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Effects of cryopreservation of recalcitrant *Amaryllis belladonna* zygotic embryos on vigor of recovered seedlings: a case of stress 'hangover'?

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Cryopreservation is the most promising long-term storage option for recalcitrant (i.e. desiccation-sensitive) seed germplasm; however, its effects on the vigor of recovered seedlings are unclear. This study looked at the vigor of seedlings recovered from partially dried (D) and cryopreserved (C) recalcitrant zygotic embryos (ZEs) of *Amaryllis belladonna*. Seedlings recovered from fresh (F), D- and C-embryos were regenerated in vitro, hardened-off ex vitro and then exposed to 12 days of watering (W) or 8 days of water deficit (S), followed by 3 days of re-watering. Seedling vigor was assessed in terms of physiological and growth responses to the imposed water stress. Compared with F-embryos, partial dehydration and cryopreservation reduced the number of embryos that produced seedlings, as well as the subsequent in vitro biomass of these seedlings. DW- and CW-seedlings (i.e. seedlings recovered from dried and fresh ZEs that were watered for 12 days) exhibited lower CO_2-assimilation rates and abnormal root growth. Stomatal density was also lower in C-seedlings. DS- and CS-seedlings were exposed to persistent low leaf water and pressure potentials and unlike FS-seedlings, displayed signs of having incurred damage to their photosynthetic machinery. CS-seedlings were less efficient at adjusting leaf water potential to meet transpirational demands and more susceptible to persistent turgor loss than DS- and FS-seedlings. DS-seedlings performed slightly better than CS-seedlings but drought-induced seedling mortality in both these treatments was higher than FS-seedlings. These results suggest that seedlings recovered from partially dried and cryopreserved embryos were less vigorous and more susceptible to hydraulic failure than those from fresh ZEs.

Introduction

Cryopreservation (i.e. storage at ultra-low temperatures, usually −196°C) is the most promising long-term storage option for recalcitrant (i.e. desiccation-sensitive; Roberts 1973) seed germplasm, which otherwise cannot be stored for any useful period of time (Berjak and Pammenter 2004). Unlike the success achieved with somatic (e.g. Mycock et al. 1995) and nucellar embryos (e.g. Kartha 1985), and even zygotic embryos (ZEs) from orthodox seeds (e.g. Gagliardi et al. 2002), recovery after cryopreservation of recalcitrant ZEs/embryonic axes (EAs) very seldom results in the production of callus-free plants, rid of morphological abnormalities (Mycock 1999). In fact, a number of studies (e.g. Dumet et al. 1997, Sershen et al. 2007, Steinmacher

Abbreviations – ANOVA, analysis of variance; Chl, chlorophyll; CP, cryoprotection; EAs, embryonic axes; FW, fresh weight; Gly, glycerol; md, midday; LN, liquid nitrogen; PSI, photosystem I; PSII, photosystem II; pd, predawn; RGR, relative growth rate; SEM, scanning electron microscope; ZEs, zygotic embryos.