



## V.4 Conservation of Germplasm of Strawberry (*Fragaria* Species)

B.M. REED and K.E. HUMMER<sup>1</sup>

### 1 Introduction

The genus *Fragaria* L. is a member of the Rosaceae and subfamily Rosoidea. The base chromosome number is  $x = 7$ , and the genus includes 17 generally accepted diploid, tetraploid, hexaploid, and octoploid species (Table 1). This genus is native to temperate locations in the northern hemisphere and South America. There are no endangered species at this time. *Fragaria* can be found at elevations from sea level through 4000 m. *Fragaria vesca* L., the wood strawberry, is the most extensively distributed species of this genus. Hybridization between *Fragaria* and a related genus *Potentilla* is possible, although most hybrids are sterile.

The common cultivated strawberry, *F. x ananassa* Duch., is grown in most of the arable regions of the world (Hancock et al. 1990). This crop is economically significant, with world production close to 2 000 000 metric tons (Scott and Hancock 1989). Consumption of this fruit has increased by 10 to 15% during the last 10 years (Hancock and Luby 1993). One of the parents of the cultivated strawberry was *F. chiloensis*. The parental *F. chiloensis* stock was brought to France from Concepcion, Chile, by Lt. Col. Amédée François Frézier, sent by King Louis XIV to study the Spanish colony. Frézier was careful to secure vigorous plants with selected individuals bearing the largest fruit. By choosing these he inadvertently selected primarily pistillate flowering forms. Only five plants survived the 6-month trip and were planted in the King's gardens. The runners of these plants were distributed throughout Europe by avid botanists, horticulturists, and nurserymen. The other parent *F. virginiana*, which was brought to England in the late 1500s from the Virginia Colonies, probably by Sir Francis Drake (Wilhelm and Sagen 1974) and had already spread to the botanical gardens of Europe so it was available to supply pollen to the pistillate Chilean strawberry. *F. x ananassa* was first recognized and described by Antoine Duchesne in France in 1766. He suggested that large fruited clones of *F. chiloensis* L. from Chile, crossed with pollen from *F. virginiana*, known as the Carolina strawberry, produced the large fruited strawberry which was called the "Pineapple" strawberry or "Pine" for short (Wilhelm and Sagen 1974). Duchesne's recognition of *F. x ananassa* was the beginning of strawberry crop

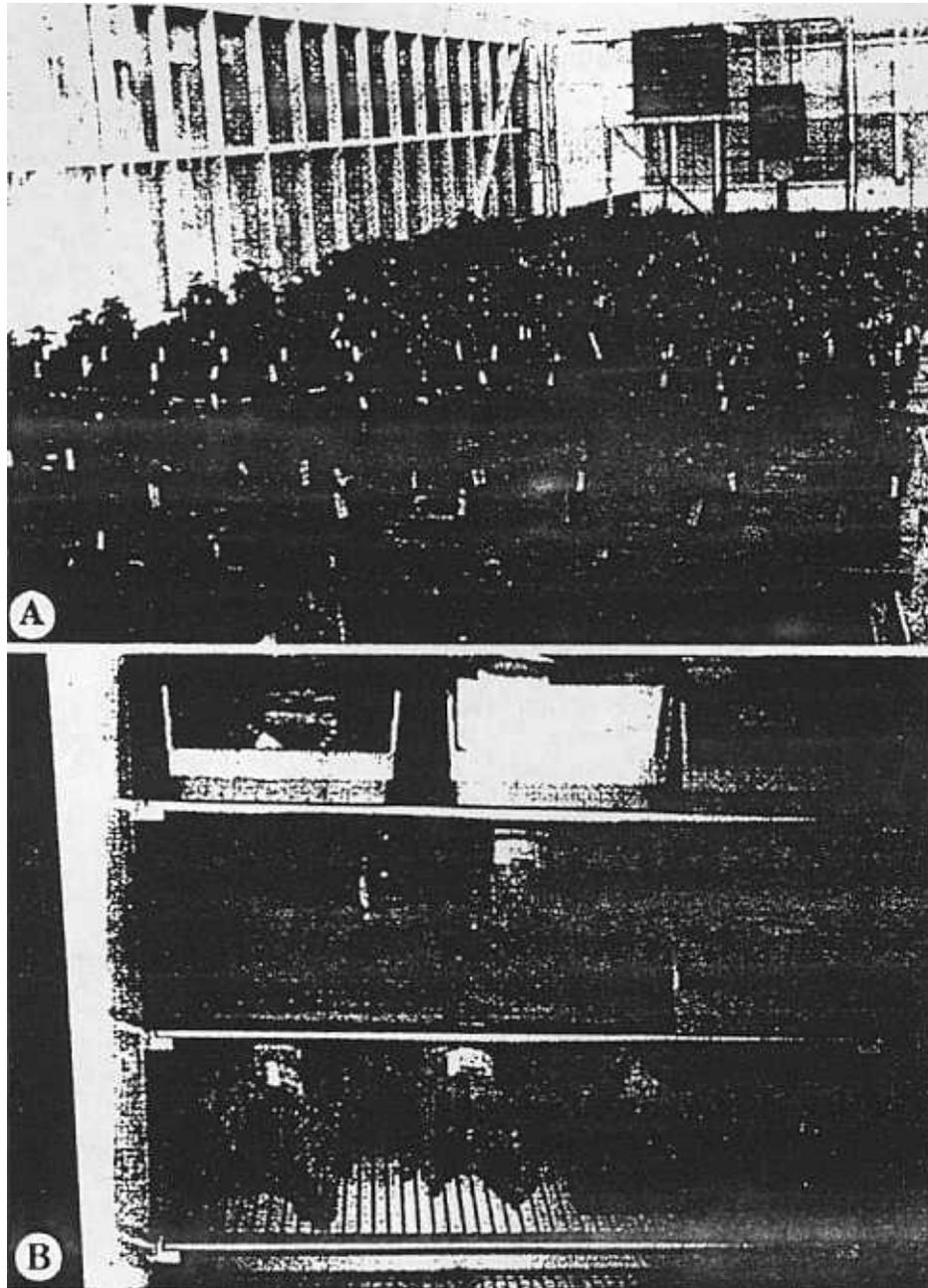
<sup>1</sup>National Clonal Germplasm Repository, USDA/ARS 33447 Peoria Road, Corvallis, OR 97333-2521 USA

