EFFECTS OF SUNFLOWER (HELIANTHUS ANNUUS L.) POLLEN STORAGE CONDITIONS ON POLLEN VIABILITY AND PROGENY Mdh1 ALLELIC FREQUENCY.

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SUMMARY

Storing pollen for long periods could have advantages in germplasm maintenance because it would allow preservation of a large sample of genetic diversity in a very small area. This study tested different storage conditions on viability and gene frequency changes of progeny of crosses from stored pollen. Pollen was collected into glass vials from 1986 field grown plants. Samples were then dried for four hours and placed into storage in a refrigerator, a refrigerator freezer, and into liquid nitrogen for 220 to 360 days. Heads of CMSHA 89 grown in the greenhouse during the winter of 1986 - 1987 and in the field during 1987 were pollinated with pollen from the various storage media. Seed set varied from 140 to 0 percent of seed set from crosses with fresh pollen. Fresh pollen (check) produced an average 26 percent seed set. Pollen stored in liquid nitrogen produced seed set greater than the check through about 360 days. Refrigerator-stored pollen lost viability over the period from 220 to 360 days in storage (110 to 17 percent of check), as did freezer stored pollen (47 to 0 percent of check). Malate dehydrogenase isozyme locus Mdh1 was evaluated for changes in allele frequency of progeny from liquid nitrogen and refrigerator stored pollen. No significant allele frequency changes were observed.

INTRODUCTION

Short-term storage of sunflower pollen has been used in breeding programs. Fick (1978) reported storing sunflower pollen up to 3 months without apparent loss of viability. Putt (1941), quoting early work on storing pollen for breeding purposes, stated Arnoldava used pollen which had been stored for up to 11 months. We regularly used undried pollen stored in a refrigerator for over three months in crosses during the following greenhouse season.

Other researchers have reported storing pollen of various species. These include azalea (Widrlechner, 1986), Solanum spp. (Towell, 1981), hops (Haunold et al., 1985), alfalfa (Hanson et al., 1972 and Collins et al., 1973), pearl millet (Hanna et al., 1983), tree species (Copes, 1985), soybean, cotton, corn, oat, rye, sorghum & wheat (Collins et al., 1973) and tobacco (Bajaj, 1977).

Long-term pollen storage would be useful for applications besides breeding. Our main interest is in long-term storage and maintaining integrity of germplasm. If successful, pollen storage would allow us to maintain an infinite number of

potential individuals in a relatively small space. Frank et al. (1982) stored sunflower pollen successfully in liquid nitrogen (LN) for up to 4 years. He suggested that maintaining sunflower germplasm in LN would be advantageous, primarily to allow a more free exchange of germplasm without quarantine restrictions. The disadvantages presently are lack of knowledge about the genetics of populations coming from stored pollen and the inability to regenerate plants directly from pollen. This paper provides a progress report in long-term pollen storage investigations.

MATERIALS AND METHODS

Bulk pollen from various sources was collected from 1986 field-grown sunflower plants. Pollen was kept in 1.8 ml glass vials with screw type lids for storage in the refrigerator and refrigerator freezer. Pollen for LN storage was placed into 1.8 ml polypropylene cryotubes. Pollen was first placed into a desiccator (caps left off the tubes) for four hours before placing the containers into the storage facilities. LN-stored pollen was immersed directly into LN without preconditioning.

CMSHA 89 and HA 89 inbred seed were planted at two different times in the 1986-1987 winter greenhouse and then again in the 1987 field. Fresh pollen was collected from HA 89 and used to pollinate CMSHA 89 heads as checks. Receptive CMSHA 89 greenhouse heads were divided into 5 sections. Pollen from each of the three storage facilities (about 220 day-old pollen for the first greenhouse planting and then again 250 day-old pollen for the second), fresh pollen and a no pollen check were applied to a section, respectively. LN-stored pollen was allowed to thaw in the refrigerator for 24 hours before pollination. Heads of field-grown plants were covered with cotton pollination bags at late bud. Pollen (about one-year old) was applied to individual receptive bagged heads as described above. Heads were left bagged until physiological maturity. Individual head sections from the greenhouse or field-grown heads were hand threshed and X-ray pictures taken of the achenes. Filled and unfilled achenes were counted and percent seed set determined from at least two greenhouse produced heads and at least 4 field produced heads. The "nonortho" procedure from MSTAT was used to analyze the data (Nissen et al., 1985).

