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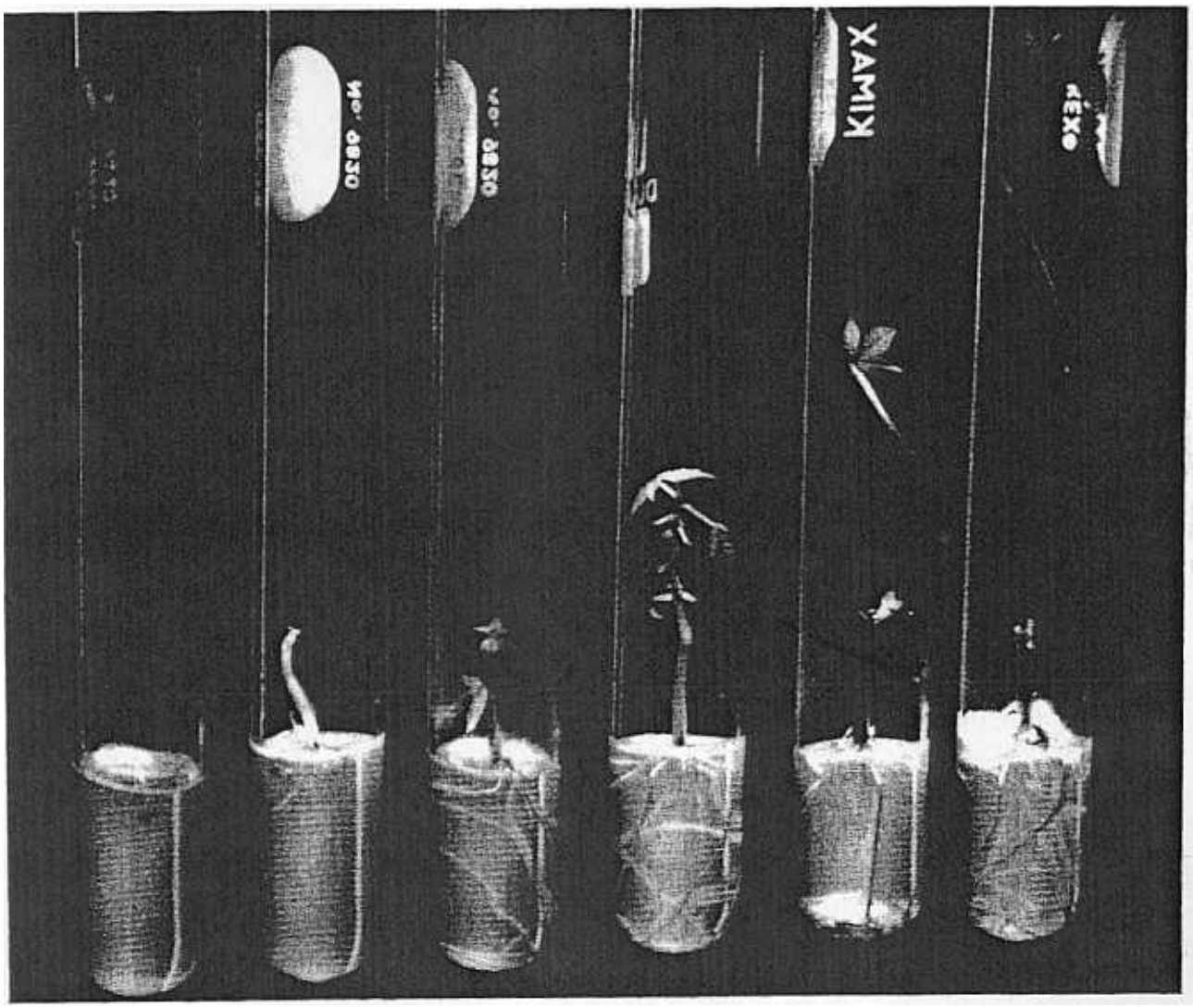

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Techniques for medium- and long-term storage of pear (*Pyrus* L.) genetic resources