**Table 1.** *Fragaria* species, ploidy, and distribution. (Hummer, unpubl.; Luby et al. 1991)

Name and ploidy level	Distribution
<b>Diploid</b>	
<i>F. daltoniana</i> Gay	Himalayas
<i>F. hayatai</i> Makino	Japan
<i>F. iinumae</i> Makino	Japan
<i>F. mandshurica</i> Staudt	China
<i>F. nilgerrensis</i> Schldl. ex Gay	S.E. Asia
<i>F. nipponica</i> Makino	Japan
<i>F. nubicula</i> Lindley ex Lacaite	Pakistan, Nepal
<i>F. vesca</i> L.	N. hemisphere
<i>F. v. subsp. americana</i> (Porter) Staudt	N. America
<i>F. v. subsp. bracteata</i> (A.A. Heller) Staudt	Western N. America
<i>F. v. subsp. californica</i> (Cham. & Schldl.) Staudt	California
<i>F. v. var. crinita</i> (Rydb.) C. Hitchc.	N. America
<i>F. v. subsp. vesca</i>	Europe, Asia
<i>F. viridis</i> Duchesne	Europe, Asia
<b>Tetraploid</b>	
<i>F. moupinensis</i> (Fr.) Card.	China
<i>F. orientalis</i> Los.-Losinsk.	W. Asia
<b>Hexaploid</b>	
<i>F. moschata</i> Duchesne	N. Europe
<b>Octoploid</b>	
<i>F. chiloensis</i> (L.) Duchesne	N. and S. America
<i>F. c. subsp. pacifica</i> Staudt	Pacific Coast, N. America
<i>F. iterupensis</i> Staudt	Juril Island
<i>F. virginiana</i> subsp. <i>virginiana</i> Duchesne	N. America
<i>F. v. subsp. glauca</i> (S. Watson) Staudt	N. America
<i>F. v. subsp. platypetala</i> (Rydb.) Staudt	N. America
<i>F. x ananassa</i> Duchesne	
<i>F. x. a. var. cuneifolia</i> (Nutt. ex Howell) Staudt	Western N. America
<i>F. x bringhurstii</i> Staudt	California
<i>F. x. hagenbachiana</i> Lange ex Koch	

improvement through breeding. Since that time strawberry breeding programs have developed in many countries throughout the world as the crop has developed to significant economic production.

The United States ranks first in world strawberry production (Hancock et al. 1990). The value of the total production of fresh market and processing strawberries for the USA in 1989 was \$520 million (Anonymous 1990). California produces 80% of the US crop. Japan, Poland, Italy, Russia, France, Mexico, and Spain are also significant world producers, while Israel, Chile, Brazil, Peru, and Argentina are looking to increase their strawberry production. Conservation of selected cultivated genotypes is significant for future strawberry improvement. Cultivated and wild *Fragaria* germplasm are preserved ex situ as seeds, and clonally as plants in fields, under screen, in greenhouses, as in vitro cultures, and as cryopreserved tissues (Fig. 1). The choice of preservation



**Fig. 1.** Storage of *Fragaria* collections at the National Clonal Germplasm Repository, Corvallis, OR. **A** Screenhouse collection. **B** In vitro cold storage. (Reed and Hummer, unpubl.)

technique depends on the purpose of the collection, available technology and expertise, and funding. In situ preservation, while providing future possibilities of conservation of *Fragaria* species in designated native locations, may not save the highly vulnerable genetic diversity on the fringe of the species ranges.

## 2 Germplasm Storage Methods

### 2.1 Seed and Pollen Storage

Seed of most *Fragaria* species are resistant to desiccation and cold and can survive for many years. Although Scott and Draper (1970) observed that the germination of several *Fragaria* seed lots stored at 1 to 4 °C for 23 years was 89% or greater, strawberry seed germination can be irregular. It may be improved by stratifying the seed for 3 months (Bringhurst and Voth 1957) or exposing the seed to light (Scott and Draper 1967). Seed storage is excellent for preserving collections of species populations and lines from specific crosses in breeding programs. However, because outcrossing is prevalent in some species, seed does not exactly reproduce the specific parental genotypes, clonal germplasm preservation techniques must be implemented to conserve important cultivars and selections.

*Fragaria* germplasm can also be stored as pollen when preservation of a specific genotype is not important. A high percentage of pollen stored in closed vials at –20 °C will remain viable for at least several years (Hancock et al. 1990). Strawberry pollen or anther fragments have been cryopreserved in liquid nitrogen for 2 years with high viability (65–75%) following air drying for 24 h (Craddock 1987).

### 2.2 Field Gene Banks

*Fragaria* germplasm throughout the world is commonly preserved in field gene banks as growing plants. Field plantings are of greatest use for evaluation of descriptive traits and qualities, morphological verification of taxonomic and horticultural identity, and as a backup for other germplasm storage techniques. This simple preservation method has some disadvantages, such as periodic replanting and naturally spread viruses or viruslike diseases. Virus diseases are present wherever cultivated strawberries are grown and cause major losses in the quantity and quality of the crop (Converse 1987).

