PRESERVATION METHOD—DORMANT BUDS

NLGRP CLONAL DB MALUS SLOW-FREEZING DORMANT BUDS 1

12/28/2020

File Name
NLGRP_CLONAL_DB_MALUS_SLOW-FREEZING-DORMANT-BUDS_1.pdf

Authors
Gayle Volk (gayle.volk@usda.gov)*, Remi Bonnart (remi.bonnart@usda.gov), Katheryn Chen (katheryn.chen@usda.gov)

*Method PDF contact person

National Laboratory for Genetic Resources Preservation, 1111 S. Mason St., Fort Collins, CO 80521

Study Reason
Preservation

Introduction
Dormant bud cryopreservation is an efficient system of preserving important genetic material of some temperate woody crops. This is a standard method for cryopreserving *Malus* dormant buds at NLGRP. In this procedure, budwood is collected, processed into 35 mm sections, desiccated, slow cooled, and stored in the vapor phase of liquid nitrogen. These materials can be utilized for a variety of recovery protocols, including grafting, antimicrobial forced bud development, and more.

Source of Plant Material
Dormant budwood twigs are harvested from the previous season's growth of field-grown apple trees. In the USDA, budwood is collected from trees grown at PGRU in Geneva, NY in January, ideally after temperatures have been below 0 ºC for at least several consecutive days.

Plant Material Description
The harvested twigs (5-7 mm diameter) have about 10 nodal sections each and a total of 17-20 twigs (170-200 nodal sections) per accession. When source material is limited, smaller volumes of material may be processed. Budwood is packaged in bundles and sent by overnight courier to NLGRP. Budwood is kept at -5 ºC in sealed plastic bags for up to a month until it is used for cryopreservation.

Plant Material Cleanliness
Field materials are not tested for pathogens.

Plant Material Preparation/Pretreatment
Material is kept in -5 ºC storage, except when it is being processed. Dormant budwood is cut into 35 mm long sections with a single node centered on the segment. The dry base and the terminal bud on the branch are discarded. For mass-processing, budwood is cut using a bandsaw with the appropriate safety precautions.

Dehydration
The budwood moisture content must be reduced from field moisture contents to 25-30% MC (fresh weight basis, fwb) for each accession that will be cryopreserved. Ten randomly selected segments are used to establish moisture content (MC_i). These are individually

weighed immediately after segments are cut (FW_i), and again after they have dried three days in an oven at 60 to 100 ^{0}C (DW_i). The following equation is used to calculate the initial moisture content: $MC_i = ((FW_i - DW_i) / FW_i) \times 100\%$. The moisture content is then averaged for those 10 segments (MC_{avg}). Initial moisture is typically 45-55% (fwb).

The remaining material is weighed as a group immediately after segments are cut (FW_g). Once MC_{avg} is established, the following equation is applied to calculate the dry weight of the group: $DW_g = FW_g \times (1 - (MC_{avg} / 100\%))$.

The segments are spread out in a mesh tray or mesh bag and left to desiccate at -5 ^{0}C and low relative humidity (about 35%). The group is re-weighed (FW_x) every 1 to 7 days to determine the moisture content: $MC_x = ((FW_x - DW_g) / FW_x) \times 100\%$. Desiccation is complete when material reaches 25-30% MC (fwb).

Packaging
When the nodal sections are predicted to have a 25-30% moisture content, they are placed into polyolefin tubes (10 nodal sections per tube). Nodal sections are packaged into polyolefin tubes, typically ¾ inch or ½ inch diameter, depending on the diameter of the nodal sections. A total of 17 polyolefin tubes are prepared for each accession, material permitting. The tubes are heat-sealed, then labeled with the accession number, NSSL inventory number, taxon, cultivar name, barcode and date. They are then placed into metal cryo-boxes for slow-cooling.

Cryopreservation Method
The boxes containing packaged tubes are placed into a programmable cooler and are cooled from -5 ^{0}C to -30 ^{0}C at a rate of 1 ^{0}C per hour, then held at -30 ^{0}C for 24 hours. The cryo-boxes are then transferred to the vapor phase of liquid nitrogen (LNV), typically in an LNV transfer tank.

Storage Conditions
The LNV transfer tank with processed material is taken to the NLGRP secure vault where the long-term storage tanks are located. Dormant buds are stored above the liquid nitrogen, in the vapor phase. Each box is transferred to a specific location within the cryo-tanks, and that location is recorded in the GRIN-Global database.

Comments
N/A

References
Forsline PL, Towill L, Waddell J, Stushnoff C, Lamboy W, McFerson JR. 1998. Recovery and longevity of cryopreserved dormant apple buds. J. Amer. Soc. Hort. Sci. 123(3):365-370.

Jenderek MM, Forsline P, Postman J, Stover E, Ellis D. 2011. Effect of geographical location, year, and cultivar on survival of Malus sp. dormant buds stored in vapors of liquid nitrogen. HortScience 46:1230-1234.

Towill LE, Forsline PL, Walters C, Waddell J, Laufmann J. 2004. Cryopreservation of Malus germplasm using a winter vegetative bud method: Results from 1915 accessions. CryoLetters 25(5):323-334.

Volk GM, Waddell J, Bonnart R, Towill L, Ellis D, Luffman M. 2008. High viability of dormant Malus buds after 10 years of storage in liquid nitrogen vapour. CryoLetters 29(2)89-95.

Appendices
N/A