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Summary

The security of genetic resource collections requires their conservation by several techniques. Duplicate collections of field-grown pear genetic resources are now conserved in medium- and long-term storage using cold rooms or liquid nitrogen dewars. Ten percent (169 accessions) of the primary pear field collection held at the National Clonal Germplasm Repository are kept as *in vitro* cultures in 4°C storage in polyethylene tissue culture bags. *In vitro* cultures were stored under refrigerated temperatures for from 8 months to 4.7 years, with mean storage of 2.75 years. Apices for more than 50 pear accessions are stored in liquid nitrogen. More than 100 additional genotypes have been screened for survival following cryopreserved storage. As an added security precaution, cryopreserved pear apices are stored in liquid nitrogen at the National Seed Storage Laboratory, Fort Collins, Colorado as a remote long-term base collection.

Introduction

Primary collections of pear genetic resources are commonly held in field genebanks (Hummer 1993; Hummer and Sugar 1998). These field trees allow confirmation of their morphological identity. In addition the performance of the genotypes can be evaluated for resistance to pests and diseases. The response of the trees to the environmental conditions can also be compared. Unfortunately field trees are vulnerable to environmental catastrophes such as high wind, rain, drought, freezing or above-average temperatures, pest and disease outbreaks. For secure conservation of these collections, alternative medium- and long-term storage methods such as refrigerated *in vitro* cultures or cryogenically preserved apices have been developed. These alternative techniques may cost less and have the advantage of the safety of remote laboratory storage (Epperson *et al.* 1997). The field genebank at NCGR, Corvallis consists of one tree for each of more than 1500 genotypes. This reduction from the two trees originally planted was instituted as a cost-saving measure.

In vitro culture of *Pyrus* species and cultivars is well studied and many genotypes can be micropropagated (Cheng 1979; Lane 1979). Several laboratories have studied reduced-growth storage of pears (Wanas *et al.* 1986; Wilkins *et al.* 1988; Moriguchi *et al.* 1990; Moriguchi 1995). Pears were first stored *in vitro* at NCGR, Corvallis in 1984 (Gunning and Lagerstedt 1985). Initially, the medium-term storage consisted of 20 x 100 mm tubes held at 4°C in darkness. Tubes were replaced with polyethylene tissue-culture bags in 1989 (Reed and Chang 1997).

Cryopreservation as a long-term storage method for *in vitro* cultures became feasible in 1990 following the development of two techniques applicable to pear apices (Dereuddre *et al.* 1990b; Reed 1990). Cryopreservation of *in vitro* pear apices using slow freezing was developed by Reed (1990). Cold acclimatization (CA) and slow cooling resulted in a high rate of regrowth (55% to 95%) for cryopreserved shoot tips of four *Pyrus* species including 75% regrowth for *P. koehnei*, a subtropical species. Pear plantlets were acclimatized to cold for 1 week (22°C 8 h light, –1°C 16 h dark) and then apices were cooled in PGD cryoprotectant (10% each polyethylene glycol [MW 8000], glucose and DMSO in liquid medium) at 0.1°C/min to –40°C before they were exposed to liquid nitrogen and thawed for 1 min in a 40°C water bath.

Dereuddre *et al.* (1990a, 1990b) developed the encapsulation-dehydration technique with the tips of axillary shoots of pear. Shoot tips from plantlets, cold-hardened for 2 months, were encased in alginate-gel beads, precultured in a liquid medium with 0.75M sucrose for 18 h, and dehydrated in an airflow cabinet for 2 to 6 h. The beads were cooled rapidly by direct immersion in liquid nitrogen and rewarmed slowly in air at room temperature. High rates of survival (80%) were obtained after dehydration for 3 h; 40% of the surviving shoot tips produced new plantlets. The best results (80% shoot recovery) were obtained using a 0.75M sucrose preculture and 4 h dehydration (20% residual water) (Scottez *et al.* 1992). Niino and Sakai (1992) obtained about 70% shoot formation for three pear cultivars with a modified encapsulation-dehydration method. Niino *et al.* (1992) successfully applied the vitrification method to pears, obtaining 40-72.5% shoot formation rates.

