Fruit Varieties Journal 46(2):98-102 1992

Cold Storage of Strawberries In Vitro: A Comparison of Three Storage Systems

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Abstract

Three storage systems used for strawberry *in vitro* germplasm conservation were compared for plant health, culture longevity and frequency of contamination following storage at 4°C. Storage systems using 16 mm glass tubes, 6 x 6 x 9 cm plastic boxes and 5 chamber plastic bags were analyzed for longevity and contamination rates. Ninety-six accessions cold hardened and stored in polyethylene bags showed a greater average longevity than 127 non-hardened plantlets stored in plastic boxes. After 12 months, 76% of accessions in bags remained viable in storage in contrasts with 42% in boxes. Storage of 130 *Fragaria* plantlets in 16 mm glass tubes resulted in 20% survival after 24 months of storage. The contamination rate for tubes was 47%, boxes 30% and bags 10%. Contamination in bags was confined to a single plant in one chamber of a five chamber bag, however, contamination in boxes and tubes usually involved all plants in that container. The bag system was superior to boxes and tubes for preserving stored plants and excluding contamination and provides a good choice for germplasm conservation.

Introduction

Temperate fruit crops including *Fragaria* are stored by many different *in vitro* storage systems with variations of container size, storage temperature or media modifications to improve survival rates (9). Because only a few individual plants of each accession are held in repositories, losses may be severe if the storage technique proves unreliable (11). Apple shoot cultures stored on solid medium with hormones in 25 x 150 mm tubes at 1° or 4°C for one year showed no loss of viability (4). Defoliated *in vitro* apple and *Prunus* shoots submerged in agar medium without growth regulators survived three to four years (3). Wilkins et al. (11) found 4°C to be a good storage temperature for a number of woody genera in tubes or jars on solid medium with hormones. *Pyrus* survived at high levels after one to two years at 4°C in 25 x 150 mm tubes with solid media (10). Cold storage methods for *in vitro* strawberries range from tubes with liquid medium and filter paper bridges (6) to tubes and jars (2) or gas-permeable bags containing solid media (9). In this study we compared three storage systems for resulting plant health, longevity in storage and contamination rates for groups of *Fragaria* germplasm.

Materials and Methods

Plantlets were multiplied on basal medium composed of MS salts and vitamins (7) pH 5.7, with (per liter): 170 mg sodium phosphate (monobasic), 80 mg adenine sulfate, 1 mg BA (N^6-benzyladenine), 1 mg IAA (indole-3-acetic acid), 0.01 mg GA_3 (gibberellic acid) and 6 g agar (Difco-Bacto agar, Difco, Detroit, MI). Growth room conditions were 16 hr (25 $mol \cdot m^{-2} \cdot S^{-1}$) days at 25°C. Cold storage was at 4°C in the dark in a walk-in cold room. Each system of storage was used during a different time period but used the same storage and growth room conditions.

1. **Tube system:** *Fragaria* tissue culture plantlets (130 accessions) were stored in 16 x 100 mm glass tubes. Single plantlets were transferred to 16 x 100 mm glass tubes (Corning Glass

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Works, Corning, NY) with five ml basal medium without growth regulators and capped with plastic caps, grown for two weeks, sealed with Parafilm (American Can Company, Greenwich, CN) and stored. Each accession was stored as two cultures in individual tubes.

2. Box system: Plantlets of 127 *Fragaria* accessions were stored in plastic boxes. Sixteen plantlets of each accession were transferred to a Magenta GA7 (Magenta Corporation, Chicago, IL) plastic box (6 x 6 x 9 cm) containing 40 ml basal medium without growth regulators and grown for two weeks. The box was sealed with Parafilm to decrease dehydration and contamination and stored.

3. Bag system: Plantlets of 96 accessions were multiplied on the basal medium stated above except that the gelling agent was a combination of 3 g agar (Bitek agar, Difco, Detroit, MI) and 1.25 g Gelrite (Chemical Dynamics Corp., South Plainfield, NJ) per liter. Plantlets were transferred to bags (CultuSAK, Becton Dickinson, Lincoln Park, NJ) which were than heat sealed with an impulse sealer. Cultures were grown for one week in the growth room and one week under cold-hardening conditions of 8 h, 22°C days and 16 h, -1°C nights before storage (1, 9). For each accession, five plantlets were stored, each in an individual section (15 x 150 mm) of a five-section bag with ten 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 (9).

