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Artificial Seed Production from Encapsulated Micro Shoots of *Saintpaulia ionantha* Wendl. (African Violet)

[1]N. Daud, [2]R.M. Taha and [2]N.A. Hasbullah
[1]Faculty of Science and Technology,
Sultan Idris University of Education, 35900 Tanjong Malim, Perak Darul Ridzuan, Malaysia
[2]Institute of Biological Sciences, Faculty of Science, University of Malaya, 50603 Kuala Lumpur, Malaysia

Abstract: Artificial seeds were produced from encapsulated micro shoots of *Saintpaulia ionantha* Wendl. (African violet). The production of artificial seeds of this species gave ideal beads based on firmness, texture, size and shape of beads. The percentage of germination from encapsulated micro shoots influenced by the concentrations of sodium alginate and calcium chloride ($CaCl_2.2H_2O$) used. It was found that among the concentrations tested, 3% sodium alginate and the exposure to 100 mM $CaCl_2.2H_2O$ solution for 30 min had produced optimal beads with firm, clear, round and uniform size and suitable for handling. It was also observed that micro shoots obtained from optimization of encapsulation matrix showed the highest percentage of germination (84%). Encapsulated micro shoots exposed for 30 min in 100 mM $CaCl_2.2H_2O$ solution gave the optimal time of hardening process. The findings suggested that the encapsulation method for micro shoots could be used as an alternative to artificial seed derived from somatic embryos of *Saintpaulia ionantha*.

Key words: Synthetic seeds, *Saintpaulia ionantha*, encapsulated micro shoot, tissue culture

INTRODUCTION

The encapsulation of somatic embryos to produce artificial seeds or synthetic seeds was first proposed by Murashige (1977). According to Redenbaugh *et al*. (1987), synthetic seeds consisting of somatic embryos were suggested as a powerful tool for propagation of plant species. However, the production of synthetic seeds by encapsulating vegetative parts such as shoot tips shoots primordial or axillary buds had been used in recent years as more suitable alternative to somatic embryos (Sarkar and Naik, 1997). The seed consists of tissues derived from vegetative parts or somatic embryos and artificial endosperms. The vegetative parts and somatic embryos were coated by encapsulation matrix i.e., sodium alginate, subsequently formed the artificial endosperm. Besides sodium alginate, polyethyleneimine (Kersulec *et al*., 1993) or chitosane (Tay *et al*., 1993) were also used as encapsulation matrix. The condition of an artificial endosperm could provide nutrients and growth regulators that were essential for plantlet development from encapsulated parts (Nieves *et al*., 1998).

The synthetic seed is currently considered as the most effective and efficient alternate technique for propagation of commercially important plant species that had problems in seed propagation and plants produced non-viable seeds or without seeds. Its potential advantages include stabilities during handling, potential for long terms storage without losing viability, transportation and planting directly from *in vitro* to field conditions and higher scale at a low cost production (Ghosh and Sen, 1994). Few studies had been done on the production of synthetic seeds from encapsulated vegetative parts of several plant species such as shoot tips of banana (Ganapathi *et al*., 1992), nodal segments of Cassava (Danso and Ford-Floyd, 2003), shoot buds of *Morus indica* (Bapat and Rao, 1987) and axillary buds of *Daucus carota* (Kitto and Janick, 1985) with different degree of successes.

Saintpaulia ionantha Wendl. (African violet) is considered as one of the most popular ornamental indoor plants species. The production of synthetic seed was useful since this species produce a tiny and non-viable seeds. To our knowledge, there was no published report on the production of artificial seed from encapsulated micro shoots of *Saintpaulia ionantha*. Thus, we would like to report a method for encapsulation of micro shoots to produce ideal beads for this species. Therefore, the aim of this work was to determine the optimum concentration of encapsulation matrix (sodium alginate solution and $CaCl_2.2H_2O$ solution). At the same time, to determine the

Corresponding Author: Nor Azlina Binti Hasbullah, Institute of Biological Sciences, Faculty of Science, University of Malaya, 50603 Kuala Lumpur, Malaysia Tel: +603-79677121, +6012-9199852 Fax: +603-79674178

optimal duration of exposure to $CaCl_2.2H_2O$ solution and the percentage of germination in order to produce ideal beads.

