germination rates for all types of encapsulated propagules.

9. Survival rates for germination of encapsulated micro shoots, globular and cotyledonary somatic embryos was optimum when the encapsulation matrix was composed of Ca-free MS with 3.0 % sucrose supplemented with 2.0 mg/l BAP and 0.5 mg/l NAA. The lowest survival of germinated synthetic seeds was observed when the encapsulation matrix was composed of distilled water.

10. Synthetic seeds of *G. jamesonii* were able to be stored at 4 ± 1^{o}C. Each encapsulated propagule showed different response on germination depending on the duration of storage. Storage for 180 days allowed encapsulated micro shoots to germinate. However, encapsulated globular and cotyledonary somatic embryos were capable to germinate when stored up to 90 days only.

11. Long term storage of synthetic seeds reduced the viability and germination potentials. Encapsulated micro shoots germinated at 93.3 ± 0.5% when not stored under low temperature. The germination rate reduced to 14.3 ± 0.7% when the synthetic seeds were stored for 180 days at 4 ± 1 ^{o}C. Germination rates of encapsulated globular somatic embryo reduced from 49.0 ± 0.8% (0 day storage) to 21.7 ± 1.0% (90 days storage) while germination of cotyledonary somatic embryo decreased from 67.3 ± 0.7% (0 day storage) to 32.3 ± 0.5% (90 days storage).

12. Experiments testing the effect of storage on germination of synthetic seeds at 25 ± 1^{o}C were also conducted. After 20 days of storage, all synthetic seeds were desiccated, dehydrated and lost their viability to germinate.