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Effects of partial dehydration of recalcitrant *Haemanthus montanus* zygotic embryos on vigour of recovered seedlings

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Abstract

Cryopreservation is the most promising route for the long-term conservation of recalcitrant seed germplasm. Partial dehydration is a standard pre-treatment for the cryopreservation of zygotic embryos or embryonic axes excised from recalcitrant seeds since it reduces the likelihood of lethal ice-crystal generation during cooling. However, there is presently little to no understanding of how pre-conditioning treatments such as partial dehydration imposed at the embryonic stage are translated or manifested during subsequent *in* and *ex vitro* seedling growth. The present study assessed the vigour of seedlings recovered from partially dried (D) zygotic embryos, excised from recalcitrant *Haemanthus montanus* (Baker) seeds. Seedlings recovered from fresh (F) and partially dried (D) embryos *in vitro*, were hardened-off *ex vitro*, and subsequently subjected to either 42 days of watering (W) or 42 days of water deficit (S). The adverse effects of partial dehydration on seedling dry mass accumulation observed after 60 days *in vitro* growth did not disappear with an extension of the *in vitro* growth period but did appear to be reversible during *ex vitro* growth. A water stress during *ex vitro* growth dominated over the effects of embryo pre-treatment with relative growth rates in FS-seedlings (recovered from fresh embryos and subsequently stressed) and DS-seedlings (recovered from dried embryos and subsequently stressed) being statistically comparable. D- and F-seedlings responded typically to the water stress but DS-, compared with FS-seedlings, appeared to have incurred permanent damage to their photosynthetic machinery, were exposed to lower predawn water potentials, were less efficient at adjusting leaf water potential to meet transpirational demands, did not exhibit signs of osmotic adjustment, failed to adopt growth patterns that reduce transpirational water loss, and were more susceptible to persistent turgor loss. It was therefore not surprising that *ex vitro* seedling mortality occurred in more DS- than FS-seedlings. These results suggest that partial dehydration of recalcitrant *H. montanus* zygotic embryos, even when not followed by cooling, can reduce the vigour and drought tolerance of recovered seedlings.

Keywords: Cryopreservation; *Haemanthus montanus*; Recalcitrant; Seedling vigour; Water stress; Zygotic embryos

1. Introduction

Partial dehydration, in reducing explant heat capacitance and mass, can facilitate faster cooling rates and reduce the formation of lethal ice-crystals during the cryopreservation of seed tissues (Wesley-Smith et al., 2001), and has become a standard pre-treatment for the cryopreservation of zygotic embryos or embryonic axes excised from recalcitrant seeds (Pence, 1992; Sershen et al., 2007; Steinmacher et al., 2007). Seedlings recovered from partially dehydrated, cryopreserved orthodox zygotic embryos or embryonic axes have been reported to be morphologically similar to those generated from control axes, going on to produce flowers and fruit in the field (Gagliardi et al., 2002). However, partial dehydration represents a source of physicochemical damage in recalcitrant zygotic germplasm (Walters et al., 2001), with a number of studies showing seedlings recovered from partially dried and/or cryopreserved recalcitrant zygotic germplasm to exhibit abnormal phenotype (Pence, 1992; Wesley-Smith et al., 2001) and/or reduced growth (Sershen et al., 2007; Steinmacher et al., 2007).

There is at present little to no understanding of how stresses imposed at the embryonic stage are translated or manifested during subsequent *ex vitro* seedling growth, in recalcitrant-seeded species. Reports in orthodox-seeded species suggest that there exists within developing embryos (zygotic and somatic)

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