Each group of *Fragaria* accessions stored contained a representative sample of germplasm. Accessions included *F. chiloensis* (L.) Duch.; *F. moschata* Duch. cvs. Capron and Profumata di Tortona; *F. virginiana* Duch.; *F. vesca* L. and hybrids; and *F.* x *ananassa* Duch. cvs. Aberdeen, Aliso, ArKing, Atlas, Beaver, Blakemore, Cambridge Favorite, Canoga, Climax, Dabreak, Dana, Deutsch Evern, Dover, Earlibelle, Fairfax, Florida Belle, Fou Chu, Francesco, Fresno, Himiko, Kaiser's Samling, Kaoling, Klondike, Komsomalka, Kurume, Kurume 103, Marshall, Massey, Marsyalakya, Perle de Prague, Pocahontas, Podnyaya Zagorya, Primella, Robinson, Rubin, Senga Pantagruella, Sierra, Sunrise, Surecrop, Tufts, Vantage, Zefyr, and unnamed selections.

At three month intervals the health of *in vitro* plantlets in cold storage was rated on a scale of 0 to 5 (0 dead, 1 poor, 2 fair, 3 good, 4 very good and 5 excellent). Plants rated 1 or 2 have few green leaves and only the newest growth alive, for those rated 3, most tissues are still living but may be etiolated and those rated 4 or 5 are mostly green and in a condition similar to when they were originally stored. Contaminated cultures were noted and discarded.

Results and Discussion

This study examines the longevity and contamination rates of *Fragaria in vitro* cultures cold stored using three different storage systems. The systems vary in several parameters such as size and composition of the container, gelling agent and cold hardening. The differences in these systems reflect conditions found in storage systems presently in use for plant germplasm throughout the world.

Storage in polyethylene bags with cold hardening and Gelrite as part of the medium was examined over a two year period. Of the original 96 accessions stored in bags, 99% remained in storage after six months and 47% at 15 months (Fig. 1). These levels are 12 to 15% higher than those stored in boxes for the same time periods. At each three month inventory a larger percentage of accessions stored in bags remained in good condition than did those in boxes. The mean health rating

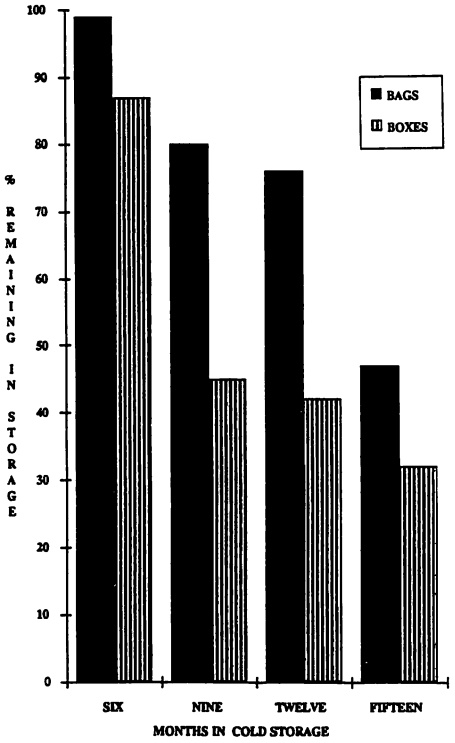

Figure 1. Percentage of uncontaminated *Fragaria* accessions with ratings of 3 or better, remaining in storage after 6, 9, 12 and 15 months in CultuSak polyethylene bags or Magenta GA7 boxes. Originally 127 boxes and 96 bags were stored.

of plants remaining in storage in bags was 3.7 at nine months and 2.5 at 15 months. Five percent of the individual accessions stored in bags maintained ratings of three for 24 months or more. Over the 15 month period no accessions were lost due to contamination. Contamination of a single chamber in a five chamber bag occurred in 10-15% 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, unpublished).

Cultures in boxes were stored for up to 15 months. Of the original 127 accessions stored, the percentage of uncontaminated cultures with ratings of three or better declined to 87% at six months and to 32% at 15 months (Fig. 1). The health ratings of cultures remaining in storage in boxes declined from 3.5 at nine months to 2.5 at 15 months. Contamination of boxes (30%) was primarily fungal, occurred at twice the level of that in bags and usually resulted in the loss of accession. Contamination was not always evident until repropagation was attempted. A greater amount of replication of accessions would decrease the number lost but would greatly increase the storage space 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 has also been reported in storage jars (5) and tubes (8).

Storage of cultures in bags and boxes was compared with an existing collection in 16 x 100 mm tubes. Of the 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 it reduced the survival rate to 20%. No data was available at the 9, 12 or 15 month time periods. This system had been in use for many years but this study found that it was not reliable due to high contamination rates throughout the storage period.

