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Long-term storage of *Pennisetum glaucum* (L.) R. Br. pollen

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Summary. Pearl millet [*Pennisetum glaucum* (L.) R. Br.] pollen has been successfully stored for 2,615 and 2,911 days at −18° and −73 °C, respectively, and continues to be viable. Viability of pollen stored at −73 °C appears to be little affected either by pollen storage moisture contents below 7.2% or by storage in glass vial or zip-lock plastic bag containers. Pollen moisture content appears to be more critical for maintaining viability at −18 °C than at −73 °C. Glass vials appear to be more desirable for longer term (>3 years) storage at −18 °C.

Key words: Pearl millet – Pollen viability – Freezing pollen – Pollen moisture content

Introduction

Pollen storage is a valuable tool in a plant breeding program because it makes recurrent and breeding lines available on an as-needed basis regardless of flowering response and planting date. Rapid exchange and use of germ plasm between scientists nationally and internationally is facilitated by pollen storage. Pollen storage makes possible maintenance of germ plasm from specific genotypes for future crossing. It also may have some potential for long-term germ plasm storage, especially of unique genotypes. The overall advantage of pollen storage is that it makes germ plasm immediately available for crossing onto female parents, without having to continually plant recurrent parents, hoping they will flower at the same time as the female parents.

Long-term storage of Gramineae pollen has been less successful than storage of pollen from *Pinus*, *Pyrus*, and *Vitis* species. Viability was maintained in maize (*Zea mays* L.) pollen for 180 days (Nath and Anderson 1975) and 363 days (Barnabas and Rajki 1976) at −196 °C. Another study showed that maize pollen stored at 13% pollen moisture maintained the highest viability after 7 days in −196 °C storage (Barnabas et al. 1988). Viability of ryegrass (*Lolium multiflorum* Lam) pollen was maintained for 39 days at −20 °C by mixing the pollen with powdered polyvinylpyrrolidone to stabilize the water content (Nietzsche 1970). Rye (*Secale cereale* L.) pollen retained seed-setting capability after storage for 7 days at −196 °C (Collins et al. 1973). Longevity of sugarcane (*Saccharum spontaneum* L.) pollen was increased from 40 min to 14 days at storage temperatures just above freezing and 100% relative humidity (Moore 1976). About 21% germination was maintained in napier grass (*Pennisetum purpureum* Schum.) pollen stored at 4 °C and at 90%–100% relative humidity for 7 days (Aken'Ova and Chheda 1970).

Viability was maintained for 186 days at room temperature (16°–35 °C) in pearl millet [*Pennisetum glaucum* (L.) R. Br.] pollen by mixing it with a lycopodium diluent (Vasil 1962). Pollen collected from field-grown pearl millet plants lost viability after 1 day at 27 °C and after 3 weeks at 4 °C (Cooper and Burton 1965). Less than 5% seed set was obtained from pearl millet pollen stored at 4 °C for 7 days and at 14 °C for 21 days (Pokhriyal and Mangth 1979). Pearl millet pollen dried to 7.5% moisture and stored at −73 °C for 185 days gave 100% seed set (Hanna et al. 1983). In later studies, viability of pearl millet pollen was maintained for 1089, 461, and 816 days at −73 °C, −18 °C, and 5 °C, respectively (Hanna et al. 1986). These showed that pearl millet pollen with less than 7% moisture at storage time was the most favorable for maintaining long-term viability.

The objective of this research is to develop a practical method for long-term storage of pearl millet pollen. This paper reports on the progress being made toward meeting this objective.

Materials and methods

Pollen was collected from field-grown pearl millet inbreds Tift 23DB, Tift 18DB, Tift 23DBE, Tift 23BE, Tift 186, Tift B39, 81B, and a brown midrib (bmr) breeding line obtained from Dr. J. Axtell, Purdue University. Glassine bags were placed on inflorescences the day before pollen collection, and the mouth of the bag was folded over and secured with a paper clip to prevent pollen loss. At 1,100 h, pollen was collected, bulked, screened through a 450-μm sieve to separate anthers from pollen, placed in a 1-mm layer on a glass plate, and dried for 1 h in a convection oven at 38 °C. Dried pollen (0.5 ml) was placed in 7.5 cm × 7.5 cm plastic zip-lock bags or sealed in 4-ml glass vials. Some pollen was also dried in a forced air oven at 38 °C in the original glassine collection bags. The glassine bags with dried pollen were folded and placed in 10 cm × 14 cm zip-lock bags. Pollen in storage containers was then placed in an ultralow temperature freezer cooled to −73 °C, in a freezer at −18 °C, or in a refrigerator at 5 °C. The pollen in glassine bags was stored only at −18 °C and 5 °C because of space limitations.

Pollen moisture content was determined from a 0.5-g pollen sample on a moisture balance September 2, 1989. Moisture content was not determined on all samples because of limited pollen supply.