Aphids, leafhoppers, and nematodes can transmit various virus and viruslike diseases in strawberries (Converse 1987). Thus, once a pathogen-negative clone has been produced, it could be maintained either with the regular application of a systemic insecticide or without the presence of insect vectors, or the clone will become reinfected. *Fragaria* germplasm collections can be stored as potted plants under insect-proof screens to reduce the risk of virus contamina-

tion (Hummer 1991). Aphids must not be allowed to survive near the pathogen-negative strawberries. This ideal can be accomplished on a practical level in a screenhouse setting with an active integrated pest management program to observe and react to insect vectors or a chemical control program. Periodic retesting for virus is performed on stock plants stored in screenhouses. Screenhouse storage must allow for natural seasonal acclimation of the plants and the acquisition of the chilling hours needed for normal bud break. Pots may need to be protected from extreme cold as well since roots can be killed at warmer temperatures than the upper portions of the plant. Containerized strawberries must be regularly and carefully monitored to insure that runners from one clone do not become established in the pot of another clone. Identity of plants in screenhouses can be checked by chemical analyses or morphological examination in field trials (Hummer 1991). Flowers and fruit are generally removed from primary collections of strawberries under screen, to improve runnering for propagation, and to reduce the possibility of contamination from seedling genotypes.

### 2.3 In Vitro Storage and Cryopreservation

Micropropagation is an excellent germplasm storage technique for strawberry clones. Plantlets of most genotypes can be readily cultured in vitro and stored under refrigerated conditions for several years. Plantlets are retrieved from cold storage, recultured, and acclimatized to greenhouse conditions. Studies examining field performance of micropropagated strawberry subclones (Swartz et al. 1981; Moore et al. 1991) have documented epigenetic differences, such as increased runnering in field-grown plants following in vitro culture. Moore et al. (1991) concluded that differences observed in Olympus were probably "transient responses to the micropropagation environment, not genetic changes" and reputed clonal differences in yield were not substantiated in field trials. Swartz et al. (1981) examined tissue cultured transplants of Earliglow, Redchief, and Guardian for off-types. They suggested that changes in performance were caused by increased vigor and that off-types could be traced to an original off-type meristem from which they were produced (Swartz et al. 1981). No changes in cultivar-specific properties were found with in vitro depot plants grown on phytohormone-free medium (Jungnickel 1988). Storage techniques for in vitro *Fragaria* at the USDA-ARS National Clonal Germplasm Repository in Corvallis (NCGR-Corvallis) use minimal levels of cytokinins and auxins for culturing and no phytohormones in storage media. Off-types have not been observed in plants following in vitro storage of *Fragaria* germplasm (Reed, unpubl.).

Plant materials can be cultured and preserved as callus. The higher frequency of mutation in callus cultures growing with strong plant growth regulator concentrations makes this technique useful for inducing somaclonal variation and developing new genotypes, but callus cultures are not appropriate for preservation of specific cultivars.

Meristems can be excised from runners or from in vitro plantlets and preserved in liquid nitrogen (Sakai et al. 1978; Kartha et al. 1980). Research on cryogenic preservation of *Fragaria* meristems is ongoing at several locations; at present this technique is not used for routine long-term conservation in any world gene bank.

#### 2.4 Status of *Fragaria* Germplasm

There are no currently endangered *Fragaria* species. Rather, locations representing distinct ecotypes or perhaps varieties are experiencing habitat changes due to human encroachment. As development of beach-front property occurs in California, many sites no longer support wild strawberries. In Chile, changes caused by forestry and farming have affected wild strawberry populations at some forest edge and coastal locations (Scott Cameron, pers. comm.).

The germplasm base for the cultivated strawberry is quite narrow. Cultivars-introduced from North American breeding programs since 1960 are derived from 53 founding clones (Sjulin and Dale 1987). A narrow germplasm base can predispose a crop to genetic vulnerability to diseases, pests, and environmental stresses. *Fragaria* is host to many pathogens and pests, including fungi, bacteria, viruses, nematodes, insects, and arthropods (Maas 1984). Some of these diseases are becoming major obstacles to maintaining or developing production levels of the cultivated strawberry. Development of resistant cultivars is a commonly used strategy to overcome production difficulties in regions where diseases, such as red stele, verticillium, or anthracnose have become established. The continued development of genetically resistant cultivars requires the availability of a broad spectrum of germplasm, both within and among species.

One of the strategies proposed by Sjulin and Dale (1987) to increase the genetic diversity in strawberry includes using germplasm from wild *Fragaria* species. Luby et al. (1991) list desired traits that could be derived from wild *Fragaria* germplasm collected during recent USDA-sponsored collection expeditions to obtain wild material from many sites throughout Chile and the Northern Rocky Mountains of the USA. *Fragaria* species have also recently been collected from China, Japan, and Pakistan. Collections in Canada, Minnesota, Michigan, Kentucky, North Carolina, Washington, Oregon, and California have been made by public and private breeders in the USA. (Luby et al. 1991).

In 1956, George Darrow traveled through Chile to collect native Chilean strawberries for the USDA and some clones that he collected showed promise for his breeding program. He made crosses and eliminated the majority of the plant material that he collected because of lack of funds to preserve the germplasm. Because breeders were again emphasizing the need for wild material, two more USDA plant-collecting expeditions were sent to Chile in 1990 and 1992. This *Fragaria* germplasm is housed at the NCGR-Corvallis. Although only a portion of the recently collected material may be initially incorporated into the present breeding efforts throughout the world, the remaining genotypes will be preserved for future use.

### 3 Conservation/Cryopreservation

#### 3.1 Review of Germplasm Conservation of Strawberry

Active strawberry research programs exist in Australia, Belgium, Canada, China, Czechoslovakia, Denmark, England, Egypt, France, Finland, Germany, Ireland, Israel, India, Italy, Japan, Lebanon, Poland, Russia, Scotland, The Netherlands, South Africa, Spain, Sweden, and the USA (Moore 1991; Maas and Galletta 1993). Most research collections are stored as plants in the field while some are preserved under screen or in vitro. Specific germplasm collections are located at gene banks in the USA, Canada, Denmark, India, and Germany (Bettencourt and Konopka 1989).

