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RESEARCH PAPER

Production of Artificial Seeds of *Carica papaya* L. var Eksotika

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Abstract

Artificial seed production technique for *Carica papaya* L. var Eksotika is considered as a valuable alternate technology of propagation. Limited availability of the elite genotypes apprehends the utility of the *C. papaya* L. var Eksotika plants, for industrial use and ecological improvement. Therefore, development of an efficient method for *C. papaya* L. var Eksotika artificial seeds production by encapsulation of micro shoots in sodium alginate matrix were produced. Artificial seeds of *C. papaya* L. var Eksotika were produced by encapsulated micro shoots of *C. papaya* L. varieties Eksotika propagated *in vitro*. The production of artificial plant seeds of this species offered ideal beads based upon stiffness, texture, size and shape of beads. It was found that 4% sodium alginate and harden in two hundred millimolar $CaCl_2.2H_2O$ solution produced optimum beads with stiff, clear, round and homogeneous size for 30 minutes among the concentrations tested.

Keywords: Micro shoots; Encapsulation; Artificial seeds

Abstrak

Teknik pengeluaran benih tiruan *Carica papaya* L. var Eksotika dianggap sebagai teknologi pembiakan alternatif yang berharga. Penghasilan genotip elit adalah terhad bagi memenuhi keperluan tanaman *C. papaya* L. var Eksotika, untuk kegunaan industri dan peningkatan ekologi. Sehubungan dengan itu, pembangunan kaedah yang berkesan untuk pengeluaran benih tiruan *C. papaya* L. var Eksotika oleh enkapsulasi pucuk mikro dalam matriks natrium alginat telah dihasilkan. Benih tiruan dihasilkan oleh pengkapsulan pucuk mikro bagi *C. papaya* L. varieti Eksotika. Pengeluaran benih tiruan spesies ini memberikan manik yang ideal berdasarkan ketegangan, tekstur, saiz dan bentuk manik. Didapati bahawa di antara kepekatan yang diuji, 4% natrium alginat dan rendaman ke dalam 200 mM larutan $CaCl_2.2H_2O$ selama 30 minit telah menghasilkan manik yang optimum dengan saiz yang kukuh, jelas, bulat dan seragam.

Katakunci: Pucuk micro; Enkapsulasi; Biji benih tiruan

INTRODUCTION

In many tropical countries, papaya is considered one of the most important fruit crops. It is traditionally propagated by seeds and is therefore inhibited by problems such as seed dormancy and highly priced seedless type of papaya making it essential to search for an alternative propagation method (Mani et al., 2016). Artificial of *C. papaya* L. var Eksotika seeds make a promising technique for transgenic plant life propagation, non-seed plant production, and plant lines with seed propagation problem. Artificial plant seed propagation broadens the horizon of plant biotechnology (Pond and Cameron, 2017). The technology provides methods for the preparation of seedling analogues from the micropropagules such as axillary shoots and apical shoot tips.

Effective coatings of gelling agents such as alginate, agar, and carboxyl methyl cellulose contain micropropagules (Hecht and Srebnik, 2016). In a number of plant varieties, such as pineapple, banana and rice, encapsulation of micro shoots and subsequent collection of complete plantlets was reported. These kinds of evidence have shown that the production of artificial plant seeds is potentially useful for the large - scale propagation of advanced species hybrids.

Artificial plant seed technology can only be successful with efficient upstream production of micropropagules as well as downstream germination protocols for the high percentage of plant revitalization among the importants of plant tissue culture products. Various micropropagules were recently considered for the production of artificial plant seedling; however, the main recommendations were somatic embryos and axillary shooting. The present study represents different aspects of synthetic seed plant production and revitalization in *C. papaya* L. var Eksotika. The aim of this work was to study the production of synthetic seeds of *C. papaya* L var Eksotika from micro shoots obtained *in vitro*.

MATERIALS AND METHODS

Induction of Micro Shoots

Tissue culture method was used in this study. Explants were obtained from eight-week-old aseptic seedlings. Micro shoots of *C. papaya* L. var Eksotika was obtained from stem, leaf and root explants cultured on MS medium supplemented with 2.0 mg/l BAP. The stem cultures were maintained in the culture room at 23 ± 2°C for 16 hours light and 8 hours dark photoperiod.

