Duration of culture of grapevine (*Vitis vinifera*) microcuttings on medium with zeatin riboside affects shoot tip recovery after cryopreservation

Zvjezdana Marković [1, 2, *], Philippe Chatelet [2, 3], Isabelle Sylvestre [3], Jasminka Karoglan Kontić [1], Florent Engelmann [3, 4]

[1] University of Zagreb, Faculty of Agriculture, Svetošimunska cesta 25, 10 000 Zagreb, Croatia
[2] INRA UMR AGAP, Equipe DAVEM, 2 place Viala, 34060 Montpellier, France
[3] IRD, UMR DIADE, 911 Avenue Agropolis, BP 64501, 34394 Montpellier cedex 5, France
[4] Bioversity International, Via dei Tre Denari 472/a, 00057 Maccarese, Rome, Italy

1. Introduction

Cryopreservation is the only method currently available for the safe, cost-effective, long-term conservation of vegetatively propagated plant species such as grapevine. Cryopreservation protocols have been established for shoot tips sampled from grapevine *in vitro* plantlets (Plessis *et al.* 1993; Wang *et al.* 2000; Zhao *et al.* 2001; Matsumoto and Sakai 2003).
An important parameter for successful cryopreservation is the physiological state of the plant material, and buds taken at different levels on the shoots of the stock plants could differ in this regard. It can then be advisable to preculture microcuttings to obtain axillary shoots from which terminal buds can be sampled, constituting a more homogeneous explant population. A cytokinin can be used at this stage to achieve quicker and more homogenous shooting from the microcuttings. Zeatin riboside (ZR) has been shown to stimulate bud proliferation in grapevine (Goussard 1987). As culture duration on ZR medium could affect the physiological state of the plant material, we investigated the effect of this parameter on grape shoot tip recovery after cryopreservation.

2. Material and methods

2.1 Plant material

The plant material employed in this study consisted of *in vitro* plantlets of grapevine (*Vitis vinifera*) cultivar 'Portan', (initial material courtesy of the late Dr A. Bouquet) obtained from the INRA grapevine germplasm field repository in Vassal, France.

2.2 Methods

In vitro culture. *In vitro* plantlets were cultured on half-strength MS medium (Murashige and Skoog 1962) containing 20 g/l sucrose and 7 g/l agar. They were kept without subculture for 2 months at 26 ± 1 °C under a 16 h light/8 h dark photoperiod, with a light intensity of 3000 lux until they were approximately 12 cm long. The plantlets were then dissected in microcuttings consisting of stem fragments of approx. 1.5 cm with one bud, which were placed on culture medium containing 1 µM ZR, and kept on this medium for 4 to 16 weeks with transfer to new medium every 4 weeks. Control samples consisted of shoot tips, which were not cultured on medium with ZR. Mean explants number per treatment was 10.

Cryopreservation. The droplet-vitrification technique was employed for shoot tip cryopreservation. After excision, shoot tips were placed for 24 h on a medium containing 0.3 M sucrose, treated for 20 min with a loading solution containing 1.2 M glycerol + 0.4 M

sucrose, then with cold (0 °C) half-strength PVS2 vitrification solution (Matsumoto and Sakai 2003) for 30 min, dehydrated with full-strength PVS2 for 25, 50 or 75 min, cooled rapidly in liquid nitrogen in PVS2 droplets placed on aluminium foils, rewarmed rapidly by immersion of foils in an unloading solution containing 1.2 M sucrose for 20 min, then transferred for recovery on half-strength MS based with 1 µM BA (Wang *et al.* 2003).

3. Results

Recovery of cryopreserved shoot tips was achieved after up to 8 weeks of culture on ZR medium (Table 1). No regrowth was obtained after longer culture durations. The highest regrowth percentages (40-45 %) were noted with shoot tips cultured on ZR medium for 4 or 8 weeks, which had been treated with PVS2 for 75 min. Regrowth of cryopreserved shoot tips was rapid and direct (Fig. 1). Extended culture duration on ZR medium induced callogenesis during the development of microcuttings (Fig. 2).

Table 1. Effect of culture duration on medium with ZR and of duration of exposure to PVS2 on recovery (%) of grapevine shoot tips after cryopreservation.

Culture duration on ZR medium (weeks)	Recovery (%) **Duration of exposure to PVS2 (min)**		
	25	**50**	**75**
0	33	6	20
4	18	20	40
8	0	30	45
12	0	0	0
16	0	0	0

Figure 1: Regrowth of cryopreserved shoot tip treated with PVS2 for 75 min.

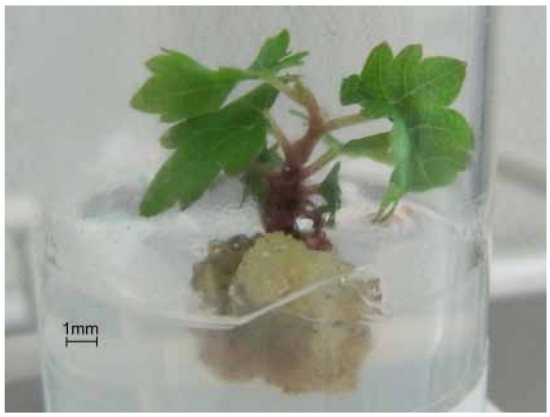

Figure 2: Extended culture duration of shoot tips on ZR medium induced callogenesis.

4. Discussion

This study demonstrated that microcutting culture duration on ZR affected shoot tip recovery percentage after cryopreservation. Shoot tips should be sampled on microcuttings cultured on ZR medium for 0-8 weeks. Although a stimulatory effect of preculture cannot be undoubtedly demonstrated from these experiments, it can be remarked that the highest recovery percentages obtained in this study were achieved after one or two preculture cycles on ZR-containing medium.

Only intermediate recovery was obtained after cryopreservation in these preliminary experiments. Improved results may be achieved notably by modifying PVS2 treatment duration, or by using other vitrification solutions such as PVS3 or recently developed alternative solutions (Kim *et al.* 2009).

5. Acknowledgements

The authors thank André Peyrière for initial plant material multiplication. This work has been financed by the National Foundation for Science, Higher Education and Technological Development of the Republic of Croatia (ZM) and supported in part by the ARCAD project, a flagship programme of Agropolis Fondation (IS).

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Markovic Z., Chatelet P., Sylvestre Isabelle, Karoglan Kontic J., Engelmann Florent (2011)

Duration of culture of grapevine (Vitis vinifera) microcuttings on medium with zeating riboside affects shoot tip recovery after cryopreservation

In : Grapin A. (ed.), Keller E.R.J. (ed.), Lynch P.T. (ed.), Panis B., Revilla Bahillo A. (ed.), Engelmann Florent (ed.). Cryopreservation of crop species in Europe : COST Action 871 : CryoPlanet : proceedings of the final meeting

Bruxelles (BEL) ; Luxembourg : COST Office ; European Union, 145-147

COST Action 871 : CryoPlanet : Cryopreservation of Crop Species in Europe : Final Meeting, Angers (FRA), 2011/02/08-11

ISBN 978-92-898-0051-8