Improved techniques for medium- and long-term storage *in vitro* save time and labour costs and increase the security of germplasm collections. This study compared three methods for medium-term storage of 46 *Pyrus* genotypes. We also describe techniques for cryopreservation of *Pyrus* apices for long-term storage.

Materials and methods

In vitro *cold storage*

Three storage conditions were tested in a medium-term storage experiment initiated with 46 pear genotypes from the NCGR, Corvallis *in vitro* collection. Shoots were multiplied on Cheng's medium (Cheng 1979) with (per L): 1 mg N^6-benzyladenine, 3 g agar (Bitek, Difco, Detroit, MI) and 1.25 g Gelrite (Schwizerhall, South Plainfield, NJ) at pH 5.2. Cultures were grown in Magenta GA7 culture vessels (Magenta Corp., Chicago, IL) at 25°C under a 16-h photoperiod

with 25 $mE \cdot m^{-2} \cdot s^{-1}$ irradiance provided by cool white fluorescent bulbs.

Shoots (2-3 cm) from *in vitro* cultures were stored in the dark using three treatments: upright, base submerged, 4°C; defoliated, topped and 3/4 submerged, 4°C; upright, base submerged, 1°C. Five plantlets of each accession were stored per treatment, each in an individual section (15 x 150 mm) of a five-section bag (Gardner Enterprises, Willis, TX) with 10 ml of medium per section. Bags contained a firmer medium (3 g agar and 1.5 g Gelrite/L) than that used in boxes and tubes to compensate for the small amount of water lost through the semi-permeable bag walls (Reed 1991). Every four months the bags of plants were inventoried and rated as a whole on a scale of 1 to 5, with a rating of 5 = excellent condition (green shoots, leaves present), 4 = good (elongated shoots, shoot tips healthy), 3 = fair (some browning, some shoot tip necrosis), 2 = poor (much browning, most shoot tips necrotic), and 1 = very poor (viability questionable, brown, shoots necrotic) (Reed 1992). Approximately 3 months into the experiment the 4°C cold room malfunctioned and warmed to 32°C for several hours. Data were collected at 4-month intervals. Data were analyzed by ANOVA as a completely randomized design and means separated by LSD with MSTAT software (Michigan State University 1988).

Cryopreservation of apices

Over 100 *Pyrus* genotypes at NCGR, Corvallis were screened for regrowth following slow freezing and liquid nitrogen treatment using the slow-freezing cryopreservation method. Plantlets were given 1 week of cold acclimatization (CA) in an incubator with 22°C, 8-h days (3 mE $m^{-2} \cdot s^{-1}$) and –1°C 16-h nights before cryopreservation. Using the method developed by Reed (1990), 25 apices were pretreated for 2 days in the CA incubator on Cheng medium with 5% DMSO and 0.25 g/L additional Gelrite (total 3 g agar, 2.5 g Gelrite), then transferred to 0.25 ml liquid medium in 1.2-ml plastic cryotubes. One millilitre of the cryoprotectant PGD was added gradually over a 30-min period. Meristems were allowed to equilibrate at 4°C for 30 min after which the cryoprotectant was drawn down to 1 ml, 20 apices were frozen at a rate of 0.1°C/min to –40°C and then plunged into liquid nitrogen; 5 control apices were rinsed and plated on recovery medium. Frozen apices were thawed for 1 min in 45°C water, then 1 min in 22°C water, rinsed in liquid medium, and plated on to recovery medium.

Genotypes with greater than 40% shoot formation in the initial tests were cryopreserved and shipped to the National Seed Storage Laboratory (NSSL), Fort Collins, CO for long-term storage. Each genotype shipped required 150 apices (25 apices per vial): 25 apices to be thawed before shipping in Corvallis, 25 to be thawed at NSSL after storage, and the remaining 100 to be stored in liquid nitrogen at NSSL (Reed *et al.* 1998a, 1998b).

