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Cryopreservation of grapevine (*Vitis vinifera* L.) *in vitro* shoot tips

Research Article

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Abstract: In this work, we compared the efficiency of encapsulation-dehydration and droplet-vitrification techniques for cryopreserving grapevine (*Vitis vinifera* L.) cv. Portan shoot tips. Recovery of cryopreserved samples was achieved with both techniques; however, droplet-vitrification, which was used for the first time with grapevine shoot tips, produced higher regrowth. With encapsulation-dehydration, encapsulated shoot tips were precultured in liquid medium with progressively increasing sucrose concentrations over a 2-day period (12 h in medium with 0.25, 0.5, 0.75 and 1.0 M sucrose), then dehydrated to 22.28% moisture content (fresh weight). After liquid nitrogen exposure 37.1% regrowth was achieved using 1 mm-long shoot tips and only 16.0% with 2 mm-long shoot tips. With droplet-vitrification, 50% regrowth was obtained following treatment of shoot tips with a loading solution containing 2 M glycerol + 0.4 M sucrose for 20 min, dehydration with half-strength PVS2 vitrification solution (30% (w/v) glycerol, 15% (w/v) ethylene glycol, 15% dimethylsulfoxide and 0.4 M sucrose in basal medium) at room temperature, then with full strength PVS2 solution at 0°C for 50 min before direct immersion in liquid nitrogen. No regrowth was achieved after cryopreservation when shoot tips were dehydrated with PVS3 vitrification solution (50% (w/v) glycerol and 50% (w/v) sucrose in basal medium).

Keywords: *Encapsulation-dehydration • Droplet-vitrification • Shoot tip size*

1. Introduction

Grapevine is one of the economically most important fruit worldwide [1]. Its long cultivation has resulted in the development of large numbers of cultivars adapted to a wide diversity of climates. Many grapevine cultivars are now endangered and international efforts aiming at preserving grapevine biodiversity have been undertaken. Although field collections play a preeminent role in grapevine conservation programmes, maintenance of plant genetic resources only in field collections is risky, as valuable germplasm can be lost because of pests, diseases and various calamities such as adverse weather conditions [2,3]. Today, biotechnology offers a broad range of techniques, which allow optimizing plant genetic resource conservation.

Among these techniques, cryopreservation (liquid nitrogen [LN], -196°C) is a highly suitable and efficient tool for long-term storage of plant germplasm, requiring minimum space and maintenance [2]. The cryopreservation protocols developed recently do not require controlled cooling, thereby allowing cells and shoot tips to be cryopreserved by direct transfer to LN. The development of simple and reliable cryopreservation methods should allow much borader use of cryopreserved cultured cells, shoot tips and somatic embryos [4]. Among the techniques developed, encapsulation-dehydration, vitrification and droplet-vitrification are the most frequently used for cryopreservation of shoot tips.

Encapsulation-dehydration is based on the technology developed for producing artificial seeds

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