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Effects of Salicylic Acid and Encapsulation on Enhancing the Resistance of Embryonic Axes of Persian lilac (*Melia azedarach* L.) against Cryopreservation

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ABSTRACT

Embryonic axes of Persian lilac (*Melia azedarach* L.) encapsulated into calcium alginate beads with sucrose (0.75 m) and salicylic acid (0, 50, 200 µM) were subjected to cryopreservation with dehydration and freezing in liquid nitrogen. In this case 200 µM salicylic acid significantly enhanced the percentage of viability of encapsulated embryonic axes. The role of salicylic acid in increasing tolerance to dehydration is discussed.

Key Words: Cryopreservation; *M. azedarach* L.; Encapsulation; Salicylic acid

INTRODUCTION

Melia azedarach L. (Persian lilac) is a native tree of Asiatic Middle East. *In vitro* conservation of germplasm is often realized by conditions, which reduce the rate of tissue growth to a minimum either by low temperature, withdrawing a nutrient from the medium or by adding a growth retardant. Encapsulation of tissues within alginate beads (artifical seed) by restricting their respiration reduces considerably their growth (Brodelius *et al*., 1982) and then allows their stockage in this condition.

For the long-term conservation of plant germplasm, cryopreservation is actually the valuable technique since in the conditions of liquid nitrogen (LN) freezing the metabolism ceases to function, tissues are maintained without growth and genetic alterations do not take place during the very long period of stockage. But tissues must withstand the drastic conditions of the technique; this means to undergo the high dehydration necessary in most cases. For this reason, the use of pretreatments is necessary. For these pretreatments sucrose, which increases the tolerance to dehydration and therefore contributes to maintaining the tissues' viability (Dumet *et al*., 1993) is greatly preferred. Pretreatment with abscisic acid may also increase tolerance to dehydration (Senaratna *et al*., 1989). In cryopreservation, complementary to these pretreatments, encapsulation within alginate beads before dehydration was shown to be beneficial to the technique by increasing considerably the resistance of plant tissues to desiccation and freezing (Dereuddre *et al*., 1990). Generally tissues, which have low water content, such as meristematic tissues and embryonic axes, are more resistant to the stresses of these techniques (Radhamani & Chandel, 1992; Janeiro *et al*., 1996).

In this research, the effect of salicylic acid (SA) natural substance implicated regularly in plant responses to physical and biological aggressions (Raskin, 1992) and encapsulation studied on embryonic axes submitted to the stresses of cryopreservation. This work was undertaken with embryonic axes of *M. azedarach* L). Persian lilac wood is desirable and it is used at industry especially in making woody equipments. Difference parts of this plant are used in medicinal and pharmaceutical.

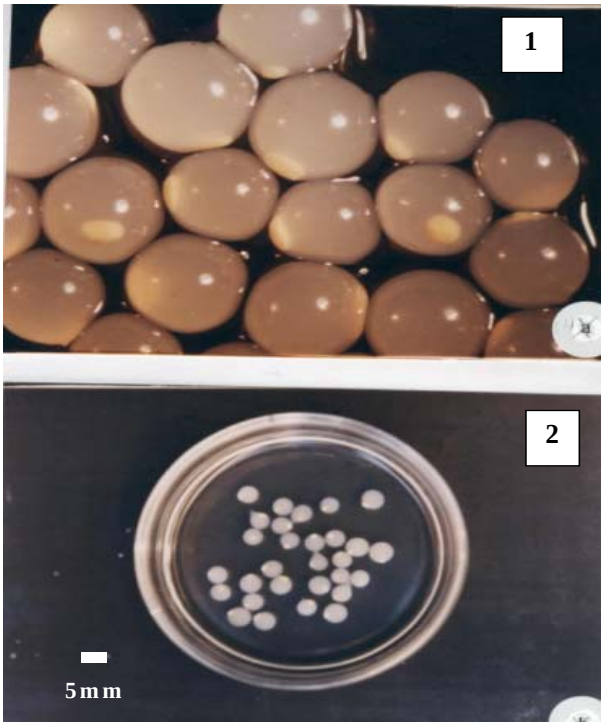
MATERIALS AND METHODS

Embryonic axes were isolated from the seeds of ripe fruits collected from the region of Mazandaran, Iran. Seeds were disinfected for 1 min in ethanol 70% (v/v) and 1% sodium hypochlorite (w/v) for 15 min. After, embryonic axes were excised and placed in the culture medium for experimentation. All experiments were done in MS medium (Murashige & Skoog, 1962) (basal salt mixture & vitamins) supplemented with glutamine (2 mg L^{-1}).