Results and discussion

In vitro *cold* storage

Pyrus shoots can be stored in good condition *in vitro* at 1°C or 4°C for 1-5 years depending on the genotype. *Pyrus* accessions of the general collection (82 genotypes sampled) were stored in tissue culture bags in the dark at 4°C. Storage duration ranged from 8 months to 4.7 years with a mean storage time of 2.7±1.1 years. A range of species and cultivars was tested in this study (Table 1). Twenty-one genotypes remained rated at 2 or above for 4-5 years, 18 for 2-4 years, and only seven for less than 2 years. Ratings were usually similar for a genotype over the three treatments tested but 'Citron d'Ete', 'Colonel Wilder, 'Fondante de Charneau' and several *Pyrus* species did significantly better ($P>0.05$) at 1°C than with either 4°C treatment. Over all 46 genotypes sampled, no significant differences were seen among the treatments for the first 1.3 years (Fig. 1). At 2 years the submerged treatment had significantly lower ratings than the 1°C treatment, and from 2.3 to 5 years, 1°C ratings were significantly higher than either 4°C treatment.

After 1 year most of the 46 genotypes remained in storage with ratings greater than 2 (data not shown). A rating of 2 indicates a declining condition and a need for repropagation. Little change occurred between 2 and 3 years for plantlets at 4°C, while those at 1°C declined slightly. At 4 years more accessions remained in storage at 1°C than at 4°C (data not shown). Many *P. communis* cultivars and a dozen other *Pyrus* species stored for 2-5 years in this study indicate the suitability of medium-term *in vitro* storage for *Pyrus* germplasm. Most earlier studies considered shorter storage times. Wanas *et al.* (1986) stored shoot tips of *P. communis* L. var. *caucasica* on basal medium at 4, 8 and 12°C with a 16-h photoperiod. All treatments depressed growth for 12-18 months, but survival was highest at 4°C. Mannitol or increased sucrose concentration in the medium did not produce acceptable survival rates at 4 or 28°C. Wilkins *et al.* (1988) stored *P. pashia* D. Don on multiplication medium at 4 or 10°C with a 16-h

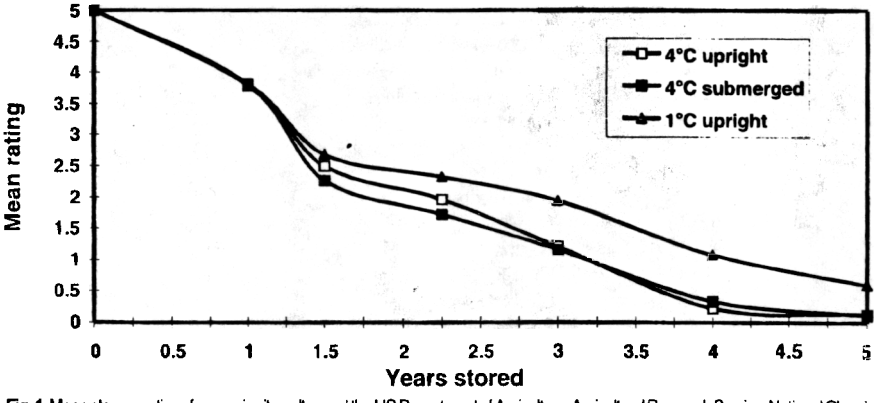

Fig. 1. Mean storage ratings for pear *in vitro* cultures at the US Department of Agriculture, Agricultural Research Service, National Clonal Germplasm Repository, Corvallis over a 5-year period. All cultures (one bag with five plants for each treatment) were stored in total darkness and either stored upright at 1°C, upright at 4°C, or 3/4 submerged in the medium at 4°C. Ratings: 5 = excellent condition (green shoots, leaves present), 4 = good (elongated shoots, shoot tips healthy), 3 = fair (some browning, some shoot tip necrosis), 2 = poor (much browning, most shoot tips necrotic, should be repropagated), 1 = very poor (viability questionable, brown, shoots necrotic), 0 = dead.

photoperiod with 100% survival after 12 months. Moriguchi *et al.* (1990) found that *P. communis* cvs. 'La France', 'Bartlett' and 'La France' × 'Bartlett' had high survival at 5°C with a 16-h photoperiod and at 1°C in darkness after 20 months, but survived poorly at 10 and 15°C in light.

