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Slow Growth Storage of Encapsulated Germplasm of *Coffea arabica* L.

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ABSTRACT

Germplasm conservation of encapsulated shoot buds of *Coffea arabica* was attempted. Shoot buds excised from *in vitro* plantlets were encapsulated in calcium alginate beads and stored on different substrates at 15, 20 and 25°C for 4, 8 and 12 months. Viability when encapsulated shoot buds were retrieved from storage conditions was evaluated. Substrate containing growth regulator-free 1/2 MS salts, 1% sucrose and 10 mg L^{-1} ABA was found to be the most effective in suppressing growth of encapsulated *Coffea arabica* shoot buds. The most suitable temperature for storage with all substrates tested was 20°C. However, if ABA is added to the substrate, a good storage is also possible at 15°C. Storage at 25°C was not recommended since buds germinated during storage and showed low percent of conversion into plantlets. The approach of the present investigation may be helpful for preservation of *Coffea arabica* germplasm.

Key Words: Slow growth; Germplasm storage; Encapsulation; Calcium alginate; *Coffea arabica*

INTRODUCTION

Coffea arabica is an important beverage crop grown in the tropical areas. It is one of the recalcitrant species whose seeds cannot be stored under normal conditions and must be planted soon after collection. Seeds cannot also be dried or frozen without losing viability (Ford-Lloyd & Jackson, 1986). Ellis *et al.* (1990) have grouped coffee under intermediate category for seed behavior because seed viability lasts for only short periods. The gene bank of Central Research Institute (CCRI) comprises over 350 genotypes of Arabian coffee, 15 types of robusta coffee and 17 different species of coffee (Naidu & Sreenath, 1999). The germplasm of this plant is maintained under field conditions, which requires large areas, intensive labour and the collection of plants is exposed to environmental risks. Hence, *in vitro* preservation methods offer an alternative means for maintaining gene banks.

For *in vitro* conservation, the preservation protocols should reflect maximum survival rate as well as genetic stability with minimum subculture frequency. *In vitro* plant cultures are usually stored by one of two approaches: slow growth or cryopreservation (Wilkins & Dodds, 1983; Withers, 1986). Slow growth could be achieved by reducing the culture temperature, modifying the culture medium usually by the addition of osmotic or hormonal inhibitors or the omission/ reduction of some factors which are usually essential for normal growth (Ford-Lloyd & Jackson, 1986; Dodds & Roberts, 1995). Reports on the successful preservation of several species using slow growth techniques have been published (Kartha, 1981; Grout, 1995 a, b; Bonnier & Tuyl, 1997; Romano & Martins-Loucao, 1999; Negri & Standardi, 2000; Hao & Deng, 2003). Yet, for coffee there are a few reports of *in vitro* germplasm preservation (Bertrand-Desbrunais *et al.*, 1991; Dussert *et al.*, 1997; Naidu & sreenath, 1999).

Studies on *in vitro* germplasm conservation using alginate-encapsulation techniques have been reported for only a few species. Maruyama *et al.* (1997) described the germplasm conservation of three forest trees using alginate-encapsulated shoot tips stored at 12-25°C for 6-12 months. Castillo *et al.* (1998) reported that encapsulated papaya somatic embryos survived storage for 85 days at 10°C under low light whereas un-encapsulated (control) embryos failed to germinate after storage. Pattnaik and Chand (2000) stored encapsulated buds of six mulberries for 60-90 days at 4°C without losing regenerative capacity. Soneji *et al.* (2002) stored synthetic seeds containing axillary buds of pineapple at 4°C. These buds remained viable without sprouting for 45 days.

The present study involves investigation of slow growth conditions for the storage of *Coffea arabica* germplasm encapsulated in calcium-alginate beads. Water or half-strength MS (Murashige & Skoog, 1962) nutrient salts in combination with reduced temperature, low sucrose concentration with or without the addition of abscisic acid (ABA) as a growth retardant were examined.

MATERIALS AND METHODS

Plant materials. Shoot tips (1.5 cm) were cut in early spring from *Coffea arabica* plant grown in the Botanic Garden of Botany Department, Faculty of Science, Ain Shams University, Cairo, Egypt. Shoot tips were washed in running tap water for 30 minutes, surface sterilized with 10% sodium hypochlorite for 15 minutes and washed three times with sterile distilled water. Sterilized shoot tips were placed vertically on initiation medium consisted of MS salts and B_5 vitamins (Gamborg *et al.*, 1968) supplemented with 8 µm benzyladenine and 1 µm naphthalene acetic acid. The

pH of the medium was adjusted to 5.6 ± 0.1 before the addition of 0.8% bacteriological agar (Meron, Marine Chemicals, Cochin, India). Media were sterilized by autoclaving for 15 minutes at 121°C. Plant materials were incubated at 27°C and 16 h photoperiod with light intensity of 35 µmol m^{-2} s^{-1}. Six weeks later, apical and axillary buds (3 mm) were excised from micro-shoots produced and used as explants for encapsulation.