MATERIALS AND METHODS

This study was conducted at Laboratory B2.5, Institute of Biological Sciences, Faculty of Science, University of Malaya, Kuala Lumpur, Malaysia in the year 2004. The standard tissue culture methods were used for this study. Leaf explants of *Saintpaulia ionantha* were obtained from *in vitro* (aseptic) plantlets (3-months-old). Leaf explants were cultured on MS medium (Murashige and Skoog, 1962) supplemented with 30 g L^{-1} sucrose and 2.5 g L^{-1} gelrite added with different concentrations of Thidiazuron (TDZ). The pH of the medium was adjusted to 5.8 prior to autoclaving at 121°C for 21 min. All cultures were incubated under the culture room conditions of light intensity (1000 lux), temperature at 25±1°C and 70-80% relative humidity with 16/8 h light/dark photoperiod. Micro shoots approximately 3.0 mm in length were induced directly from leaf explants after 6 weeks in culture. The micro shoots were isolated from the surface of the leaf explants and encapsulated as below.

Encapsulation: Encapsulation matrix consisted of sodium alginate solution at various concentrations (1-5%) was prepared in MS basal medium solution (pH 5.8) added with 30 g L^{-1} sucrose. For hardening process (i.e., an ion exchange reaction between Na^+ in sodium alginate solution with Ca^{2+} in the $CaCl_2.2H_2O$ solution resulting in the formation of insoluble calcium alginate), different concentrations of $CaCl_2.2H_2O$ solution were prepared in MS basal medium solution.

For encapsulation purposes, micro shoots were individually dipped for a few seconds into sodium alginate solution. Single micro shoot and alginate mixture was picked up by sterile pipette 5 mm (internal diameter). Single coated micro shoot was then dropped into 25-125 mM of $CaCl_2.2H_2O$ solution in a beaker which was placed on a magnetic stirrer. Each drop containing a single micro shoot was expected to produce individual beads with 5 mm in diameter. Each bead was maintained in $CaCl_2.2H_2O$ solution for certain duration of exposure for hardening process. After 30 min (as a control), the beads were washed three times with autoclaved MS basal medium and retrieved using a nylon mesh. The experiments were performed to optimize the encapsulation matrix. Subsequently, the beads were cultured or germinated on MS basal medium for determination of germination percentage.

Germination conditions: For germination purposes, the beads were maintained under the culture room conditions of light intensity (1000 lux), temperature at 25 ±1°C and 70-80% relative humidity with 16/8 h day/night photoperiod. Twenty five seeds were used per treatment. Data were collected after 6 weeks of germination.

RESULTS

Micro shoots obtained directly from leaf explants were cultured on MS medium (Murashige and Skoog, 1962) with 30 g L^{-1} sucrose and 2.5 g L^{-1} gelrite supplemented with 0.2-1.0 mg L^{-1} TDZ. The micro shoots were isolated from the surface of the leaf explants after 6 weeks in culture (Fig.1a).

Observations were made after 30 min in $CaCl_2.2H_2O$ solution for hardening process. However, it was found that encapsulated micro shoots showed different degree of successes based on ideal beads produced (Table 1). In the present study, micro shoots were successfully encapsulated in 3-5% sodium alginate solution and dipped in 100-125 mM $CaCl_2.2H_2O$ solution. The alginate matrix formed capsules around the micro shoots, thus has produced beads which were firm, clear, round and uniform in size; represented by (++++) (Fig. 1b). The solid beads formed were suitable for handling. Sodium alginate solution at 3-5% and dipped into 25-75 mM $CaCl_2.2H_2O$ solution obtained beads with uniform size, isodiametric and solid and represented by (+++). The beads have short tail on the surface of capsules. The beads that formed in 2% sodium alginate solution and harden in 25-50 mM $CaCl_2.2H_2O$ gave malformed sizes, too soft to handle and very fragile, represented by (+), while those maintained in 75-125 mM $CaCl_2.2H_2O$, (represented by (++)) gave solid texture beads and some beads formed clusters. Lower concentrations of sodium alginate (1%) resulted in the formation of very poor beads, i.e., failure for micro shoots to coat and at the same time, no beads or capsules were produced in all concentrations of 25- 125 mM $CaCl_2.2H_2O$.

