Cryogenic Storage of Olive Pollen[1]

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Abstract

The possibility of using liquid nitrogen storage conditions (—196°C) for the storage of olive *(Olea europaea)* pollen was demonstrated. Pollen from 10 cultivars was rapidly frozen and thawed. Germination tests were performed on 15% sucrose, 1% agar plates. Significant differences between unfrozen control samples and frozen pollen samples were not found. However, significant cultivar by treatment interactions were observed.

A number of methods are currently being used for the preservation of economically important plants, including storage of seed and whole plants for clonal crops. An alternative that merits serious consideration as a method for long-term storage of plant germplasm is the storage of pollen. A number of investigators have explored the possibility of short and medium term pollen storage with various fruit crops (3, 5, 6).

Recently, there has been considerable interest in the possibility of storing seeds at liquid nitrogen temperatures (−196°C) (11, 12). At this temperature metabolic activities are almost absent including chemical and biochemical processes that could reduce viability (1). Therefore, seeds or other plant tissues that have been frozen in liquid nitrogen (LN_2) may be stored without significant changes in viability levels for long periods of time, at a relatively modest cost. Unfortunately, losses of viability may be encountered during the process of freezing or thawing the samples (15). Pollen from several species have been stored via liquid nitrogen including *Lupinis poluphyllus* (9), *Pyrus communis* (9), *Rhododendron catawbiense* (9), *Vitis vinifera* (8), *Phoenix dactylifera* (13), *Medicago sativa* (2), *Humulus lupulus* (4), *Prunus species* (7), and *Solanum* species (14).

Fruit tree pollen has proven to be a durable plant tissue as shown by Griggs, et al. (3). In addition, a larger number of gametophyte genotypes may be more compactly stored as pollen than as seed or scionwood. Therefore, an experiment was designed to determine whether olive pollen could be easily frozen to −196°C and thawed without loss of viability.

Materials and Methods

Pollen from 10 randomly selected olive *(Olea europaea)* cultivars was collected during the summer of 1982. The pollen was cleaned through a 120 mesh screen and refrigerated for about 1 week over $CaSO_4$ until used. A control (unfrozen) pollen sample from each cultivar was compared to the liquid nitrogen frozen pollen sample.

Pollen samples from the 10 cultivars were rapidly frozen to −196°C in 1.5 ml plastic microfuge tubes by direct plunge into liquid nitrogen. Cryoprotectant solutions were not used due to the possible difficulties of maintaining pollen viability in the cryoprotectant media and isolating the pollen from the media for later use. All samples were held for one hour at −196°C to ensure complete freezing and then thawed rapidly in 37°C water. The measured freezing and thawing rate within the tubes was about 900°C/min.

Three replicates of frozen and control pollen from each cultivar were assayed for germination on opposite halves of agar petri plates. 15% sucrose in 1% agar was used for the pollen germination medium (5). 1%, rath-

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Table 1. ANOVA for LN_2 freezing of olive pollen.

Source	D.F.	M.S.	F	Probability of > F
Cultivar	9	570	11.70	0.00
Error 1: Replicate (Cultivar)	20	49		
Treatment*	1	114	1.21	0.30
Cultivar x treatment	9	94	4.83	<0.01
Error 2: Cultivar x Replicate	20	20		

*Treatments were control and frozen.

er than 2%, agar was used to ensure sufficient moisture for germination. Pollen grains were considered to be viable if pollen tube growth was greater than one pollen grain diameter. Between 500 to 700 pollen grains were counted for each replicate of control and frozen samples. A multiway analysis of variance was performed on the data via the BMDP8V statistical program and residuals were tested for normality.

Results and Discussion

There was no significant difference between the LN_2 frozen samples and the unfrozen controls as determined by ANOVA (Table 1). Therefore, the LN_2 freezing was not generally detrimental to pollen germination. However, the effects of cultivar and cultivar by treatment (control or frozen) were significant at the 1% and 5% levels. LN_2 freezing reduced pollen germination for 2 specific cultivars (cultivar by treatment).

Mean germination values of frozen and unfrozen samples for 10 cultivars are presented in Table 2, along with differences between control and frozen means. Negative values indicate decreased viabilities for the frozen samples. The average germination of the liquid nitrogen frozen samples was 2.8% less than the unfrozen controls. Decreased pollen germination for several cultivars, including Cordovil with a loss of 21.5%, was observed. Some of the frozen samples had greater viability than the unfrozen controls (probably due to random variation). While pollen viability levels sufficient for successful pollination remained after freezing (greater than 35% for all cultivars), the viability decrease observed for Cordovil indicates that additional testing of pollen from olive cultivars to be cryogenically stored will be needed before pollen samples from those cultivars can be routinely stored via liquid nitrogen freezing. Olive pollen is not as durable as some of the other types of pollen that we have tested (7, 8) and the difference is reflected in the decreased germination

Table 2. Germination (%) for control and LN_2 frozen olive pollen.

Cultivar	Control	Frozen	Difference (Frozen-Control)
Dolce de Morocco	64.4	64.4	0.0
Balady	41.8	40.4	−1.4
Salome	32.6	37.5	4.9
Cordovil	60.6	39.1	−21.5
Mission	66.2	67.0	0.8
Verdeal	56.5	47.3	−9.2
Zoragi	59.4	52.3	−7.1
Menara	58.1	61.7	3.6
Bouquetier	49.7	51.6	1.8
Lecci	54.9	55.0	0.1
Treatment means	54.4	51.6	−2.8

$LSD_{.05} = 7.53$.

of Cordovil, Verdeal, and Zoragi. No differences were observed in length or morphology between pollen tubes of control or LN_2 frozen samples.

This experiment demonstrates the possibility of using liquid nitrogen freezing for storage of olive pollen. Olive pollen from late blooming cultivars could be retained with moderate viability losses for crosses to early blooming cultivars one or two years later. In addition, liquid nitrogen storage is convenient, inexpensive, and safe. The pollen can be used immediately after thawing since no cryoprotectants were needed for olive pollen storage.

The possibility of true long-term storage (more than five years) would be especially valuable for germplasm preservation. However, some caution could be required when using this system for preservation of pollen from particular cultivars, since pollen of one cultivar in this experiment had much decreased viability after freezing. Cryogenic storage of pollen would be most suitable for the maintenance of gene pools rather than specific genotypes. This experiment was conducted with *Olea europaea* cultivars. Therefore, the application of this methodology in germplasm storage programs may require the testing of other *Olea* species for retention of viability after liquid nitrogen storage. Additional studies may be needed to determine if alternative methods for handling the pollen before and after storage will improve viability. The addition of dessicants to minimize moisture absorption may be especially useful.

Stanley and Linskens (10) have itemized the numerous advantages of testing pollen viability *in vitro*. However, initiation of other experiments to test the stylar penetration and seed setting ability of frozen pollen is desirable.

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