Research on in vitro storage of strawberry cultures was first reported by Mullin and Schlegel (1976) using liquid medium with filter paper bridges. Plantlets were maintained at 4 °C for up to 6 years with medium added every 3 months. Damiano (1979) reported the storage of 19 genotypes grown on solid medium and stored at 2 °C. These cultures survived for up to 27 months without transfer, depending on the genotype. Storage of mother plants used in virus-free strawberry production was successful on basal medium under weak illumination for 12–18 months (Boxus 1976). A strawberry germplasm collection at the Freidrich-Schiller Universitat has now been maintained for more than 17 years in vitro with yearly transfers on phytohormone-free medium (Jungnickel 1988). More recent studies by Reed (1991, 1992) use gas-permeable heat-sealable polyethylene bags for germplasm storage. Bettencourt and Konopka (1989) report that strawberries are stored in vitro at facilities in Belgium, Germany, Ireland, Sweden, Italy, and the United States. Many additional countries with strawberry research programs have developed in vitro collections since 1989 (Hummer, unpubl.).

Sakai et al. (1978) cryopreserved excised runner apices of Hokowase, using a cryoprotective solution of DMSO and sucrose with slow freezing to –20 or –30 °C before plunging in liquid nitrogen. Later, Kartha et al. (1980) developed a two-step method for Redcoat strawberry involving a 2-day preculture on 5% DMSO medium, addition of a cryoprotectant, and slow freezing at a controlled rate with rapid thawing. The importance of cryopreserved storage has been emphasized (Bajaj 1991); however, some authors (Withers 1986; Hancock et al. 1990; Sullivan 1991) consider cryogenic preservation as experimental because few cryopreserved plants have been analyzed for trueness to type.

#### 3.2 Cold Storage of In Vitro *Fragaria* at the USDA National Clonal Germplasm Repository (NCGR-), Corvallis, OR

At NCGR-Corvallis, some *Fragaria* germplasm has been stored at refrigerated temperatures since 1983. Plantlets were stored in glass tubes and plastic boxes until 1989 (Gunning and Lagerstedt 1985) when gas-permeable tissue-culture bags became available. These bags provided an opportunity for contamination-free medium-term storage (Reed 1991, 1992).

### 3.2.1 Methodology

Plantlets were multiplied on NCGR-FRA, a modified MS (Murashige and Skoog 1962) medium pH 5.7 (Reed 1991). Cold storage was at 4 °C in the dark in a walk-in cold room. Duration of storage differed but the same storage and growth room conditions were used.

*Tubes.* Single plantlets were transferred to 16 × 100 mm glass tubes with 5 ml agar-solidified basal medium without growth regulators and capped with plastic caps, grown for 2 weeks, sealed with Parafilm and stored. Each accession was stored as two cultures in individual tubes.

*Boxes.* Sixteen plantlets of each accession were transferred to Magenta GA7 plastic boxes (6 × 6 × 9 cm) with 40 ml basal agar-based medium without growth regulators and grown for 2 weeks. Boxes were sealed with Parafilm to decrease dehydration and contamination and stored.

*Bags.* Plantlets were transferred to bags (CultuSAK, Becton Dickinson, Lincoln Park, NJ) which were then heat-sealed with an impulse sealer. Cultures were grown for 1 week in the growth room and 1 week under cold-hardening conditions of 8 h, 22 °C day and 16 h, -1 °C night before storage (Angelo 1939; Reed 1991). For each accession, ten plantlets were stored, each in an individual section (15 × 150 mm) of a five-section bag with 10 ml per section of basal medium without growth regulators. A firmer medium (3 g agar and 1.5 g Gelrite per liter) compared to that in boxes and tubes was used in the bags to compensate for the low level of water loss through the bag walls (Reed 1991).

Cultures were ranked for condition at 3-month intervals on a scale of 0–5: 0, dead, brown; 1, etiolated, pale tan, no green color; 2, etiolated, pale green color; 3, etiolated, retaining medium green color; 4, not etiolated, medium green color; 5, not etiolated, dark green color. Cultures with a rating of 2 or 1 are considered to be at the end of their storage life and are removed for repropagation.

### 3.2.2 Results

Longevity and contamination rates were compared for *Fragaria* in vitro cultures cold stored using three different storage methods. These conditions were chosen to reflect storage systems in use for plant germplasm throughout the world.

Storage in polyethylene bags with cold hardening and Gelrite as part of the medium was examined at three month intervals over a 2-year period. At each three month inventory, bags with all plants rated 2 or lower on a scale of 1–5 were removed for repropagation. Of the original 96 accessions stored in bags, 99% remained in storage after 6 months and 47% at 15 months (Fig. 2). These levels are 12 to 15% higher than those of plants stored in boxes for the same time periods. At each 3-month inventory a larger percentage of accessions stored in bags remained in good condition than did those in boxes. Five percent of the individual accessions stored in bags maintained high condition ratings for 24