Table 1: The effect of different concentrations of sodium alginate and $CaCl_2.2H_2O$ on bead formation

$CaCl_2.2H_2O$ (mM)	Sodium alginate (%)			
	2.0	3.0	4.0	5.0
25	+	+++	+++	+++
50	+	+++	+++	+++
75	++	+++	+++	+++
100	++	++++	++++	++++
125	++	++++	++++	++++

+: Ununiform in size, too soft and very fragile, ++: Beads formed clusters, +++: Uniform in size, isodiametric and solid, ++++: Firm, clear, round and uniform in size

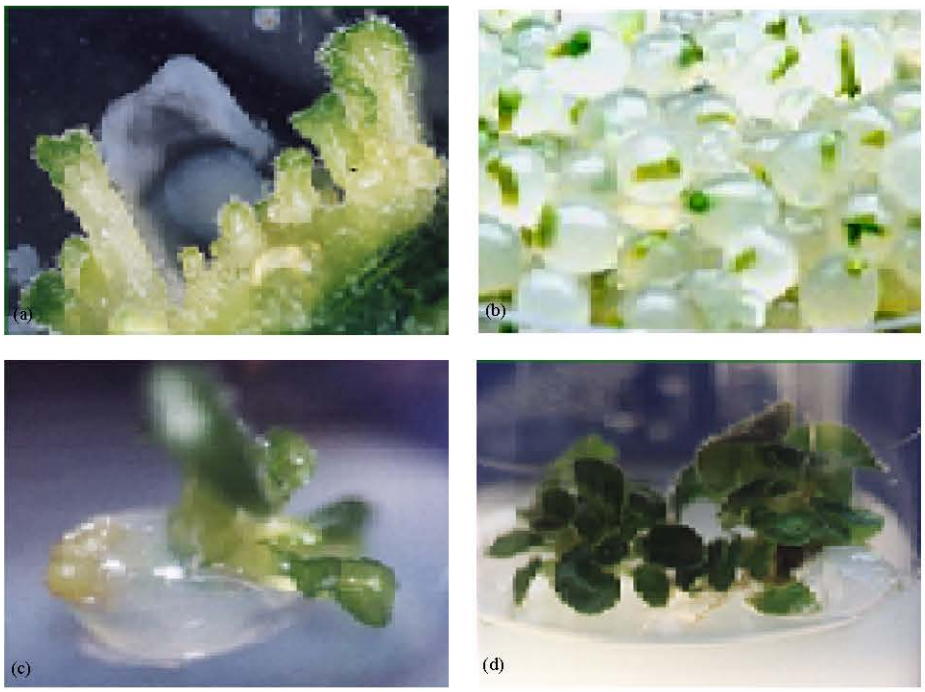

Fig. 1: (a) Micro shoots of *Saintpaulia ionantha*, (b) micro shoots encapsulated in alginate matrix, (c) the germination of encapsulated micro shoot cultured on MS basal medium and (d) The plantlets regenerated from micro shoot on MS basal medium after 10 weeks in culture

Further experiments were carried out in order to determine the optimal concentration of encapsulation matrix. The six concentrations tested include (++++) as presented in Table 1. To evaluate the germination of encapsulated micro shoots, the capability of the micro shoots to break the gel and to continue normal growth to the emergence of shoots or/and roots were observed (Table 2). The encapsulated micro shoots did not germinate at the same time. Micro shoots encapsulated with 3% sodium alginate solution and harden in 100 mM $CaCl_2.2H_2O$ exhibited the highest germination percentage, i.e., 40% during the first germination and 84% after 6 weeks in culture (Fig. 1c). Micro shoots encapsulated with 3% alginate matrix in 125 mM $CaCl_2.2H_2O$ gave germination percentage as 32% for first germination and increased to 76% after 6 weeks in culture. Both capsules germinated during the first 5 days of culture. The germination percentage of micro shoots encapsulated with 4% sodium alginate and soaked in $CaCl_2.2H_2O$ (100 mM) solution was reduced to 28% after 7 days and 72% after 6 weeks in culture, whereas micro shoots encapsulated in 4% sodium alginate with 125 mM $CaCl_2.2H_2O$ gave 16% germination after 9 days in culture and 28% after 6 weeks of culture. The germination of encapsulated micro shoots in 5% sodium alginate with 100-125 mM $CaCl_2.2H_2O$ dropped to 16 and 12% for the first 14 days whilst 24 and 16% after 6 weeks in culture.

