

## **Cryopreservation of Dormant Apple Buds**

**USDA-ARS National Laboratory for Genetic Resources Preservation (NLGRP), Fort Collins, CO**

**USDA-ARS Plant Genetic Resources Unit (PGRU), Geneva, NY**

### Harvest dormant budwood

Dormant budwood twigs are harvested from the previous season's growth of field-grown apple trees. In the USDA, budwood is derived from trees grown in Geneva, NY in January, ideally after temperatures have been below 0°C for a few days. 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 are packaged in bundles and sent by overnight courier to NLGRP. Budwood is kept at -5°C in sealed plastic bags for up to a few weeks until it is used for cryopreservation.

### Cut dormant budwood into sections

Dormant budwood is cut into 35 mm long sections with a single node centered on the segment. The terminal bud on the branch is discarded. For mass-processing, budwood is cut using a band saw with the appropriate safety precautions.

### Determine moisture content

The budwood moisture content must be reduced from field moisture contents (43 to 53%) to 25 to 30% moisture content (fresh weight basis, fwb) for each accession that will be cryopreserved. First, the initial moisture content of 10 nodal sections is calculated by measuring the fresh weight and dry weight (after 3 days in an oven at 100°C) and the following equation is used to calculate the initial moisture content:  $(FW-DW)/FW \times 100\%$ . The total fresh weight of the remaining nodal sections is measured and the desired ending weight is calculated based on a desired moisture content of 25-30% (fwb).

### Desiccate and package

The remaining original nodal sections are weighed and placed into plastic net-lined trays and desiccated in a chamber at -5°C and 35% relative humidity. The buds are re-weighed every 1 to 7 days to determine if the target moisture content of 25 to 30% has been achieved.

When the nodal sections are predicted to have a 25-30% moisture content, they are placed into polyolefin tubes (10 nodal sections per tube). The diameter of the selected polyolefin tubes is dependent upon the thickness of the nodal sections. A total of 17 polyolefin tubes are prepared for each accession, material permitting. The tubes are heat-sealed.

The tubes of dormant buds are labeled with the accession number, NSSL inventory number, taxon, cultivar name, bar code and date. They are then placed into cryoboxes for slow-cooling. The boxes are placed into a programmable cooler and are cooled from -5°C to -30°C at a rate of 1°C per hour, and are then held at -30°C for 24 hours. The cryoboxes are then transferred to the vapor phase of liquid nitrogen.

The transfer tank with the cryoboxes containing the tubes of nodal sections is taken to the NLGRP vault where the long-term storage tanks are located. Each cryobox is transferred to a specific location within the cryotanks that is recorded in the GRIN-Global database. One or two polyolefin tubes containing nodal sections with cryopreserved apple buds are retrieved from the cryotanks for viability assessments. These tubes are transferred to 2°C and warmed for 24 hours. Either 10 or 20 nodal sections will be warmed and grafted.

Graft buds onto rootstocks for viability assessment

Bare-root apple seedling rootstocks (one growing season old) are purchased from a nursery. They are potted into cone-tainers (a little less than a quart-size). The potted rootstocks are grown in the greenhouse for 3-4 weeks before budding. The rootstocks should be actively growing and leafed out nicely.

Cryopreserved nodal sections are shipped by overnight courier to Geneva, NY. They are placed into moist peat moss and allowed to rehydrate at 2-4°C for 14-21 days. Then they are patch-budded onto the seedling rootstocks, with two cryopreserved buds on each seedling rootstock. The grafting rubbers are removed and the tops of the rootstocks are cut above the grafted buds after 3 weeks. Viability data are collected after 8 to 10 weeks on each of the two buds per rootstock. Buds are scored as alive if they are pushing out, growing, green, and forming a shoot.

The viability data for each grafted accession are added to the GRIN-Global database. Accessions are considered successfully cryopreserved if there are at least 40% viable buds predicted to be in each polyolefin tube (4 out of 10 bud sections have viable buds) and if there are at least 60 buds predicted to be viable in LNV for the accession.