

Title Medium and long-term storage of *Rubus* germplasm
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Citation ISHS Acta Horticulturae 777:91-98. 2008.
Keywords: blackberries; cold storage; cryopreservation; in vitro; raspberries

Abstract

The United States Department of Agriculture, Agricultural Research Service, National Clonal Germplasm Repository at Corvallis, Oregon preserves genetic resources for *Rubus*. The in vitro collection includes about 200 accessions. In vitro cold storage of these accessions is at 4°C with 12 h of low light. Storage facilities for germplasm collections vary, but one week of cold acclimation followed by 4°C storage in the dark or with a photoperiod is acceptable for most *Rubus* germplasm when quarterly evaluation inventories are used to determine timing of repropagation. A reduced-nitrogen medium extends room temperature storage to nine months and is a useful alternative for cold sensitive and tropical genotypes which typically only survive for a short time in cold storage. Meristems of 34 cold-acclimated genotypes of *Rubus* (blackberry and raspberry) were successfully cryopreserved by slow cooling through optimization of cryoprotectants, cooling rates and cold acclimation. Alternating low temperatures as a cold acclimation (CA) treatment improved recovery of shoot tips cryopreserved by slow freezing. The length of the CA required varied from 1 to 10 weeks and was genotype dependent. Cryopreserved *Rubus* shoot tips produced shoots directly from either apical meristems or axillary buds, but not from callus. Shooting increased and callus formation decreased when IBA was eliminated from the recovery medium. Shoot tips of 25 genotypes in 9 *Rubus* species were successfully cryopreserved using encapsulation-dehydration with recovery of 60–100%. Four genotypes of 3 species were tested using PVS2 vitrification with 71% average regrowth. A protocol for cryopreservation of *Rubus* germplasm should include a CA period of 2–10 weeks and recovery on auxin-free medium. These studies confirm that all three cryopreservation protocols may be used for cryopreservation of a wide range of *Rubus* genetic resources.