Table 2: The effect of different concentrations of sodium alginate (%) and $CaCl_2.2H_2O$ (mM) on percentage germination of encapsulated micro shoots on MS basal medium

Sodium alginate (%) and $CaCl_2.2H_2O$ (mM)	Germination (Days)[1]	Germination (%)[2]	Germination (%)[3]
3%; 100 mM	5	40.0±1.2	84.0±1.6
3%; 125 mM	5	32.0±1.0	76.0±1.4
4%; 100 mM	7	28.0±1.2	72.0±1.3
4%; 125 mM	9	16.0±0.8	28.0±0.9
5%; 100 mM	14	16.0±0.8	24.0±1.2
5%; 125 mM	14	12.0±0.8	16.0±0.8

[1]No. of days taken for the first encapsulated micro shoots culture to germinate, [2]Percentage of first germination, [3]Percentage of germination after 6 weeks

Based on the results obtained, it was obvious that the concentration of sodium alginate and hardening agent ($CaCl_2.2H_2O$ solution) influenced the frequency of bead germination. At higher concentrations of sodium alginate (4-5%), beads formed were harder but probably suppressed the ability of shoots and roots to emerge. Lower concentration of sodium alginate solution (2%) was not suitable for encapsulation because the bead formed without a definite shape, resulting in reduction of germination. In the present study, it was observed that 3% sodium alginate maintained in 100 mM $CaCl_2.2H_2O$ solution for encapsulation was the optimal concentration to produce artificial seeds from encapsulated micro shoots. The beads started to germinate after 5 days in culture with 40% germination rate and developed into

Table 3: The relationship between percentage of germination and exposure duration to 100 mM of $CaCl_2.2H_2O$ solution of encapsulated micro shoots after 6 weeks in culture on MS basal medium

Exposure time to $CaCl_2.2H_2O$; 100 mM (min)	Germination (%)
10	52.0±0.8
30	84.0±1.6
50	80.0±1.6
70	76.0±1.4
90	72.0±1.3

plantlets 10 days later (Fig. 1c). The shoots developed into normal plantlets on MS basal medium (Fig. 1d).

Based on the results, the duration of exposure to $CaCl_2.2H_2O$ for hardening process affected the frequency of germination from encapsulated micro shoots after 6 weeks in culture. The hardening process for 30 min gave the best result and referred to as optimal period (Table 3). The percentage of germination for encapsulated micro shoots were low when the hardening period was shorter, i.e., 10 min or longer than 30 min. Sodium alginate (3%) was found to produce sufficient hard beads while still maintaining the micro shoots integrity.

