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Published in:
CryoLetters

Publication date:
2010

Document version
Publisher's PDF, also known as Version of record

Citation for published version (APA):
Vogiatzi, C., Grout, B. W. W., & Toldam-Andersen, T. B. (2010). Understanding the cryosurvival of cold-hardened, winter apple buds - critical water content and the role of non-differentiated secondary primordia. *CryoLetters*, 31(2), 182-183.

**UNDERSTANDING THE CRYOSURVIVAL OF COLD-HARDENED,
WINTER APPLE BUDS – CRITICAL WATER CONTENT AND THE ROLE OF
NON-DIFFERENTIATED SECONDARY PRIMORDIA**

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Dormant bud cryopreservation is important for genetic conservation as it has the benefits of simplicity and avoids expensive in vitro culture yet, for temperate fruit crops e.g. apples and pears, survival can be highly variable within, and between, accessions, cultivars and seasons.

To investigate events during the first part of the cryopreservation protocol (a prolonged dehydration step) winter-collected explants of three apple cultivars were stored for 1-2 weeks at -40C, allowing water content to reduce from c.45 to c.30% of fresh weight, widely reported as suitable level for subsequent preservation. Using exotherm analysis as a diagnostic tool it was clear that between cultivars significant differences existed in the freezing pattern of bulk water in the dehydrated bud. These related consistently to post-cryopreservation survival.

The acclimation history of the explants may be of significance in this regard as bulk water in apple buds is known to increase during the endodormant period [2]. A comparable examination of buds recovered from liquid nitrogen showed similar differences, suggesting that a high, freezable water content at this stage was linked to low survival. Additionally, published survival data typically presents bud outgrowth in some way but ignores the contribution of secondary buds. This study demonstrated that where the primary bud meristem does not survive cryopreservation then secondary bud development and meristem outgrowth can contribute significantly (up to 50%) to overall survival.

1] Toldam-Andersen TB, Nygaard TB and Krogholm KS (2007) *Adv Hort Sci* **21**, 193-197

2] Faust M, Liu D, Line MJ and Stutte GW (1995) *Acta Horticulturæ* **395**, 113-118