World Applied Sciences Journal 30 (3): 330-334, 2014
ISSN 1818-4952

DOI: 10.5829/idosi.wasj.2014.30.03.14028

Effect of Pretreatment Methods of Dormant Pear Buds on Viability after Cryopreservation

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Abstract: This study aimed to develop alternatives of using several cryoprotectants on four pear cultivars with a view to improve the viability of the dormant buds. Have used different cryoprotectants such as Honey, PVS2, PVS3, PVS4, Towill, IPBB-1 for cultivars: Talgarskaya Krasavitca, Nagima, Zhazdyk, Lesnaya Krasavitca. Each varieties on the moisture content of the buds are divided into two moisture control (MC) subgroups natural moisture of the buds and dried to 30% moisture. To determine viability of bud tissues we conducted histological analysis and Triphenyltetrazolium chloride (TTC) staining. The application of these solutions to cryoprotectants viability of dormant buds showed that the PVS3 results were better than other cryoprotectants.

Key words: Cryopreservation · Liquid nitrogen · Pear · Freezing · Dormant buds · Moisture · Cryoprotectants

INTRODUCTION

Cryopreservation in liquid nitrogen is one option for long-term preservation of fruit crop genetic resources. It is possible to freeze and store in super-low temperatures different plant organs as seeds, pollen, shoot tips and dormant buds. The system has been tested with many fruit species, eg *Malus, Amelanchier, Morus, Prunus* and others [1]. Cryopreservation of dormant buds seems to be the best among other alternatives because it is simple, cheap and reliable method (clonal integrity is maintained) and grafted trees can be forced into early flowering. Dormant vegetative buds from diverse species can be preserved using cryopreservation [2].

Authors [3, 4] provided one of the first studies showing that winter twigs of poplar (*Populus sieboldi*) and willow (*Salix koriyanagi*) could survive low temperatures if slowly cooled prior to immersion in liquid nitrogen. Later, methods were developed, resulting in the observed 80% to 100% of restoring the viability of cryopreserved dormant buds Malus, Amelanchier, Crataegus, Sorbus and Prunus. The most important factor identified to date for successful cryopreservation of dormant buds is the extent of cold acclimation that the species can attain and the extent of acclimation that the material possesses when collected. Another factor that is important for optimizing recovery for some species is the extent of desiccation obtained before cooling [3].

We studied pear dormant bud cryopreservation to determine which cryoprotectants are effect different cultivars of pear.

In order to improve the viability for cryopreservation of the buds to freeze raised a number of experiments to optimize the cryopreservation of dormant overwintering buds.

MATERIALS AND METHODS

Plant Materials: Cold hardened branches (~1M) of field-grown pear trees were collected after the ambient temperature was below -10 °C for two weeks (mid-January) at the Kazakh Pomological Garden Institute of Horticulture and Viticulture near Almaty, Kazakhstan. We used local cultivars of pear: Talgarskaya Krasavitca, “Lesnaya Krasavitca”, “Nagima” and “Zhazdyk”.

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Fig. 1: Middle segments of dormant buds

Cryopreservation Protocol: Scion wood collected as noted above was cut into 2 cm segments with single buds, located in the middle of the segment (Fig. 1) and placed in (5ml) cryovials (2-5 segments). Segments were dehydrated for two weeks at -5 °C in a controlled climate chamber to a moisture content of 30%. Segments in tubes were then cooled at a rate of 1 °C / h to -25 °C and held at this temperature for 24 hours before immersion in liquid nitrogen at -196 ºC [4]. Rewarming was carried out in the laboratory at 25 °C.

Recently, techniques have been developed that result in 80% to 100% viability and recovery of cryopreserved dormant vegetative buds from *Malus*, *Amelanchier*, *Crataegus, Sorbus* and *Prunus*. The system now has been tested with a reasonable number of temperate deciduous fruit trees, including gooseberry, currant, mulberry, some cultivars of cold-hardy and cold-tender apples, a few cultivars of apricot, sweet and sour cherry, peach and 11 other species in the *Rosaceae* family [4].

Comparison of Natural Moisture Content with Dried Buds: Part of the segments were dried in a controlled moist chamber at -5 ºC to reduce the moisture content to 30% MC. Water loss from the buds was measured twice a week using a moisture hygrometer (KERN MLB 50-3). A second set of segments was held at the natural moisture content in sealed petri dishes. These two sets of segments were then tested with cryoprotectants and exposed to liquid nitrogen.

Six cryoprotectant treatments were tested for 3 hour exposure for buds with natural moisture and dried to 30% MC:

- Honey (locally sourced)
- PVS2 (30% glycerol, 15% ethylene glycol, 15% DMSO in liquid MS medium with 0.4 M sucrose, pH 5.7)

Fig. 2: Moist chamber

- PVS3 (50% glycerol + 50% sucrose)
- PVS4 (35% glycerol + 20% EG + 0.6 M sucrose)
- Towill (35% glycerol + 10% DMSO + 10% PEG-8000 + 0.4 M sucrose)
- IBBR-1 (50% glycerol, 50% glucose in liquid MS medium with 0.4 M sucrose, pH 5.7).