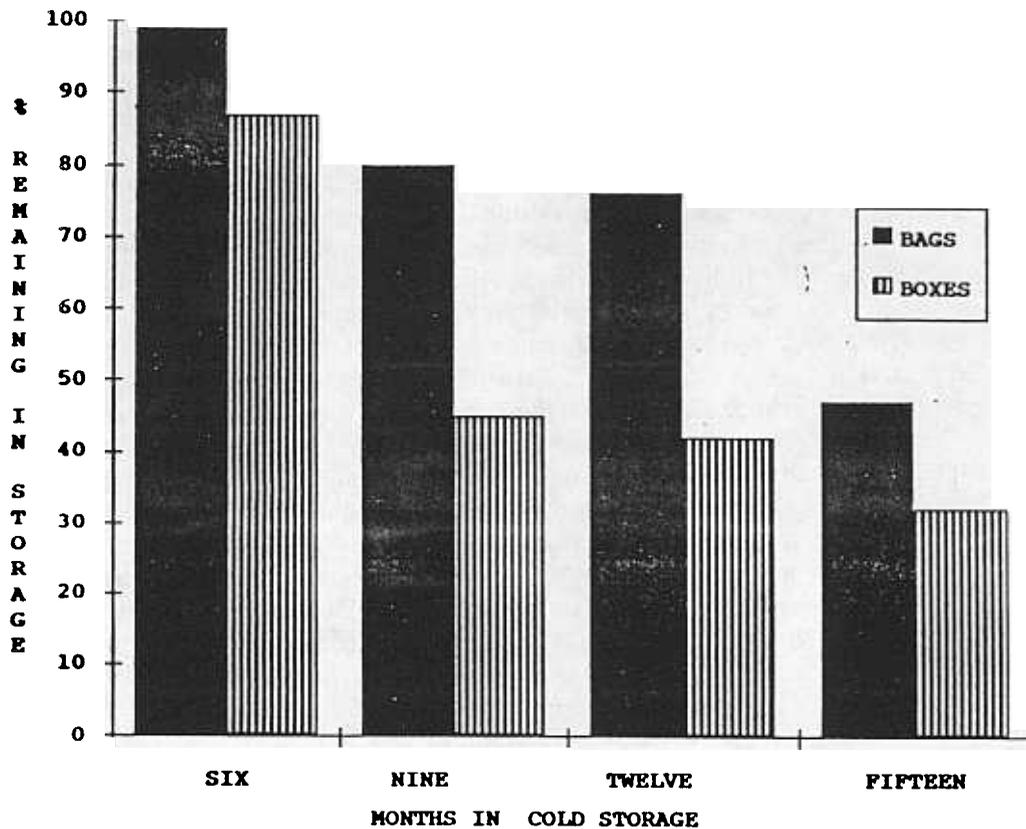


Fig. 2. Percentage of uncontaminated in bags and boxes remaining in storage during a 15-month period. (Reed 1992)

months or more. No accessions were lost due to contamination. Contamination of a single chamber in a five-chamber bag occurred in 10 to 15% of the bags due to cracks or improper sealing, but did not spread to other chambers. Newer versions presently on the market do not crack in storage (Reed, unpubl.).

Cultures in boxes were stored as long as 15 months. Of the original 127 accessions stored 87% had high condition ratings at 6 months but only 32% at 15 months (Fig. 2). Contamination of cultures in boxes (30%) was primarily fungal and occurred at twice the level of that in bags, usually resulting in the loss of the accession. Contamination was not always evident until repropagation was attempted. A greater number of boxes for each accession would decrease the number lost but would greatly increase the storage space, labor, and material costs required for the collection. Contamination was a major factor in the decline of cultures stored in boxes. Losses of large numbers of plant tissue cultures due to contamination have also been reported in storage jars (Marino et al. 1985) and tubes (Nord and Hanniford 1989).

From a collection of 130 accessions stored in tubes for 24 months, 40% were in fair or good condition (rating of 2 or 3), 31% were in very poor condition

(rating of 1), and 29% were dead. Contamination (47% of the tubes stored) was not always evident until the plants were repropagated, and reduced the survival rate to 20%. This study found that standard storage in tubes was not reliable due to high contamination rates throughout the storage period.

In general, uncontaminated *Fragaria* accessions stored well for 12 to 15 months in any of these systems (Table 2). Genotype survival varied and the length of time an individual accession remained viable in storage ranged from 6 to 24 months. Individual cultivars, Aliso, Francesco, and Pocahontas, had good or very good ratings using the bag system (rating 3 or 4) after 12 months and fair ratings (rating 2) at 15 months with 100% survival. Damiano (1979) reported 90% survival for Pocahontas at 12 months, 80% for Aliso at 16 months, and 70% for Francesco at 17 months, for cultures stored in jars or large tubes. The advantages of the bag system over that of Mullin and Schlegel's liquid medium system are that the bags minimize contamination, and do not require the time and effort needed to replenish liquid medium quarterly.

The percentage of accessions remaining in storage and contamination rates varied greatly among the three storage systems. Judging by the large percentage of accessions with very low contamination rates and high average health ratings remaining in storage after 1 year, as well as the reduced need for storage space, we

Table 2. Ratings for individual *Fragaria* accessions stored in both plastic boxes (Magenta GA7) and in gas-permeable polyethylene bags (CultuSak) at 4 °C in the dark. The rating scale is 0 = dead, 5 = excellent. Mean of ten plants per treatment. (Reed 1992)

Accession	Rating	
	Form stored	
	Box	Bag
12 months' storage		
Fou Chu	4	3
<i>F. chiloensis</i> Darrow 72	3	3
<i>F. virginiana</i>	4	4
<i>F. virginiana</i> subsp. <i>glauca</i>	4	4
Klondike	3	
Komsomalka		
Kurume 103	3	
MDUS 3022	3	
Podnyaya Zagorya	4	
Robinson		
Tufts	4	4
Vantage	4	4
15 months' storage		
Fou Chu	3	2
Komsomalka	3	3
MDUS 3022	2	3
Surecrop -	2	2

concluded that bags provided more secure and healthy storage for strawberry germplasm than either tubes or boxes. Bags have the additional advantages of easy handling, reduced storage space, resistance to breakage, ease of inventory, and safer shipping of germplasm.

The present storage system at NCGR-Corvallis employs the plastic bags described here, cold hardening for 1 week and evaluation at 3-month intervals to identify declining cultures. Over 350 *Fragaria* accessions are stored in bags. Additional studies of photoperiod and cytokinin effects are in progress.

### 3.2.3 Discussion

Researchers, breeders and certification programs have an increased interest in medium-term germplasm storage for many crop plants. The number of accessions presently in cold storage is increasing as additional specimens are collected from around the world, and cultivated genotypes are added to the germplasm core. In vitro collections will play an important part in the distribution of pathogen-negative plants throughout the world. The desire for correctly identified, pathogen-negative germplasm is increasing. Many certification programs have adopted in vitro culture as standard technique in the production of pathogen-negative plants from stock collections, and virus and viruslike pathogens do not spread between in vitro cultures. Improvements in storage containers, conditions, and media are needed to make in vitro culture less labor-intensive and more secure as a storage system for important germplasm.