Formation of Artificial Seeds

Sodium alginate solution 3% and 4% w/v were well prepared, added in sterile distilled water and MS basal medium (Trivedi, 2015). Calcium chloride dihydrate $CaCl_2.2H_2O$ solution was used as a complexion agent. The micro shoots of *C. papaya* L. were drizzled with sodium alginate and complexed in calcium chloride $CaCl_2$ for 30 minutes. Single micro shoot and alginate mixture were drawn up by using a sterile pipette. The micro

shoots were hardened by allowing them to remain $CaCl_2.2H_2O$ solution for 30 minutes. These beads have been removed and transferred into MS rinsing solution to wash out the excess $CaCl_2.2H_2O$ solution and were blotted with made sanitary tissue paper before being placed onto the culture medium. It is expected that each drop containing a single micro shoot will produce individual beads of 5 mm in diameter. The experiments were conducted to optimize the matrix of encapsulation (Ranabhatt and Kapor, 2017).

Germination of Artificial Seeds

For germination of artificial seeds, the beads were germinated on various germination medium and substrates. All the germinating substrates were autoclaved prior to use. The beads were also stored at 4°C (one to six months) prior to germination process. All samples were incubated at 25 ± 1°C, under 16-h light photoperiod of light intensity (1000 lux). Germination rate were recorded after six weeks of germination.

Data Analysis

Data obtained was analyzed using analysis of variance (ANOVA) and Duncan's Multiple Range Test (DMRT) was performed.

RESULTS AND DISCUSSION

Thirty replicates were used in each treatment. Uniform hardened encapsulated micro shoots were formed after 35 min in $CaCl_2.2H_2O$ solution. It was found that encapsulated micro shoot showed different degree of success depending on produced ideal beads (Table 1). The concentration of sodium alginate needed to encapsulate micro shoots has been declared to vary based on species (Raju et al., 2016). The present study successfully encapsulated micro shoot in a solution of 4 % sodium alginate and hardened it in a solution of 200 mM $CaCl_2.2H_2O$. The alginate matrix produced capsules around the micro shoot, resulting in solid, clear, round and uniform beads; represented by (++++) (Fig. 1B). Sodium alginate solution obtained bead with uniform size, isodiametric and solid and shown by (+++) dropped to 100 mM $CaCl_2.2H_2O$ solution. The beads that formed in 3% sodium alginate solution and hardened in 100 mm of $CaCl_2.2H_2O$ were soft to handle and very fragile, represented by (+), while those preserved in 3% sodium alginate solution and hardened in 200 mM of $CaCl_2.2H_2O$ (represented by (++)) gave solid texture beads and some beads formed clusters. Hecht and Srebnik (2016) reported that concentration of 3% sodium alginate was the most optimum to encapsulate *Ananas comosus* L. shoots.

Table 1. The effect of different concentrations of sodium alginate and $CaCl_2.2H_2O$ used in the encapsulation process of *Carica papaya* L. var Eksotika micro shoots.

$CaCl_2.2H_2O$ (mM)	Sodium alginate (%)	
	3.0	**4.0**
100	+	+++
200	++	++++

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

Advance experiments were conducted to determine the optimum encapsulation matrix concentrations. In order to assess the germination of encapsulated micro shoots (Figure 1A), the ability of the micro shoots to crack the gel and continue normal growth in shoot and root development was observed (Table 2). There was no simultaneous germination of the encapsulated micro shoot. Among the various concentrations and combinations, the best results were found when micro shoots were encapsulated with 4% sodium alginate solution and hardened with 3.500 ± 0.274 shoot germinated per explant in 200 mM $CaCl_2.2H_2O$ (Figure 1B). According to Micheli and Standardi (2016), the beads can potentially act as a nutrient reservoir that can help accelerate growth. The supplemented nutrients to the alginate matrix reduced the gel's viscosity and ability to form solid beads. Nevertheless, it was found that 30 minutes of exposure to the solution $CaCl_2.2H_2O$ was essential for complete encapsulation. Benasla and Hausler (2018) explained that both concentrations of sodium alginate and $CaCl_2.2H_2O$ played significant roles in the process of hardening and hardness of the capsules. Lowest germination rate was achieved, when artificial seed was prepared with 3% of sodium alginate and harden in 100 mM $CaCl_2.2H_2O$ solution seed bead with 0.933 ± 0.166 of shoot germinated every explants (Figure 1C). It was due to hardness in capsules and at the same time, due to anaerobic environment inside the beads, it could inhibit the micro shoot respiration. The bead hardness will depend mainly on the amount of sodium ions exchanged with calcium ions. The alginate bead's firmness thus provided better protection for the enclosed plant materials. Simultaneously, one of the significant limiting factors affecting germination could also be internal factors related to the micro shooting. Embryo quality has also been found to be one of the vital limiting factors affecting higher germination frequency, in this case micro shoots (Kesoju et al., 2016).