Three replications of three buds were used in each treatment (n=9).

After three weeks, cryovials were removed from the liquid nitrogen and placed for 24 hours in a cold room at 4 °C. Segments were removed from the cryovials, washed with distilled water and placed in a moist chamber for 5 days (Figure 2- Photograph of chamber). Segments were then immersed in warm water (25-30 ºC) for a day in the controlled climate chamber before TTC testing.

Viability Determination: Viability of buds was determined by Triphenyltetrazolium chloride (TTC) testing. A solution of 1% TTC (pH - 7.0) was prepared in 1/15M N_2HPO_4 and 1/15M KH_2PO_4 (Zerens's Buffer reference) [5, 6]. Segments were immersed in TTC solution for 24 h. Tissue sections were prepared by hand with a razor blade. Sections were viewed and photographed under a stereomicroscope "Digital Microscope".

Statistical Analysis: Buds were scored as alive or dead as determined by the TTC test. Data was analyzed by ANOVA with MYSTAT 12.

RESULTS AND DISCUSTION

There were significant differences in viability among cultivars, moisture contents and cryoprotectants. Cultivar by cryoprotectant interaction was significant as was interaction of the three factors ($P \leq 0.002$) (Table 1).

Table 1: Analysis of variance for cultivar, moisture Content (MC) and cryoprotectant treatment

Source	Type III SS	Df	Mean squares	F-ratio	p-value
Varieties	9.409	3	3.136	6.385	0.001
MC	10.454	1	10.454	21.281	0.000
Cryoprotectants	76.724	5	15.345	31.237	0.000
Varieties * MC	0.509	3	0.170	0.345	0.793 NS
Varieties * Cryoprotectants	32.258	15	2.151	4.378	0.000
MC* Cryoprotectants	3.757	5	0.751	1.529	0.188 NS
Varietes*MC* Cryoprotectants	19.968	15	1.331	2.710	0.002
Error	46.667	95	0.491		

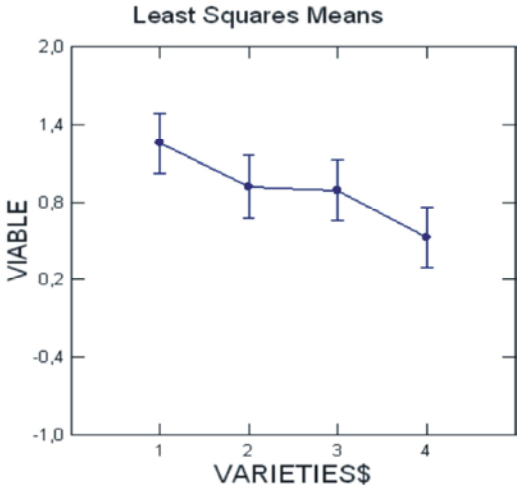

Fig. 3: Viability of pear cultivars
The four local cultivars of pear: 1-'Talgarskaya Krasavitca', 2-'Lesnaya Krasavitca', 3-'Nagima' and 4- 'Zhazdyk'

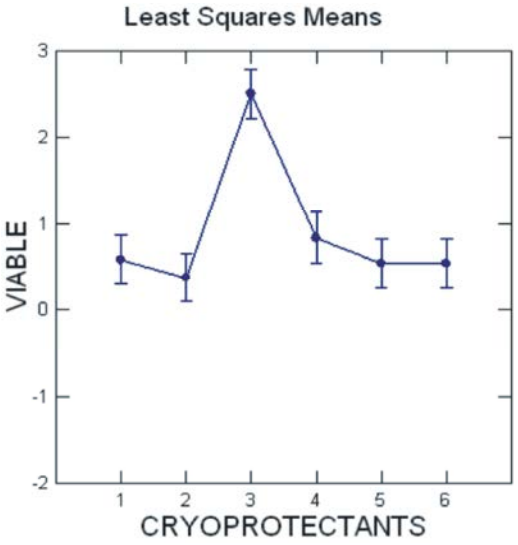

Fig. 4: Effects of different cryoprotectants
Different 6 cryoprotectants: 1- Honey, 2- PVS2, 3- PVS3, 4- PVS4, 5- Towill, 6- IBBR-1

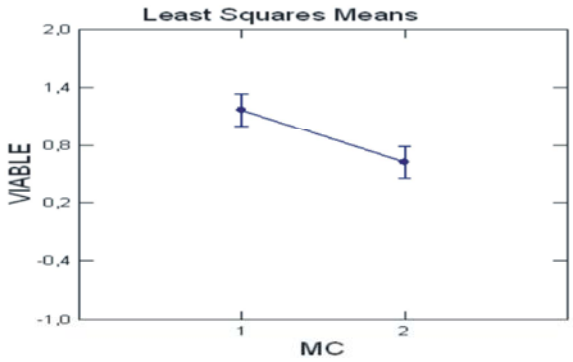

Fig. 5: Natural (1) and (2) 30% moisture effects

The pear cultivars also differed in their viability with Talgarskaya Krasavitca producing higher viability than other cultivars (Fig. 3). PVS3 cryoprotectant was significantly better than all the other cryoprotectants tested (Fig. 4). Viability was significantly higher for the natural moisture content than for the 30% dried segments (Fig. 5).