Encapsulation. Autoclaved 5% sodium alginate (Duchefa Biochemie BV, Netherlands) dissolved in ½ strength MS salts containing 2 g L^{-1} sucrose was dropped into a sterile solution of 100 mM $CaCl_2$. Explants were immediately immersed into the formed beads. Beads were left for 20 minutes during which Na^+ and Ca^{++} ions exchanged (Kinoshita & Saito, 1990). Beads containing buds were then picked up and washed three times with sterile ½ - MS liquid medium. Encapsulated buds were then transferred to 250 mL quick-fit jars containing 100 mL of either of the following substrates:

S_1 : Water containing 1% sucrose; S_2 : Growth regulator-free ½ - MS salts containing 1% sucrose; S_3 : Growth regulator-free ½ - MS salts containing 1% sucrose and ABA (10 mg L^{-1}).

All substrates were gelled with 3% agar and sterilized before use. When ABA was added to the medium, it was filter sterilized using 0.2 µm diameter filters (Flow, U.K.) and added to the medium after autoclaving.

Storage. Beads containing shoot buds were stored at 15, 20 and 25°C and 12 h photoperiod with light intensity of 10 µmol m^{-2} s^{-1}. Encapsulated buds were retrieved from storage conditions after 4, 8 and 12 months. Viability was tested by transferring buds to initiation medium and conditions mentioned earlier. Viability is defined as the percentage of buds that were able to convert into vigorous healthy shoots.

Data analysis. Four replicates, each containing eight encapsulated buds were used per treatment at the three storage temperatures on the three substrates and over three storage periods. Quantitative data were analyzed by calculating percentage of plant conversion from stored buds. The standard error of means was calculated according to Snedecor (1975).

RESULTS

In the present investigation, an approach towards growth suppression of *Coffea arabica* germplasm using alginate-encapsulated technique was attempted. Minimizing nutrient and sucrose concentrations in combination with reducing temperature with or without the addition of ABA as a growth retardant were tested.

After 4 months, storage at 20°C showed high percentage of conversion with the three substrates tested in comparison with 15 and 25°C (Table I). However, at 15°C, the highest conversion rate (80%) was recorded for S_3, the substrate containing ABA. Storage on S_1 resulted in a higher conversion rate than S_2 at all temperatures tested (Table I).

After 8 months, a decline in the survival rate of the encapsulated shoot buds was observed with all substrates and temperatures. This decline was less on S_3. Some buds had also grown out of their capsules at 25°C on S_1 and S_2 but not on S_3 (Fig. 1).

After 12 months, a notable deterioration of the stored buds maintained on S_1 and S_2 at 15°C and 25°C and less at 20°C was recorded. Germination during storage at 25°C on S_1 and S_2 continued. Buds stored on S_3 showed high rate of conversion into plantlets at all temperatures tested.

DISCUSSION

The results of the present study revealed that the survival rate of *Coffea arabica* shoot buds encapsulated in alginate beads decreased with the prolonged duration of storage with all substrates and temperatures tested (Table I). When the influence of temperature is concerned, the best temperature for storage of this germplasm is 20°C. This result agrees with Maruyama *et al*. (1997) who found that 20°C is a recommendable temperature for the storage of alginate-encapsulated shoot tips of *Jacaranda mimosaefolia*, a tropical forest tree, for 6 months. The pronounced deterioration of shoot buds and failure of storage at 15°C in this study, on substrates S_1 and S_2 could be attributed to the sensitivity of coffee, being a tropical plant, to reduction in temperature. Lyons (1973) and Blakesley *et al*. (1996) reported that tropical and subtropical plants present a lesser natural cold-resistance and temperature of 10-12°C induced physical dysfunction. Banerjee and De Langhe (1985) determined the lower tolerance limits of tropical plants to 15-18°C. However, Maruyama *et al*. (1997) reported a high percent viability (80%) of encapsulated shoot tips of *Cedrela odorata*, a forest tree, after 12 months storage at

Table I. Effect of temperature and substrates on percentage of conversion after storage of alginate-encapsulated buds of *coffea arabica* (mean ± SE)