Eighteen individual seed from each of 11 heads for each treatment of field grown plants were assayed for locus Mdh1 (malate dehydrogenase EC 1.1.1.37) (Kahler and Lay, 1985). Seed were soaked overnight and cut into quarter sections. Sections including both cotyledon and embryo tissue were ground in microtubes with extraction buffer. Extraction buffer consisted of the following: 1.0 M Tris, 0.001 M Polyvinylpyrrolodine MW 40,000, 0.016 M Diethyldithiocarbamic acid - sodium salt, 0.011 M Ascorbic acid - sodium salt, 0.10 M Sodium tetraborate (Borax). Concentrated HCl was added to buffer to 7.8 pH. About 0.15 ml of the buffer were placed in a 0.5 ml polyethylene microtube, and either stored frozen until use or used immediately. The AC (amine-citrate) buffer system (Clayton and Tretiak, 1972) was

used for the Mdh1 assay starch gel electrophoresis and was run at 70 to 75 milliamps for 5.0 to 5.5 hours. MDH stain was identical to that reported by Kahler and Lay (1985). Gel slices were stained for 20 to 40 minutes in the dark at 26.7'C, then fixed in 50 percent ethanol. Analysis of variance was used to determine significant differences in mean frequency of alleles at locus Mdh1.

RESULTS

The analysis of variance for mean seed set from crosses made with stored pollen indicated that both the effects of type of storage and of time in storage were highly significant. The interaction was not. Because the main effects are significant, we considered the individual values derived from pollen from the various storage conditions vs. time to be different and applied the Duncans multiple range test to assess the significance of those differences. Some bias may result from this assumption.

Pollen stored in LN maintained its viability throughout this trial (Table 1). At the end of the first year in storage, LN-stored pollen produced seed set not significantly different from seed set of the fresh pollen check. LN-stored pollen had higher viability than fresh pollen after 220 and 250 days in storage.

No seed was obtained from non-pollinated heads.

Refrigerator and freezer-stored pollen decreased in viability over the trial period (Table 1). After 220 days, refrigerator stored pollen produced seed set 110 percent of the check. By the end of a year in storage, seed set was only 17 percent of check. Freezer-stored pollen had reduced seed set. Only 47 percent of check was realized at the end of 220 days in storage, and was reduced to zero by the end of the year.

Allele frequencies at locus Mdh1 were not significantly different in seed from crosses from refrigerator, and LN-stored pollen (Table 2). The rare allele, Mdh1-1, was found in seed from refrigerator and LN stored pollen. The frequency of rare alleles were also statistically equal. No seed was produced on field-grown plants from crosses with freezer-stored pollen.

DISCUSSION

We do not know why more LN or refrigerator-stored pollen was more viable than fresh pollen, but suspect the greater seed set from stored pollen to be an artifact.

Apparently, storing pollen in the refrigerator freezer resulted in increased damage to the pollen compared to other storage conditions. Drying the pollen before storage extended the viability of refrigerator and freezer-stored pollen from less than 80 days (Roath and Widrlechner, 1986) to over 220 days (Table 1). We have not attempted to test additional pollen drying time on longevity of pollen for storage under any condition. We

believe there is no advantage to storing pollen in the refrigerator or freezer for periods longer than 6 months, when a higher degree of viability can be maintained by storage in LN with little additional effort or cost.

Pollen samples remain in storage and we plan to check pollen viability at the end of five years and again after ten years.

CONCLUSIONS

1. Long term storage (more than one year) in LN maintained pollen viability equal to or greater than what was found in fresh pollen.

2. Extended storage of pollen in a refrigerator or freezer can be obtained by drying the pollen for at least four hours before storing. Four hours of drying is not adequate to preserve viability of freezer-stored pollen over about 200 days.

3. Storing pollen for up to one year in either the refrigerator or in LN did not significantly change allele frequencies at the Mdh1 locus.

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Table 1. Seed set of crosses with stored sunflower pollen over time.

Storage Condition	Days in Storage	Percent of Check[1,2]
Refrigerator	220	110 abc
Refrigerator	250	76 cd
Refrigerator	360	17 ef
Freezer	220	47 de
Freezer	250	21 ef
Freezer	360	0 f
Liquid nitrogen	220	119 ab
Liquid nitrogen	250	140 a
Liquid nitrogen	360	101 bc
CV = 2.16%		

1. Mean seed set from fresh pollinated crosses = 26 %.
2. Means followed by unlike letters are significantly different at P=0.05, Duncans multiple range test.

Table 2. Frequencies of alleles Mdh1 and Mdh1-1 among progeny obtained from crosses made with stored sunflower pollen.

Storage Condition	Alleles	
	Mdh1	Mdh1-1
Refrigerator[1]	0.65 a	0.35 b
Liquid Nitrogen[1]	0.75 a	0.25 b
Fresh pollen	1.00	0.00

[1] Means followed by unlike lettered are significantly different. P=0.05, Duncans multiple range test.