DISCUSSION

It has been reported that the concentration of sodium alginate needed for encapsulation of somatic embryos or micro shoots varies depending on species (Redenbaugh *et al*., 1986). The success of retrieving complete plantlets *in vitro* from encapsulated axillary buds or shoot tips on various planting medium has been achieved in few herbaceous plants (Sharma *et al*., 1994) and woody species (Bapat *et al*., 1987). Soneji *et al*. (2002) reported that a concentration of 3% sodium alginate was the most effective to encapsulate shoots of *Ananas comosus*. This is in agreement with the present study, both species could produce ideal beads with firm coats suitable for handling when encapsulated with 3% sodium alginate solution, however, encapsulated micro shoots of *Saintpaulia ionantha* were maintained for 30 min in 100 mM $CaCl_2.2H_2O$ solution while, *Ananas comosus* were maintained in 60 mM $CaCl_2.2H_2O$. In contrast, axillary shoot buds of *Valeriana wallichii* DC. were successfully encapsulated in 6% sodium alginate and maintained in 75 mM $CaCl_2.2H_2O$ solution (Marthur *et al*., 1989). They have reported that the above concentration gave the optimal, firm and round beads after 30 min. The germination of beads from those species were also observed on MS basal medium, but generally the frequency of germination was lower compared to germination of encapsulated micro shoots from *Saintpaulia ionantha*. The higher germination percentage of encapsulated micro shoots of *Saintpaulia ionantha* obtained was probably due to the nutrients (MS salt solution) in the sodium alginate solution. In many cases, sodium alginate (encapsulation matrix) was prepared with different solution, i.e., either distilled water only, distilled water with hormones or MS solution with hormones. For example, in encapsulated shoot tips of *Musa* sp., whereby the sodium alginate were prepared in distilled water and distilled water added with 5 mg L^{-1} BAP which gave 50 and 33% germination on MS basal medium (Ganapathi *et al*., 1992). Patel *et al*. (2000) reported 81% synthetic seeds of *Solanum tuberosum* was successfully germinated when shoot tips explant were encapsulated with 0.8% sodium alginate. Meanwhile, encapsulated micro shoots of *Begonia* × *Hiemalis* Fotch. produced 90.48% germination rate (Awal *et al*., 2007) when 3% sodium alginate was used as the encapsulation matrix. Therefore, it was suggested that the successful germination of the beads was probably due to the ability of micro shoots to absorb nutrient substances in the sodium alginate solution. According to Redenbaugh *et al*. (1993), the beads can potentially serve as a reservoir for nutrients that may aid the survival and speed up growth. The nutrients supplemented to the alginate matrix reduced the viscosity and ability of the gel to form solid beads. However, it was found that 30 min exposure to $CaCl_2.2H_2O$ solution was required to achieve complete encapsulation. In contrast, a shorter (10 min) exposure time in $CaCl_2.2H_2O$ solution with 2.5% sodium alginate for encapsulating embryos of *Carica papaya* resulted in well-formed beads and gave 80% germination (Castillo *et al*., 1998). Both sodium alginate and $CaCl_2.2H_2O$ concentrations played important roles in hardening process and capsule hardness (Redenbaugh *et al*., 1991). Redenbaugh *et al*. (1993) also stated that variable methods related to encapsulation, including alginate type and concentrations, media and methods used to produce artificial seeds were responsible for germination rate of alfalfa, carrot and celery. In the present study, germination percentage was lower (52%) for 10 min exposure to $CaCl_2.2H_2O$ 100 mM solution, while 72-80% germination for more than 30 min exposure. It was due to hardness in capsules and at the same time, may be inhibiting the micro shoots respiration due to anaerobic environment inside the capsules. The hardness or rigidity of the beads or capsules mainly depends on the number of sodium ions exchanged with calcium ions. Hence, the rigidity of the alginate capsule provided better protection to the encased plant materials. At the same time, the internal factors related to micro shoots could also be one of the important limiting factors affecting germination. The quality of embryos, in this case micro shoots has also

been found to be one of the important limiting factors affecting high frequency of germination (Lutz *et al*., 1985).

CONCLUSIONS

The germination percentage of encapsulated micro shoots was affected by encapsulation matrix and duration of exposure to $CaCl_2.2H_2O$ solution. Micro shoots encapsulated with 3% sodium alginate maintained in 100 mM $CaCl_2.2H_2O$ for 30 min gave the optimal concentration and showed the highest percentage of germination i.e., 84%. The best result for hardening process was by exposure to $CaCl_2.2H_2O$ for 30 min.

In conclusion, the artificial seed production obtained from encapsulation of micro shoots can be used as a potential method to solve problems of propagation for *Saintpaulia ionantha* that have tiny seeds or produced non-viable seeds. The present study suggested that the production of uniform beads with high frequency of germination in *Saintpaulia ionantha* would be useful for cloning and mass propagation especially for commercial purposes.

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