## 3.3 Cryopreservation of *Fragaria* Germplasm at the USDA National Clonal Germplasm Repository, Corvallis, OR

The cryopreservation protocols used at the Repository are modifications of the methods of Sakai et al. (1978), Kartha et al. (1980) and Reed (1990). Although cryogenic storage of clonal germplasm is still experimental, the US National Plant Germplasm System is planning a cryogenic base collection at a site remote from the active collection.

### 3.3.1. Cryopreservation Methodology

Tissue-cultured plants were multiplied on modified MS medium. In vitro plants were cold-hardened for 1 week prior to excision of meristems. Dissected meristems (0.8 mm) were grown for 48 h on MS medium with 0.8% agar and 5% DMSO under cold-hardening conditions (Reed 1988). After 48 h, meristems were transferred to 0.25 ml of liquid MS medium in 1.2-ml plastic cryotubes on ice and the cryoprotectant PGD [a mixture of 10% each of polyethylene glycol (MW 8000), glucose, and DMSO in medium (Finkle and Ulrich 1979)] was added dropwise to 1.2 ml over 30 min. A 30-min equilibration period at  $-1^{\circ}\text{C}$  was followed by removal of excess cryoprotectant.

Samples were frozen in 1 ml of cryoprotectant at 0.8 °C/min to -40 °C in a programmable controlled-temperature cooling chamber and then immersed in LN for 1 hour. Vials were thawed in 40 °C water for 1 min then transferred to 23 °C water. The cryoprotectant was drained from the vials and replaced with liquid medium. Meristems were drained on sterile filter paper and plated on growth medium.

### 3.3.2 Results

Fifty-six *Fragaria* accessions were screened using cold hardening for 1 week, slow freezing, and PGD as the cryoprotectant (Table 3). The wide range of germplasm

**Table 3.** Cryopreservation screening of 56 *Fragaria* accessions using cold hardening for 1 week, slow freezing, and PGD as the cryoprotectant. Freezing rate was 0.8 °C/min to -40 °C followed by storage in liquid nitrogen for 1 h. (Reed, unpubl.)

Accession	Percent regrowth	General survival rating*
Albritton	30	+
Allstar	78	+++
Atlas	14	+
Beaver	60	++
Berkeley	30	+
Blakemore	0	-
Bounty	88	+++
Capron (117) <i>F. moschata</i>	0	
Capron (210) <i>F. moschata</i>	22	
Cardinal	30	
Catskill	70	++
Cavalier	100	+++
Comet	0	
Del Norte	0	
Donner	45	++
Dunlap	90	+++
Earliglow	30	+
Empire	17	
<i>F. moschata</i>	20	
<i>F. chiloensis</i> Darrow 5	38	+
<i>F. chiloensis</i> Yaquina B	88	+++
<i>F. chiloensis</i> RCP-19	38	+
<i>F. chiloensis</i> D6	33	+
Fletcher	25	+
Florida Belle	60	++
Florida Ninety	22	+
Fresno	80	+++
Garnet	0	-
Gorella	13	+
Holiday	40	+

(Contd.)

Table 3. (Contd.)

Accession	Percent regrowth	General survival rating*
Jerseybelle	30	+
Kaiser's Samling	82	+++
Kurume	70	++
Kurume 103	80	+++
Marlate	0	
Massey	91	+++
Orland	40	+
Pai Yu	0	-
Pocahontas	67	++
Podnyaya Zagorya	92	+++
Primella	25	+
Profumata di Tortona	10	+
Red Gauntlet	33	+
Redchief	70	++
Redcoat	70	++
Redstar	20	+
Robinson	80	+++
Scott	56	++
Sierra	30	+
Tennessee Beauty	11	+
Titan	60	++
Tribute	0	
Tufts (70)	20	+
Tufts (231)	20	+
Veestar	60	++
White Pine	56	++

must  
be in

\*No survival.  
+ = 1-40%.  
++ = 41-70%.  
+++ = 71-100%.

tested showed an equally wide response to cryopreservation. A frequency distribution of the genotypes by percent survival shows that 25 genotypes had greater than 50% survival from slow freezing (Fig. 3). Survival rates may also be dependent on genotypic variation and can be improved by varying the freezing speed (Table 4). To be useful for germplasm storage, any new method must produce high survival rates in diverse genotypes. Newer techniques may also prove effective for cryopreservation of strawberry germplasm (Table 5). Initial tests of vitrification methods (Yamada et al. 1991) with several *Fragaria* accessions produced varied results. Additional modifications to the currently published techniques may increase the survival of vitrified tissues. The addition of a gradual dehydration with PGD to the vitrification technique of Yamada et al. (1991) improved the survival of two *Fragaria* accessions. The alginate bead encapsulation-dehydration technique (Dereuddre et al. 1990) has also shown some survival in preliminary trials (Table 5).