For encapsulation, embryonic axes were suspended in MS medium supplemented with 3% (w/v) sodium alginate, 0.75 m sucrose and SA (0, 50, 200 µM). After 1/2 h, embryonic axes were picked individually with forceps and dropped into MS medium containing 100 mm $CaCl_2$ (w/v), 0.75 m sucrose and SA (0, 50, 200 µM). Alginate beads, each containing one embryonic axis were maintained in this medium for 30 min with slow agitation (Fig. 1). In the case of controls with non-encapsulated embryonic axes, axes were suspended 1 h in liquid MS, 0.75 m sucrose and SA in the abovementioned concentrations. Encapsulated and non-encapsulated axes were transferred to empty open petri dishes and desiccated in the air current of a laminar flow chamber for 1 h.

For cryopreservation, after dehydration, samples were transferred into sterile polypropylene tubes, which were directly immersed in LN, where they remained for 1 h. For control, a series of non-pretreated axes was placed for 1 h in LN immediately after their excision. Then frozen samples

Fig. l. Encapsulated embryonic axes (beads), 1-under of Binoculair (l0x) (scale:mm), 2-In natural size (scale: mm)



were thawed rapidly by placing the tubes into a water bath at 37°C for 2 min and transferred directly to appropriate culture medium.

After cryopreservation, samples were cultured on solid MS (Agar- agar 0.8%) with 3% sucrose. Cultures were incubated at 25°C with a photoperiod of 16 h light/8 h dark. The percentage of viability was monitored 15 days after the transfer to the culture medium and the length of axes that had germinated was measured. In this experiment, each treatment consisted of six replications and two or four times repeated. Data were analyzed using analysis of variance (ANOVA) and means were compared at $P < 0.05$.

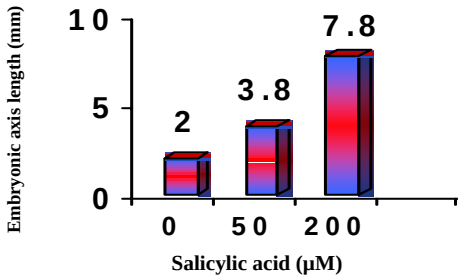
RESULTS AND DISCUSSION

Contrary to results with embryonic axes of *Camellia sinensis* (Janeiro *et al*., 1996), which withstood freezing after being submitted to partial desiccation, non-encapsulated embryonic axes of *M. azedarach*, even those preteated with sucrose and dehydrated for 1 h, did not survive the liquid nitrogen treatment. In this case, SA given in pretreatment with sucrose did not enhance the resistance of these tissues (Table I). According to the literature (Dereuddre, 1990) embryonic axes of *M. azedarach* L. encapsulated within alginate may offer a better resistance. With 0.75 m sucrose in the encapsulation medium the rate of viability after freezing reached 42% (Table I). Sucrose at high concentration must be maintained in contact with the axes during the whole time of the procedure and this is

Table I. Effect of salicylic acid on the viability of *M. azedarach* L. non-encapsulated and encapsulated embryonic axes immersed into liquid nitrogen for 1 hour. %Viability is measured 15 days after their transfer to culture medium

Salicylic acid (μ M)	Viability % of embryonic axes		
	Non-pretreated, non-encapsulated, embryonic axes	Pretreated non-encapsulated embryonic axes	Pretreated, Encapsulated embryonic axes
0	0	0	42 ±10
50	0	0	63 ±10
200	0	0	71 ±9

Fig. 2. Effect of salicylic acid in the medium of encapsulation on the growth of cryopreserved embryonic axes of *M. azearach*. Measures were done 15 days after transfer of the encapsulated axes from liquid nitrogen to the MS growth medium.



permitted by their encapsulation in alginate beads. Preculture with a high concentration of sucrose greatly increases the intracellular concentration (Uragami *et al*., 1990) and intracellular sucrose acts as the principal agent of tolerance to desiccation (Koster & Leopold, 1988). But if SA (at 50 or 200 µM) is added to alginate in complement to sucrose, the registered percentage of survey is considerably increased until 71% with 200 µM SA (Table I). From these results, it can be postulated that SA plays an interesting role in the resistance of these tissues to freezing. Exogenous application of SA may lead to an enhancement of endogenous SA (Seo *et al*., 1995), which may also play an active role in the process of resistance to desiccation. Endogenous SA is an induction signal for specific defense responses of plants and it has been showed that it acts in one of its numerous roles by causing water stress in the tissues (Shah & Klessig, 1999). Thus, embryonic axes precultured one hour and encapsulated with SA may better reflect the dehydration and freezing of cryopreservation protocols.

Concentrations of SA used in this experiment, on no account, interfered with the axes development and even a higher significant recovery of growth has been monitored on the axes, which benefited from a higher protection by SA (200 µM) during cryopreservation (Fig. 2) probably resulting from a better conservation of the tissue integrity.

To conclude, the addition of SA in the encapsulation medium may be suitable to reinforce the tolerance of tissues and more particularly for those that are sensitive to dehydration caused by conservation procedures.

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