Temperature	15°C			20°C			25°C		
Subs.	S_1	S_2	S_3	S_1	S_2	S_3	S_1	S_2	S_3
4 months	71± 3.92	65± 3.80	80± 0.11	86± 2.19	73± 2.09	81± 3.19	70± 3.17	61± 3.75	68±2.54
8 month	32± 3.12	29± 1.76	68± 3.15	60± 4.12	58± 3.78	64± 1.87	43^G± 1.87	39^G± 2.53	59±3.34
12 months	21± 2.24	20± 1.74	57± 3.68	36± 3.69	32± 1.97	59± 4.03	15^G± 2.11	12^G± 2.61	44±1.16

G: Explants showed germination during storage; S_1: Water containing 1% sucrose;.S_2: Growth regulator-free ½ - MS salts containing 1% sucrose; S_3: Growth regulator-free ½ - MS salts containing 1% sucrose and ABA 10 mg L^{-1}

Fig. 1. Germination of *Coffea arabica* shoot buds encapsulated in calcium alginate beads after storage for 8 months at 25°C on substrate containing growth regulator-free ½ - MS salts and 1% sucrose

12°C. They used a substrate of only water solidified with 1% agar for storage.

The addition of ABA to the substrate in this investigation greatly inhibited the growth of the encapsulated buds during storage while preserved the viability. The first notable result is the high percent conversion of encapsulated buds after retrieved from storage conditions especially at 15°C compared to other substrates. A high percent conversion was also noticed after 8 and 12 months at 20 and 25°C (Table I). This result implies that ABA is important for either maintaining the dormancy and/or inducing cold tolerance in encapsulated buds. The role of ABA in inducing bud dormancy has been well documented in several tree species (Walton, 1980). The exogenous application of ABA was reported to induce dormancy in woody plants and it is present in high concentrations in dormant seeds (Naidu & Sreenath, 1999). Moreover, ABA is known to induce cold tolerance in various plant species (Chen & Gusta, 1983). The result of the present investigation confirms observations of other workers. Jarret and Gawel (1991) reported that ABA (10 mg L^{-1}) effectively inhibited the growth of sweet potato germplasm *in vitro* and this effect did not diminished over a one year culture period. In addition, a combination of ABA and cold treatment improved survival after cryopreservation of different *Rubus* genotypes (Reed, 1993). Vandenbussche and De Proft (1998) used encapsulation-dehydration technique to cryopreserve shoot tips of sugar beet and reported an increase in survival rate by adding ABA to the medium. Naidu and Sreenath (1999) successfully preserved immature zygotic embryos of coffee for 2 years at 25°C on medium containing 18.9 and 37.8 µM ABA.

In addition to the influence of ABA and reduced temperature, halving the nutrient supply (1/2 - MS) with the omission of exogenous plant growth regulators, the use of water or reduced sucrose concentration, beside the use of low light intensity are all recommended means of growth suppression reviewed by several workers. Berjak *et al.* (1996) reported the survival of *in vitro* shoots of *Eucalyptus* for 6 months when maintained under constant low light intensity (4 µmol m^{-2} s^{-1}) and reduced temperature on ¼ - MS salts. Negri and Standardi (2000) stored single node shoots of apple genotypes on different media containing ½ - MS salts, at 4°C in dark conditions for 18 months.

For coffee, Bertrand-Debrunais *et al*. (1992) examined the effect of low temperature in combination with reduced sucrose concentration on a collection of coffee micro-cuttings and reported a reduction in growth and a promotion in the survival rate of cuttings at 20°C and 2% sucrose in the medium. In addition, Kartha *et al.* (1981) succeeded in preserving *Coffea arabica* rooted plantlets for two years at 27°C on a medium lacking sucrose.

Moreover, the use of alginate seems to play a beneficial role in the storage of germplasm. Brodelius *et al.* (1982) reported that the alginate bead effect on growth immobilization or minimal growth storage might be attributable to a reduction in the respiration process in encapsulated cells. Storage of alginate-encapsulated buds at temperature above freezing is an acceptable method for conservation of germplasm cultured *in vitro* (Maruyama *et al*., 1997). This technique might help as well in lengthening the time between subcultures, the frequency of which enhances the risk of genetic changes (Rani *et al*., 1995). Germplasm conservation techniques at temperature conditions above freezing represent an important alternative for many species intolerant to conventional reduced temperature storage system (generally at 4°C).

CONCLUSIONS

In conclusion, half-strength MS salts containing 1% sucrose and 10 mg L^{-1} ABA was found, in this study, to be the best substrate for suppressing growth of alginate-encapsulated shoot buds of *Coffea arabica.* When the temperature is concerned, 20°C is the most suitable for storage with all substrates. However, if ABA is added to the substrate a good storage is also possible at 15°C. Storage of encapsulated buds at 25°C is not recommended since the buds showed germination during storage and low percent of conversion into plantlets.

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