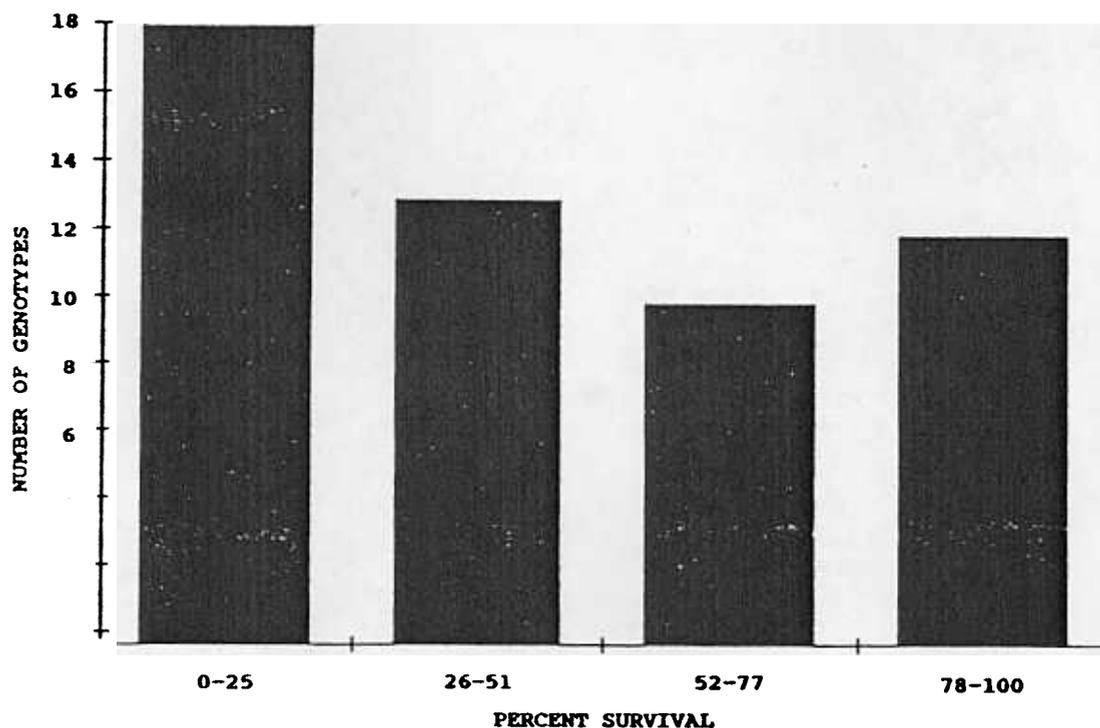


Fig. 3. Frequency distribution of survival rates of 53 strawberry genotypes following cryopreservation using cold hardening, PGD as the cryoprotectant, and slow freezing (Reed, unpubl.)

Table 4. Effect of freezing rate on the percent survival of diverse genotypes of cryopreserved *Fragaria*. (Reed, unpubl.)

Genotype	Percent survival		
	Freezing rate		
	0.3°C/min	0.5°C/min	0.8°C/min
<i>F. chiloensis</i> Darrow 72	25	10	0
<i>F. chiloensis</i> CA 1466	4	7	2
<i>F. virginiana</i> UC 10	64	58	45
LaPush Beach	60	26	
Vermilion	50	5	

Not tested

### 3. 3 Discussion

Little published information is available about actual long-term storage of cryopreserved *Fragaria* germplasm (Kantha 1985). Several *Fragaria* genotypes are stored for experimental purposes at NCGR-Corvallis, but a cryogenic base collection has not yet been established. Once research procedures become

**Table 5.** Survival of *Fragaria* accessions from cryopreservation using four different methods. (Reed, unpubl.)

Genotype	Percent survival			
	PGD → PVS2 Reed (unpubl.)	PVS2 Yamada et al. (1990)	Slow Freeze Reed (1990)	Alginate Dereuddre et al. (1990)
<i>F. virginiana</i> UC 11	60	9	35	
Guardian	–	17	25	
La Sans Rivale	23	0	0	
Profusion		0	0	

–, Not tested

routine, this base will reside as a remote back-up collection in cryogenic storage at the National Seed Storage Laboratory in Fort Collins, Colorado.

Two problems remain before routine cryogenic storage can be implemented and accepted as a standard technique for strawberries. First, the differential survival response of the widely variable *Fragaria* species to cryogenic conditions must be addressed. Continued methods development is needed to determine techniques applicable to both tender and cold-hardy genotypes. Second, a protocol for identity confirmation should be established because an insufficient amount of regenerants obtained from cryogenically treated buds or shoot tips have been analyzed (Hancock et al. 1990; Sullivan 1991). Field plantings of 20 plants each of 15 strawberry cultivars which had undergone cryopreservation and micropropagation procedures at the NCGR-Corvallis displayed no off-types (Reed, unpubl.), and plants of other species regenerated following cryopreservation have been true to type (Bajaj 1985; Towill 1988). The authors have not observed exposure to cryogenic conditions to cause an increase in mutation rate over that normally experienced in a field collection.

#### 4 Summary and Conclusions

Field gene banks are important for the evaluation of horticultural traits; screenhouses provide working and certified collections available for immediate use and direct distribution; in vitro culture in refrigerated conditions provides medium-term (several years') storage for back-up collections and increasingly provides material for plant distribution. Long-term (more than 10 years) storage of *Fragaria* species can be accomplished through seed storage; however, cryopreservation of meristems will be the key for efficient long-term preservation of clonal base collections. Protocols for clonal preservation of *Fragaria* germplasm as plants in field, greenhouse, and in vitro culture are routine in gene banks throughout the world with identity verification as a critical component in each of

these systems, though only a few locations are presently researching cryogenic techniques for the preservation of *Fragaria* germplasm.