Table 1. Effect of storage conditions[†] on the survival of pear *in vitro* cultures at National Clonal Germplasm Repository, Corvallis

Number	Accession Genotype[‡]	Years stored, by treatment A	B	C
Best storage is 4-5 years				
11.001	Admiral Gervais	5.0	5.0	5.0
228.001	Belle Lucrative	4.0	3.5	4.0
61.001	Beurre d'Amalis Panachee	3.8	4.0	4.0
115.001	Butirra Precoce Morettini	3.0	4.0	4.0
1078.001	Citron d'Ete	3.5	3.5	5.0
140.001	Clapp Favorite	5.0	5.0	5.0
142.001	Colonel Wilder	3.5	3.5	5.0
161.001	Count A.W. Moltke	3.5	3.5	5.0
181.001	Doyenne d'Alecon	3.5	3.5	5.0
230.001	Fondante de Charneau	2.3	2.3	4.5
380.001	Marchland	3.5	4.0	4.0
415.001	Nijisseiki[§] x OH (decline susceptible)	3.5	3.5	4.0
418.001	Notaire Lepin	3.0	3.0	4.0
1518.001	Poirier Fleurissant Tard	4.0	4.0	4.0
491.001	Rosee de Juillet	4.0	4.0	4.0
657.001	*P. betulifolia* Bunge	3.5	3.5	5.0
1276.001	*P. calleryana* Decne.	4.0	4.0	5.0
1589.001	*P. cordata* Desv.	3.5	3.0	5.0
828.001	*P. cossonii* Rheder	3.5	3.5	5.0
764.001	*P. dimorphophylla* Makino x *P.* sp.	2.5	2.5	5.0
250.001	Good Christian[¶]	4.0	4.0	4.0
Best storage is 2-4 years				
14.001	Alliance Franco-Russe	2.5	2.0	2.5
493.001	Bartlett - Rosired	2.0	2.0	2.0
549.001	Bartlett - Striped	2.5	2.5	2.5
1066.001	Bergamote Tardive de Ganze	2.3	2.0	2.3
81.001	Beurre Hardy	3.0	3.0	3.0
2146.001	G.28-119 (P-2462) (rootstock)	3.5	3.5	3.0
313.001	Kalle	3.0	3.0	2.5
1419.001	MM - 38 (rootstock)	2.5	2.5	2.5
397.001	Monchallard	3.8	2.0	2.3
1375.001	Oregon Pear Rootstock-001	2.5	2.5	2.5
434.001	Orient	2.5	2.5	2.5
596.001	Vineland 29018	2.5	2.5	2.5
650.001	*P. calleryana* x *P. fauriei* C. Schneider	3.5	2.5	3.0
631.001	*P. hondoensis* Kikuchi and Nakai	2.3	2.0	2.0
808.001	*P. koehnei*	3.0	3.0	3.5
528.001	*P. pyrifolia* cv. Shinseiki	2.0	2.0	1.3
795.001	*P. regelii* Rheder	2.0	2.0	2.3
1121.001	*P. ussuriensis* Maxim. cv. Mien Suan L	1.0	2.0	3.5
Best storage is less than 2 years				
478.001	Bartlett - Red Max	1.3	1.0	1.3
1725.001	Cascade	1.0	1.5	1.0
144.001	Columbia	1.3	1.3	1.3
2143.001	F.12-173 (P-2366) (rootstock)		1.3	1.3
248.001	Gliva Ukrainskaya	.3	1.3	
390.001	*P. elagrifolia* Pallas x *P. communis*	.3		
818.001	*P. koehnei* C. Schneider	.3		

[†] Cultures (one bag with five plants for each treatment) were stored in total darkness and either, upright at 4°C (A), 3/4 submerged in the medium at 4°C (B) or upright at 1°C (C). The 4°C cold-room malfunctioned approximately 3 months after the experiment began and heated the room to over 32°C for several hours (adapted from Reed and Chang 1997)

[‡] Cultivars are *P. communis* unless otherwise noted.

[§] *P. pyrifolia* (Burm. f.).

[¶] *P. pyrifolia* cv. Nakai x *P. communis*.

