

Cryopreservation of Vegetative Dormant Winter Buds Apple and Sour Cherry

Preservation of woody fruit crop germplasm is traditionally done in field collections. Due to high costs associated with maintaining field collections, an accession is often represented only by one or two individual trees or shrubs. As a consequence, the risk of permanent loss of germplasm, due to diseases, insects or adverse environmental conditions, is very high.

Cryopreservation of vegetative winter buds is an alternative method to preserving plant species in the field. In this method, nodal sections of scionwood are stored in liquid nitrogen vapor. Cryopreservation of the vegetative dormant winter buds involves low maintenance costs and virtually eliminates the risk of germplasm loss. At the NCGRP, this method is routinely used to preserve *Malus* sp. (apple) and *Prunus cerasus* (sour cherry) germplasm.

The dormant buds cryopreserved at the NCGRP, were obtained through collaboration with the Plant Genetic Resource Unit, Geneva, NY.

Steps in Cryopreservation of Vegetative Winter Buds

1. Dormant scions containing last season's growth are shipped overnight to the NCGRP for processing. Scions should be harvested at their peak dormancy.



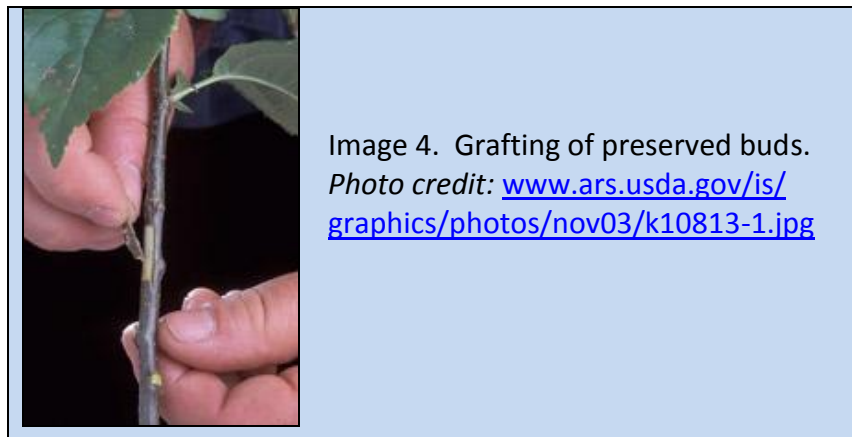
2. Scions, cut into 35 mm single bud sections, are desiccated (at -5°C) to 25-30% moisture content.



3. The desiccated one-bud sections are sealed in polyolefin tubes, slow cooled (1°C/hour) to -30°C, held at that temperature for 24 h and are immediately placed into the vapor phase of liquid nitrogen for long-term storage.



4. Before viability testing (by grafting), dormant buds are rehydrated for 12 days, at 2°C and dark conditions, in moist, sterile peat moss.
5. Viability is tested by grafting.



III. References

Chang Y, Reed BM. 2000. Extended alternating temperature cold acclimation and culture duration improve pear shoot cryopreservation. *Cryobiology* 40(4):311-322.

Forsline PL. 1996. Cryopreservation of apple and other crops in the USDA system. Second workshop on clonal genetic resources; Emerging issues and new directions. Ottawa, Ontario.

Forsline PL, McFerson J, Lamboy WF, Towill LE. 1999. Development of base and active collections of *Malus* germplasm with cryopreserved dormant buds. *Acta Hort.* 484:75–78.

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.

Katano M, Ishihara A, Sakai A. 1983. Survival of dormant apple shoot tips after immersion in liquid nitrogen. *HortSci.* 8(5):707-708.

Liu YG, Wang XY, Liu LX. 2004. Analysis of genetic variation in surviving apple shoots following cryopreservation by vitrification. *Pl. Sci.* 166(3):677-685.

Niino T, Koyaman A, Shirata K, Ohuchi S, Suguli M, Sakai A. 1993. Long term storage of mulberry winter buds by cryopreservation. *J. Seric. Sci. Jpn.* 62(5):431-434.

Oka S, Yakuwa H, Sato K, Niino T. 1991. Survival and shoot formation in vitro of pear winter buds cryopreserved in liquid nitrogen. *HortSci.* 26:65-66.

Sakai A. 1960. Survival of twigs of woody plants at -196C. *Nature* 185:393-394.

Sakai A, Nishiyama Y. 1978. Cryopreservation of winter vegetative buds of hardy fruit trees in liquid nitrogen. *HortSci.* 13(3):225-227.

Seufferheld MJ, Stushnoff C, Forsline PL, Gonzalez GHT. 1999. Cryopreservation of cold-tender apple, *Malus domestica* Borkh, germplasm. *J. Amer. Soc. Hort. Sci.* 124(6):612-618.

Stushnoff C, Seufferheld MJ. 1995. Cryopreservation of apple (*Malus* Species) genetic resources. In Y.P.S. Bajaj (ed) *Cryopreservation of Plant Germplasm I, Biotechnology in Agriculture and Forestry*, Vol. 32:87-101. Springer-Verlag, Berlin, Heidelberg.

Suzuki M, Niino T, Akihama T, Oka S. 1997. Shoot formation and plant regeneration of vegetative pear buds cryopreserved at -150 degrees C. *J. Jpn. Soc. Hort. Sci.* 66(1):29-34.

Towill LE, Forsline PL. 1999. Cryopreservation of sour cherry (*Prunus cerasus* L.) using a dormant vegetative bud method. *Cryoletters* 20(4):215-222.

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.

Towill LE, Bonnart RM. 2005. Cryopreservation of apple using non-desiccated sections from winter collected scions. *Cryoletters* 26:323-332.

Tyler N, Stushnoff C. 1988a. Dehydration of dormant apple buds at different stages of cold-acclimation to induce cryopreservability in different cultivars. *Can. J. Pl. Sci.* 68(4):1169-1176.

Tyler N, Stushnoff C. 1988b. The effects of prefreezing and controlled dehydration on cryopreservation of dormant vegetative apple buds. *Can. J. Pl. Sci.* 68(4):1163-1167.

Tyler N, Stushnoff C, Gusta LV. 1988. Freezing of water in dormant vegetative apple buds in relation to cryopreservation. *Pl. Physiol.* 87(1):201-205.

Wu YJ, Zhao Y, Engelmann F, Zhou M, Zhang M, Chen DS. 2001. Cryopreservation of apple dormant buds and shoot tips. *Cryoletters* 22(6):375-380.