The significant interaction of cultivar by cryoprotectant was evident for several of the cultivars. All four cultivars responded well and had high viability with PVS3. The rest of cryoprotectants such as Honey, PVS2, Towill, IBBR-1 they affected less low survival of dormant buds. The effect of optimal viability with different cryoprotectants depends on each plant species (Fig.4). Pear buds had the best survival when the moisture content was reduced to approximately 41% [7]. Dormant pear (*Pyrus communis* L.) buds have been successfully cryopreserved followed by recovery of meristems *in vitro* [8]. The other report of longevity of cryopreserved dormant buds has been in mulberry (*Morus bombycis* Koids.), which has been successfully stored for up to 5 years [9-11]. In our research different cryoprotectant treatments were tested for 3 hour exposure for buds with natural moisture and dried to 30% MC. Overall all four cultivars had the best viability at natural MC than 30% MC (Fig.5).

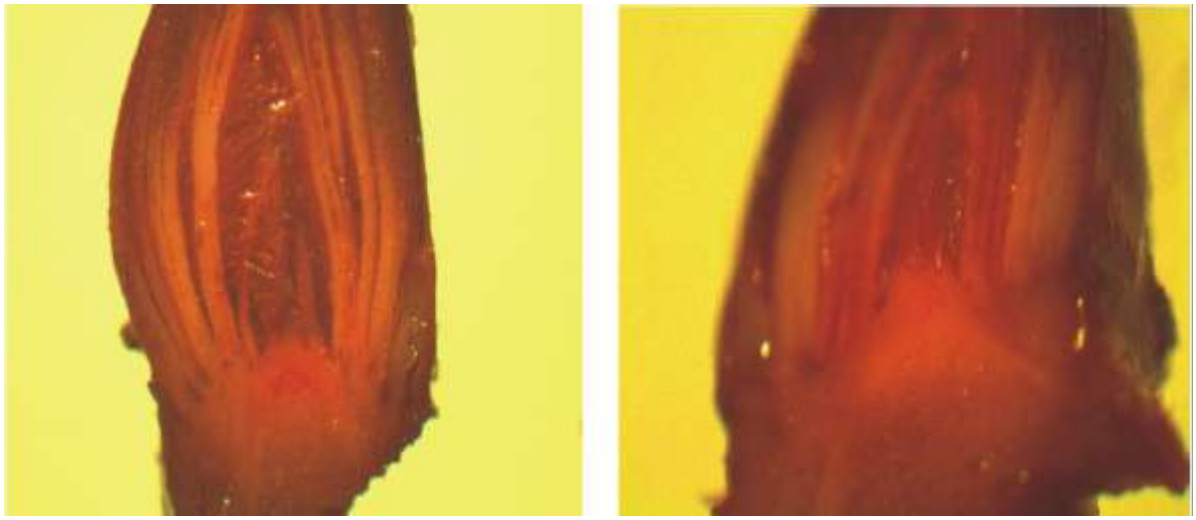

Fig. 6: Viable buds of Talgarskaya Krasavitca cryopreserved with PVS3

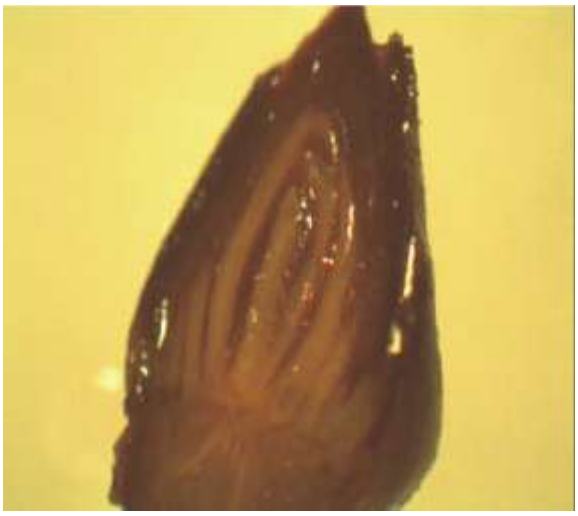

Fig. 7: Not viable buds of Talgarskaya Krasavitca cryopreserved with PVS2

A result histological analysis showed that the most suitable cryoprotectants for freezing dormant buds of pears - PVS3 and PVS4. The rest of cryoprotectants were not suitable cryoprotectants for freezing dormant buds of pears. Results of testing the viability of dormant buds by staining with 1% solution of triphenyl tetrazol chloride (TTC) shows indeed that the above effective cryoprotectants pear varieties. Living dormant buds stained pink. (Fig.6).

When the dormant buds were not painted, the color of the meristematic tissue can be determined that the pear buds died (Fig. 7).

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