In this study three 'Bartlett' cultivars ('Red Max', 'Rosired' and 'Striped') stored for 1.3 to 2.5 years with more variability among cultivars than among storage conditions (Table 1). Moriguchi *et al.* (1990) found that the Japanese pears *P. pyrifolia* (Burm.) Nakai cvs. 'Shinsui', 'Nijisseiki', 'Shinchu', 'Kosui', 'Hosui' and 'Hakataao' did not survive in storage at 5, 10 or 15°C with a photoperiod, but had 100% survival when stored at 1°C in the dark for 12 months. Adding ABA to the storage medium did not improve their survival. We found that *P. pyrifolia* cv. 'Shinseiki' stored for 1.3-2 years, and the interspecific hybrid 'Good Christian' stored for 4 years in the dark under all three treatments (Table 1). The cold-stored *Pyrus* collection now consists of 169 accessions stored upright at 4°C with a 12-h photoperiod and the mean storage time is 2.75 years Plant condition is inventoried at 4-month intervals and cultures are repropagated when the bag rating reaches 2.

Cryopreservation of apices

Initial studies of 28 cryopreserved *Pyrus* genotypes found that 61% had good survival (>40% regrowth) following slow freezing (0.1°C/min) while only 43% of the genotypes responded well to the vitrification technique (Luo *et al.* 1995). Owing to recovery differences and the logistics involved in storing large numbers of apices and genotypes, the slow-freezing technique was used to process pear apices for storage. This method allowed more flexibility in handling apices and used less toxic cryoprotectants. Vitrification or encapsulation-dehydration methods also work well for many genotypes and might be more suitable in other laboratories.

Shoots in cryopreserved-control vials thawed at NCGR and NSSL were usually similar in regrowth percentages (Table 2). Differences in regrowth between test plants and shipping controls may be attributed to many factors: variation among vials, variation in handling, personnel, growth conditions of the plants prior to freezing, culture transfer interval, type of growth medium or gel firmness, container type, size, closure, etc. In some cases the test regrowth was high while regrowth of the shipping controls was low. This anomaly was traced to a malfunctioning CA incubator which did not properly cold-acclimatize the plantlets before shipping. Because only one or two apices are needed to repropagate a genotype, the variation noted will probably not affect the ultimate recovery of the plants after thawing. Some genotypes are stored as 10 vials with 10 apices per vial. These will be thawed at 5-year intervals to track survival of the accessions in liquid nitro-

Table 2. Regrowth of test and control plants of some of the *Pyrus* accessions cryopreserved at the National Clonal Germplasm Repository and sent for long-term storage in liquid nitrogen at the National Seed Storage Laboratory

Accession Number	Genotype	Regrowth (%)[†] A[‡]	B	C
Pyrus communis L. cultivars				
549.001	Bartlett -Striped	48	55	19
2150.001	Doyenne du Comice - Regal Red	75	10[§]	27[§]
298.001	Itala Pirovano	80	30	52
2491.001	Louise Bonne d'Avanches Panachee	60	50	88
397.001	Monchallard	65	50	25
420.001	Nouveau Poiteau	50	30	36
1518.001	Poirier Fleurissant Tard	100	79	92
Cultivars of other species				
1121.001	Mein Suan Li[¶]	76	95	65
1233.001	Nijisseiki[†] x Old Home	63	50	75
1433.001	OPR255[‡]	95	20[§]	16[§]
2034.001	Shi-Hua-Li OP seedling	73	95	95
Species representatives				
1310.001	*P. betulifolia* Bunge	50	25	80
1428.001	*P. calleryana* Decne.	70	71	54
1276.001	*P. calleryana*	78	50	28
989.001	*P. communis* var. *pyraster* L.	75	80	79
802.001	*P. hondoensis* Kikuchi & Nakai	57	80	42
803.001	*P. hondoensis*	75	71	88
842.001	*P. mamorensis* Trabut	59	60	83
876.001	*P. pashia* Buch.-Ham. ex D.Don	65	45	75
877.001	*P. pashia*	60	0	40

[†] 20 apices were frozen for the initial test (screen) 150 apices frozen for shipping (25/tube) including: 25 frozen-control.