## References

- Angelo E (1939) The development of cold resistance in strawberry varieties. Minn Agric Exp Sta Tech Bul 135: 7-19
- Anonymous (1990) Agricultural Statistics. US Dep Agric, Gov Printing Office, Washington, DC
- Bajaj YPS (1985) Cryopreservation of germplasm of potato (*Solanum tuberosum* L.) and cassava (*Manihot esculenta* Crantz): Viability of excised meristems cryopreserved up to 4 years. Indian J Exp Biol 23: 285-287
- Bajaj YPS (1991) Storage and cryopreservation of in vitro cultures. In: Bajaj YPS (ed) Biotechnology in agriculture and forestry, vol 17. High-tech and micropropagation I. Springer, Berlin Heidelberg New York, pp 361-381
- Bettencourt EJ, Konopka J (eds) (1989) Directory of germplasm collections 6.II Temperate fruits and tree nuts. Int Board Plant Genet Resour, Rome
- Boxus P (1976) The production of virus-free strawberry by in vitro culture. Acta Hort 66: 35-38
- Bringhurst R, Voth V (1957) Effect of stratification on strawberry seed germination. Proc Am Soc Hort Sci 92: 369-375
- Converse R (1987) Detection and elimination of virus and viruslike diseases in strawberry. In: Converse R (ed) Virus diseases of small fruits. USDA Agric Handbook No 631: 1-100
- Craddock WJH (1987) Cryopreservation of pollen. MS Thesis, Oregon State University, 77 pp
- Damiano C (1979) Cold storage of in vitro strawberry cultures and the resumption of multiplication. Ann Ist Sper Fruttic 10: 53-58
- Dereudde J, Scottez C, Arnaud Y, Duron M (1990) Resistance d'apex de vitroplants de poirier (*Pyrus communis* L. cv. Beurré Hardy) enrobés dans l'alginate, à une deshydratation puis à une congélation dans l'azote liquide: effet d'un durcissement préalable au froid. CR Acad Sci Paris 310: 317-323
- Finkle BJ, Ulrich JM (1979) Effects of cryoprotectants in combination on the survival of frozen sugarcane cells. Plant Physiol 63: 598-604
- Gunning J, Lagerstedt HB (1985) Long-term storage techniques for in vitro plant germplasm. Proc Int Plant Prop Soc 35: 199-205
- Hancock JF, Maas JL, Shanks CH, Breen PJ, Luby JJ (1990) Strawberries (*Fragaria*) In: Moore JN, Ballington JR Jr (eds) Genetic resources of temperate fruit and nut crops II. Acta Hort 290: 489-546
- Hancock JF, Luby JJ (1993) Genetic resources at our doorstep: the wild strawberries. Science 260(5133): 141-147
- Hummer K (1991) *Fragaria* at the National Clonal Germplasm Repository at Corvallis, Oregon. In: Dale A, Luby JJ (eds) The strawberry into the 21st century. Timber Press, Portland, OR, pp 106-107
- Jungnickel F (1988) Strawberries (*Fragaria* spp. and hybrids). In: Bajaj YPS (ed) Biotechnology in agriculture and forestry, vol 6. Crops II. Springer, Berlin Heidelberg New York, pp 38-103
- Kartha KK (1985) Cryopreservation of plant cells and organs. News Int Assoc Plant Tissue Cult 45: 2-15
- Kartha KK, Leung NL, Pahl K (1980) Cryopreservation of strawberry meristems and mass propagation of plantlets. J Am Soc Hort Sci 105(4): 481-484
- Luby J, Hancock J, Cameron JS (1991) Expansion of the strawberry germplasm base in North America. In: Dale A, Luby JJ (eds) The strawberry into the 21st century. Timber Press, Portland, Oregon, pp 66-75
- Maas J (1984) Compendium of strawberry diseases. Am Phytopathol Soc, St. Paul, MN
- Mass J, Galletta G (1993) eds 2nd Int Strawberry Symp Acta Hort 348: 1-520
- Marino G, Rosati P, Sagrati F (1985) Storage of in vitro cultures of *Prunus* rootstocks. Plant Cell Tissue Organ Cult 5: 73-78

- Moore J (1991) The strawberry into the 21st century: a symposium overview. In: Dale A, Luby JJ (eds) *The strawberry into the 21st century*. Timber Press, Portland, Oregon
- Moore P, Robbins J, Sjulín T (1991) Field performance of olympus strawberry subclones. *Hortsci* 26(2): 192–194
- Mullin RH, Schlegel DE (1976) Cold storage maintenance of strawberry meristem plantlets. *HortScience* 11(2): 100–101
- Murashige T, Skoog F (1962) A revised medium for rapid growth and bioassays with tobacco tissue cultures. *Physiol Plant* 15: 473–497
- Nord SM, Hanniford GG (1989) In vitro storage of *Pelargonium x domesticum*. *Am Soc Hortic Sci Annu Meet Program and Abstr* 158 (Abstr): 78
- Reed BM (1988) Cold acclimation as a method to improve survival of cryopreserved *Rubus* meristems. *Cryo Lett* 9: 166–171
- Reed BM (1990) Survival of in vitro-grown apical meristems of *Pyrus* following cryopreservation. *HortScience* 25: 111–113
- Reed BM (1991) Application of gas-permeable bags for in vitro cold storage. *Plant Cell Rep* 10: 431–434
- Reed BM (1992) Cold storage of strawberries in vitro: a comparison of three storage systems. *Fruit Var J* 46(2): 98–102
- Sakai A, Yamakawa M, Sakata D, Harada T, Yakuwa T (1978) Development of a whole plant from an excised strawberry runner apex frozen to  $-196^{\circ}\text{C}$ . *Low Temp Sci Ser B* 36: 31–38
- Scott D, Draper A (1967) Light in relation to seed germination of blueberries, strawberries and *Rubus*. *HortScience* 2(3): 107–108
- Scott D, Draper A (1970) A further note on longevity of strawberry seed in cold storage. *HortScience* 5(5): 439
- Scott D, Hancock JF (1989) Strawberry cultivars and worldwide patterns of strawberry production. *Fruit Var J* 42: 102–108
- Sjulín T, Dale A (1987) Genetic diversity of North American strawberry cultivars. *J Am Soc Hortic Sci* 112: 375–386
- Sullivan JA (1991) Improvement of strawberry using tissue culture techniques. In: Dale A, Luby JJ (ed) *The strawberry into the 21st century*, Timber Press, Portland, OR pp 84–90
- Swartz H, Galletta G, Zimmerman R (1981) Field performance and phenotypic stability of tissue culture-propagated strawberries. *J Am Soc Hortic Sci* 106(5): 667–673
- Towill L (1988) Survival of shoot tips from mint species after short-term exposure to cryogenic conditions. *HortScience* 23: 839–841
- Wilhelm S, Sagen J (1974) *A history of the strawberry*. Univ California, Berkeley
- Withers LA (1986) In vitro approaches to the conservation of plant genetic resources. In: Withers LA, Alderson FG (eds) *Plant tissue culture and its agricultural applications*. Butterworths, London, pp 261–276
- Yamada T, Sakai A, Matsumura T, Higuchi S (1991) Cryopreservation of apical meristems of white clover (*Trifolium repens* L.) by vitrification. *Plant Sci* 78: 81–87