Etchepare et al., (2015) stated that several encapsulating agents from which agar, alginate, gelrite and polyacrylamide are essential have been tested. Nevertheless, due to its solubility at room temperature and its ability to create fully permeable gel with calcium chloride $CaCl_2$, it is advised that the most suitable encapsulating agent is sodium alginate. In this study, an efficient mechanism for encapsulating the micro shoots in *C. papaya* L. var Eksotika was successfully achieved.

Table 2. The effect of different concentrations of sodium alginate ($NaC_6H_7O_6$) and calcium chloride ($CaCl_2.H_2O$) on number of shoots germinated from synthetic seeds produced.

No	Treatment	No. of Shoots (Mean ± SE)
1	3% sodium alginate + 100 mM calcium chloride	$0.933 \pm 0.165_c$
2	3% sodium alginate + 200 mM calcium chloride	$0.967 \pm 0.131_c$
3	4% sodium alginate + 100 mM calcium chloride	$1.567 \pm 0.170_b$
4	4% sodium alginate + 200mM calcium chloride	$3.500 \pm 0.274_a$

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

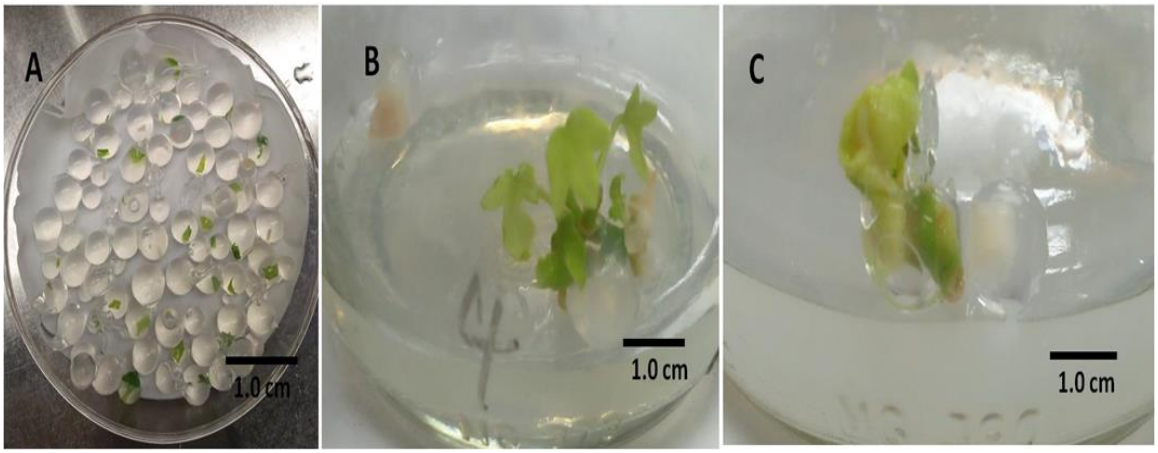

Figure 1. Encapsulated micro shoots of *C. papaya* L. (A). encapsulated micro shoot germinated on MS media without calcium added with 4% sodium alginate and harden in 200 mM $CaCl_2.2H_2O$ (B). Encapsulated micro shoot germinated on MS media without calcium added with 3% sodium alginate and harden in 100 mM $CaCl_2.2H_2O$ (C).

CONCLUSIONS

From this study, artificial seeds of *C. papaya* L. var Eksotika were successfully produced. Among the different concentrations and combinations, the optimum result was observed when 4% sodium alginate + 200 mM calcium chloride used in seed bead with 3.500 ± 0.274 of shoot germinated per explants. Lowest germination rate was achieved, when synthetic seed was prepared with 3% sodium alginate + 100 mM calcium chloride in seed bead with 0.933 ± 0.165 of shoot germinated per explants. The current research recommends that *C. papaya* L. var Eksotika produce uniform beads with high germination frequency. It would be particularly useful for commercial purposes for cloning and mass propagation of this plant species.

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