In general, if contaminants were excluded, *Fragaria* accessions stored well for 12 to 15 months using any of the systems described in this study. A great deal of variation existed among genotypes and the length of time an individual accession remained viable in storage ranged from 6 to 24 months. Mean health ratings were used to judge the condition of the collection as a whole, but as declining plants were removed for repropagation the mean ratings reflected only those remaining. Thus the percentage of accessions

remaining in storage must also be considered when comparing systems for *in vitro* storage. Mean health ratings for plants in bags and boxes were similar at all test periods (Table 1), however, at each inventory more accessions remained in storage in bags than in boxes (Fig. 1). In general, a large decline in ratings occurred after 12 months storage for both boxes and bags and by 15 months the mean rating was near two in both systems.

Individual cultivars 'Aliso,' 'Francesco' and 'Pocahontas' all 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 and all had 100% survival. Damiano (2) reported 90% survival for 'Pocahontas' at 12 months, 80% for 'Aliso' at 16 months and 70% for 'Francesco' at 17 months, when stored in jars or large tubes. In the present study, small tubes of these cultivars survived for 24 months although they were in need of transfer (ratings of 1 or 2) and were often contaminated. Twenty-two of the cultivars tested by Mullin and Schlegel (6) were used in this study as well, but individual data are not available for direct comparison. The advantage of the bag system over that of Mullin and Schlegel's (6) liquid medium system are that the bags minimize contamination and do not require the time and effort needed to add liquid medium quarterly.

All three systems described in this study provided moderate lengths of storage for some *Fragaria* germplasm, but the percentage of accessions remaining in storage and contamination rates varied greatly. When judged by the large percentage of accessions with high average health ratings remaining in storage after one year and very low contamination rates, we concluded that the bag system provided more secure and healthy storage for *Fragaria* germplasm than either the tube or box systems. Additional advantages of the bag system include easy handling, reduced storage space, resistance to breakage, ease of inventory and a safer method for shipping germplasm.

Table 1. A comparison of ratings of some *Fragaria* accessions stored in both plastic boxes (Magenta GA7) and in gas-permeable polyethylene bags (Cultusak) at 4°C in the dark for 9 to 15 months. The rating scale is 0 = dead, 5 = excellent.

Accession	Form stored	
	Box	Bag
Nine months storage		
Aberdeen	4	4
ArKing	4	4
F. chiloensis CA 1466	3	4
F. chiloensis TDM-08 TC 36.1	3	4
Senga Pantagruella	3	4
Zefyr	3	3
Twelve months storage		
Cambridge Favorite	3	3
Fou Chu	4	3
F. chiloensis Darrow 72	3	3
F. virginiana	4	4
F. virginiana v. glauca	4	4
Klondike	3	3
Komsomalka	4	4
Kurume 103	3	4
MDUS 3022	3	4
Podnyaya Zagorya	4	3
Robinson	4	3
Tufts	4	4
Vantage	4	4
Fifteen months storage		
Fou Chu	3	2
Komsomalka	3	3
MDUS 3022	2	3
Surecrop	2	2

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Fruit Varieties Journal 46(2):102-107 1992

'Segundo,' 'Byrongold' and 'Rubysweet' Plums and BY69-1637P Plumcot— Fruits for the Southeastern United States

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Abstract

Since 1984, the USDA plum breeding program at Byron, GA has released 3 plums and a plumcot adapted to the humid southeastern United States. 'Segundo' is an early "green plum" type, ripening in early June. It is suitable for local use. It turns from yellow-green to red as it ripens and has yellow flesh. 'Byrongold' is a firm, yellow shipping plum ripening in mid-late June. 'Rubysweet' also ripens in mid-late June but has blood-red flesh with a golden-bronze skin. All these plums have very good tree health relative to older varieties. 'Segundo' is somewhat smaller, not quite as firm, and higher in acidity, soluble solids and total sugars than 'Rubysweet' and 'Byrongold.' 'Rubysweet' was rated highest by the sensory panel on the hedonic like-dislike scale, followed by 'Segundo' and 'Byrongold.' A plumcot breeding line, BY69-1637P, has also been released. This selection produces light-medium crops of tart, orange-fleshed fruit. The black skin has a very short fuzz. It was released for use in further breeding.

Most Japanese-type shipping plums (*P. salicina* Lindl. hybrids) grown in California are highly susceptible to the diseases epidemic in the southeastern United States and cannot be grown successfully. Since its inception by V. E. Prince in 1964, the USDA plum breeding program at Byron, Georgia has released 5 Japanese-type plums and 1 plumcot adapted to the humid Southeast. 'Explorer,' a mid-season black-skinned plum, and 'Robusto,' an early "green-plum," were released in 1980 (1, 2, 3). Four more recent releases have not been described in the literature. 'Segundo,' 'Byrongold' and 'Rubysweet' plums and BY69-1637P plumcot are described in this

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