[‡] All non-frozen controls were recovered at 100%. 100 apices were stored as a base collection at NSSL. A = 20 apices were frozen for the initial test (screen) at NCGR; B = 25 apices (frozen control) were regrown at NCGR before shipping; C = 25 apices (frozen control) were regrown at NSSL after shipping and a short storage period.

[§] Malfunction of CA incubator so the shipped accessions were not CA (Reed *et al.* 1998).

[¶] *P. ussuriensis* Maxim.

[†] *P. pyrsifolia* (Burm. f.) Nakai.

[‡] *P. calleryana* (Oregon Pear Rootstock selection).

gen storage. Genotypic differences are clearly apparent for both the cultivars and species representatives (Table 2). The *P. calleryana, P. hondoensis* and *P. pashia* accessions from different locales also differed in their recovery following cryopreservation. The *P. communis* cultivars tested ranged from 0 to 100% in in initial screening trials and 40 to 100% for shipping tests.

Conclusions

The genotype variability in any large primary collection makes maintenance very challenging. The large number of genotypes used in the studies reported here are representative of a broad range of world pear genetic diversity. Pear germplasm can now be reliably stored *in vitro* and in liquid nitrogen as duplicate active or base collections. These techniques are now in use at NCGR, Corvallis for *Pyrus, Ribes* and *Rubus*, and are available to genetic resource workers worldwide to develop duplicate collections for active field genebanks, or as base collections in liquid nitrogen for long-term storage.

References

Cheng, T.Y. 1979. Micropropagation of clonal fruit tree rootstocks. Compact Fruit Tree 12:127-137.

Epperson, J.E., D.H. Pachico and C.L. Guevera. 1997. A cost analysis of maintaining cassava plant genetic resources. Crop Sci. 37:1641-1649.

Dereuddre, J., C. Scottez, Y. Arnaud and M. Duron. 1990a. Effects of cold hardening on cryopreservation of axillary pear (*Pyrus communis* L. cv. Beurre Hardy) shoot tips of *in vitro* plantlets. C.R. Acad. Sci. Paris 310:265-272.

Dereuddre, J., C. Scottez, Y. Arnaud and M. Duron. 1990b. Resistance of alginate-coated axillary shoot tips of pear tree (*Pyrus communis* L. cv. Beurre Hardy) *in vitro* plantlets to dehydration and subsequent freezing in liquid nitrogen. C.R. Acad. Sci. Paris 310:317-323.

Gunning, J. and H.B. Lagerstedt. 1985. Long-term storage techniques for *in vitro* plant germplasm. Proc. Internat. Plant Propag. Soc. 35:199-205.

Hummer, K.E. 1993. Genetic resources of *Pyrus* and related genera at the Corvallis Repository. Acta Hort. 367:64-71.

Hummer, K. and D. Sugar. 1998. Pear genebank information on the Worldwide Web. Acta Hort. (in press).

Lane, W.D. 1979. Regeneration of pear plants from shoot apex-tips. Plant Sci. Lett. 16:337-342.

Luo, J., J. Denoma and B.M. Reed. 1995. Cryopreservation screening of *Pyrus* germplasm. Cryobiology 32:558.

Moriguchi, T. 1995. Cryopreservation and minimum growth storage of pear (*Pyrus* species), Pp. 114-128 *in* Biotechnology in Agriculture and Forestry, Vol. 32 (Y.P.S. Bajaj, ed.). Springer-Verlag, Berlin, Heidelberg.

Moriguchi, T., I. Kozaki, S. Yamaki and T. Sanada. 1990. Low temperature storage of pear shoots *in vitro*. Bull. Fruit Tree Res. Stn. 17:11-18.

Niino, T. and A. Sakai. 1992. Cryopreservation of alginate-coated *in vitro*-grown shoot tips of apple, pear and mulberry. Plant Sci. 87:199-206.

Niino, T., A. Sakai, H. Yakuwa and K. Nojiri. 1992. Cryopreservation of *in vitro*-grown shoot tips of apple and pear by vitrification. Plant Cell Tissue Organ Cult. 28:261-266.

Reed, B.M. 1990. Survival of *in vitro*-grown apical apices of *Pyrus* following cryopreservation. HortScience 25:111-113.

