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Cryopreservation of Rose Cultivars: Comparison of Two Encapsulation Protocols

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Abstract. Shoot tips excised from in vitro plants of *Rosa x hybrida* L., cvs. Ioana, Mariana and Vulcan were successfully cryopreserved using encapsulation-dehydration and encapsulation-vitrification methods. Shoot tips (2-3 mm in length) were excised, encapsulated in natrium alginate beads, precultured in sucrose (0.25, 0.5, 0.75 M) for 24 h and either desiccated in laminar air flow (up to 5 h) or dehydrated with a vitrification solution (up to 220 min) prior to direct immersion in liquid nitrogen. The highest shoot development (72% for cv. Vulcan, 69% for cv. Mariana and 66% for cv. Ioana) after storage in liquid nitrogen was obtained after using the encapsulation-vitrification protocol.

Keywords: encapsulation-dehydration, long-term storage, vitrification

Introduction. The conventional method for conservation of woody plants is the field collection. For long-term preservation of these genetic resources the field gene bank is not only costly (due to the constant maintenance) but also is susceptible to genetic erosion, insects, diseases and environmental stress (Engelmann, 2000; Panis and Lambardi, 2005) and the traditional rose cultivation is a complex culture (Van den Berg, 1996). The availability or development of reliable and cost effective strategies and the subsequent regeneration of the plants are basic requirements for germplasm conservation (Kaviani, 2010).

Aims and Objectives. The main objective of the study was to establish an optimized protocol for the long-term storage of rose (*Rosa hybrida* (L.), cvs. Vulcan, Ioana and Mariana) shoot tips by cryopreservation using encapsulation-based techniques.

Materials and Methods. Shoot apices (2-3 mm in length) consisting of the apical meristem covered by 2-3 leaf primordia were excised from 2 months old in vitro mother plants under a stereo microscope in sterile conditions. Encapsulation-dehydration developed by Fabre and Dereuddre (1990) is based on desiccation of the alginate coated plant material in the presence of sucrose followed by evaporative desiccation performed under laminar air flow. Encapsulation-vitrification is a combination of the encapsulation technique and the vitrification protocol and involves encapsulation of shoot apices followed by treatment with a plant vitrification solution (PVS2) (Sakai *et al.*, 1990).

Results and Discussions. Combining preculture of the beads in sucrose-containing medium with their dehydration a reduction of the water content of alginate beads was achieved to a level, which allowed shoot regrowth following cryopreservation. The highest shoot development (73% for cv. Vulcan, 69% for cv. Mariana and 66% for cv. Ioana) after storage in liquid nitrogen was achieved following preculture in 0.25 M sucrose and 180 or 220 min dehydration treatment with PVS2 depending on cultivar (*Tab. 1*) During cooling in liquid nitrogen lethal ice crystals can be formed in the cells thus, prior freezing free water needs to be reduced to avoid crystallization (Sakai, 1993). Due to the extreme dehydration of

explants most freezable water is removed from cells and vitrification of internal solutes takes place during rapid exposure to liquid nitrogen avoiding lethal intracellular ice crystallization (Engelmann, 1997). Moisture content of alginate beads is a critical factor for a successful cryopreservation and therefore is vital to dehydrate encapsulated shoot tips sufficiently to allow them to survive cooling in liquid nitrogen (Engelmann, 1997).

Conclusion. Our results indicate that the use of the encapsulation-vitrification technique was efficient for producing high rates of plant regeneration from cryopreserved shoot tips in the tested cultivars. These results indicate that *Rosa* cultivars shoot tips can withstand deep-freezing in liquid nitrogen and can regenerate whole plants.

Tab. 1

Shoot regrowth from cryopreserved shoot apices
following encapsulation-vitrification (E-V) protocol.

	Shoot regrowth after E-V (% ± S.D.)					
	Sucrose	Dehydration duration (min.)				
	(M)	60	100	140	180	220
cv. Vulcan	0.25	0	19.4 ± 2.0	41.6 ± 3.0	72.2 ± 1.5	52.7 ± 2.0
	0.50	0	30.5 ± 1.5	50.0 ± 3.6	69.4 ± 1.1	47.2 ± 1.5
	0.75	0	16.6 ± 1.0	38.8 ± 3.5	47.2 ± 2.0	38.8 ± 1.1
cv. Ioana	0.25	0	27.7 ± 1.5	50.0 ± 1.7	66.6 ± 1.0	52.7 ± 2.5
	0.50	0	22.2 ± 0.5	44.4 ± 2.3	63.8 ± 0.5	47.2 ± 0.5
	0.75	0	11.1 ± 1.1	38.8 ± 2.0	52.7 ± 2.0	50.0 ± 1.7
cv. Mariana	0.25	0	25.1 ± 1.1	30.5 ± 2.0	55.5 ± 1.5	69.4 ± 1.1
	0.50	0	19.4 ± 1.1	33.3 ± 1.0	50.0 ± 1.7	47.2 ± 1.5
	0.75	0	8.30 ± 1.0	27.7 ± 0.5	36.1 ± 1.1	41.6 ± 1.7

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