Reed, B.M. 1991. Application of gas-permeable bags for *in vitro* cold storage of strawberry germplasm. Plant Cell Repts. 10:431-434.

Reed, B.M. 1992. Cold storage of strawberries *in vitro*: A comparison of three storage systems. Fruit Var. J. 46:98-102.

Reed, B.M and Y. Chang. 1997. Medium and long-term storage of *in vitro* cultures of temperate fruit and nut crops. Pp. 76-105 *in* Conservation of Plant Genetic Resources *In Vitro*. Vol. 1 (M.K. Razdan and E.C. Cocking, eds.). M/S Science Publishers, Inc., Enfield, NH.

Reed, B.M., J. DeNoma, J. Luo, Y. Chang and L. Towill. 1998a. Cryopreserved storage of a world pear collection. *In Vitro* Cell. Dev. Biol. 33:51A.

Reed, B.M, J. DeNoma, J. Luo, Y. Chang and L. Towill. 1998b. Cryopreservation and long-term storage of pear germplasm. *In Vitro* Cell. Dev. Biol.-Plant 34(3):256-260.

Scottez, C., E. Chevreau, N. Godard, Y. Arnaud, M. Duron and J. Dereuddre. 1992. Cryopreservation of cold acclimated shoot tips of pear *in vitro* cultures after encapsulation-dehydration. Cryobiology 29:691-700.

Wanas, W.H., J.A. Callow and L.A. Withers. 1986. Growth limitations for the conservation of pear genotypes, Pp. 285-290 *in* Plant Tissue Culture and Its Agricultural Applications (L.A. Withers and P.G. Alderson, eds.). Butterworths, London, UK.

Wilkins, C.P., H.J. Newbury and J.H. Dodds. 1988. Tissue culture conservation of fruit trees. Plant Genet. Resour. Newsl. 73/74:9-20.

Résumé

Techniques de conservation à moyen terme et à long terme des ressources génétiques du poirier (Pyrus sp.)

Les ressources génétiques du poirier maintenues sous forme de collections en champs peuvent maintenant être conservées dans use chambre froid ou dans l'azote liquide. Dix pour cent (169 clones ou variétés répertoriée disponibles) de la collection de Poiriers du National Clonal Germplasm Respository sont gardé(e)s sous forme de culture in vitro à 4°C dans des sacs de polyéthyléne. Le stockage au froid des cultures *in vitro* s'échelonne de 8 mois à 4,7 années et le temp de stockage moyen est de 2,75 années. Les méristèmes de plus de 50 (clones ou variétés) de Poirier sont stockés dans l'azote liquid et même plus de 100 (clones ou variétés) ont été trié(e)s en vu de déterminer leur aptitude à la conservation au froid. Les méristèmes de Poirier cryoconservés sont stockés à long terme dans l'azote liquide au National Seed Storage Laboratory, Fort Collins, Colorado, en tant que collection de base.

Resumen

Técnicas para el almacenamiento a mediano y largo plazo de los recursos genéticos de la pera (Pyrus L.)

La seguridad de las colecciones de recursos genéticos requiere la conservación a través de varias técnicas. Los duplicados de las colecciones de los recursos genéticos de pera cultivada en el campo se conservan actualmente en almacenamiento a mediano y largo plazo utilizando cámaras frías o tanques de nitrógeno líquido. El diez por ciento (169 accesiones) de la colección base de la pera de campo se conserva en el National Clonal Germplasm Repository como cultivo *in vitro* en almacenamiento a 4°C como cultivo de tejidos y envasado en bolsas de polietileno. Los cultivos *in vitro* se han almacenado a temperaturas refrigeradas desde 8 meses hasta 4,7 años, con un promedio de 2,75 años. Los ápices de más de 50 accesiones de pera se almacenan en nitrógeno líquido. Se han seleccionado para supervivencia más de 100 genotipos adicionales, siguiendo un almacenamiento de crioconservación. Como medida de precaución adicional, los ápices de pera crioconservados se almacenan en nitrógeno líquido en el National Seed Storage Laboratory, Fort Collins, Colorado, como colección básica a largo plazo.