Viability of pollen at storage was determined by taking pollen from three containers of each treatment and pollinating cytoplasmic-nuclear male-sterile (cms) Tift 23DA. All treatments in Table 1 gave 100% seed set on Tift 23DA at storage after drying. Final viability of stored pollen reported in Table 1 was determined by taking pollen from three containers (replications) of each treatment and using it to pollinate cms Tift 85DA on September 2, 1989. Pollen stored in glass vials and zip-lock bags was first poured into glassine bags. The glassine bags with stored pollen were each placed over a cms inflorescence and shaken to distribute pollen. Seed set was determined 3 weeks after pollination. Treatments P4, P8, P15, P16, and P24 in Table 1 are the same treatments previously reported (Hanna et al. 1986) but with longer storage times.

Results and discussion

Data on the effect of pollen moisture content, storage temperature, storage container on maintaining pollen viability, and the resulting seed set on cms Tift 85DA are reported in Table 1.

Temperature appears to be important for maintaining reliable long-term viability of pearl millet pollen. P24 (vial) at −73 °C with 7.2% pollen moisture content gave 100% seed set after 2,590 days of storage, while P8 at −18 °C with 6.2% pollen moisture was not viable at 2,234 days. Both of these treatments gave 100% seed set in a previous report (Hanna et al. 1986). Although P16 at 5 °C with 8% moisture gave 100% seed set after 817 d of storage (Hanna et al. 1986), it did not produce any seed after 2,590 days in 1989. Pollen stored at moisture contents of 3.0%–7.2% at −73 °C have maintained viability from 7 to 8 years and continues to be viable. Although some pollen (P29) has maintained viability for 2,615 days at −18 °C, other pollen treatments (P8, P30, P33, P34, P40, P41, and P42) with similar moisture contents and similar storage containers as those stored at −73 °C have lost viability. None of the 5 °C treatments maintained viability in this test.

Low pollen moisture content during storage has previously been shown to be favourable for long-term storage of pearl millet pollen (Hanna et al. 1983, 1986). This study supports previous observations and further shows that pollen moisture content during storage is more critical at −18 °C and 5 °C than at −73 °C. Viability of pollen with 3.0%–7.2% moisture content at −73 °C remained viable for 7–8 years, At pollen moistures of 5.7% (P30) and 6.2% (P8), pollen was non-viable after 6 years of storage at −18 °C. Treatments P35 and P38 with moisture contents of 7.9% and 8.2%, respectively, began losing viability after 4 years, while P39 with 6.9% moisture remained viable. Low pollen moisture of 4% and 5% (P32 and P36) tended to favor maintenance of pollen viability at −18 °C. Different vials or glassine bags (replications) of treatments P35, P38, and P46 gave variable results. Each treatment had one replication that gave 100% seed set, and the remaining replications gave no seed set (P35 and P38) or less than 20% seed set (P46). The variable results could be due to this pollen being close to losing its viability, which might be expected for pollen with 7.9% and 8.2% moisture at −18 °C (Hanna et al. 1986). Another possible explanation for the variable pollen viability results at −18 °C is that a no-frost freezer was used that has defrost (or heating) cycles. Further research is needed to determine the effects of frequent heating and cooling cycles on maintaining pollen viability. Although pollen with a wide range of moisture contents was not stored at 5 °C in this study, previous research (Hanna et al. 1986) indicates that low pollen moisture content during storage is even more critical at the 5 °C storage temperature than at the −18 °C storage temperature.

These data and previous research (Hanna et al. 1986) show that pollen viability is probably not affected by plastic zip-lock bag or glass vial storage containers at −73 °C. At −18 °C, glass vials appear to be more effective storage containers at longer (> 3 years) storage times (P29, P32, P35, P36, P38, and P39) than zip-lock plastic bags (P8, P30, and P40). Viability of treatments P39 and P40 is probably influenced by container type. Both treatments are inbred 23DB, both have similar moisture content, yet P39 (vial) maintained 100% viability and P40 (plastic bag) was nonviable. The zip-lock bags apparently allow moisture, oxygen, and/or carbon dioxide to diffuse through the bag or its zip-lock seal at −18 °C (possibly due to the defrost cycle) and 5 °C. Direct storage in glassine collection bags is effective for usually less than 1 year of storage (Hanna et al. 1986). Variable results were obtained with pollen stored in glassine bags (P43 to P46) at −18 °C. Pollen for these treatments was collected on different days and may have been affected by condi-

Table 1. Seed-set on cytoplasmic-nuclear male-sterile Tift 85DA pearl millet from pollen of various inbreds stored at various pollen moisture contents in different storage containers

Treatment no.	°C temp.	Inbred	Storage			% Seed set on Tift 85DA
			Moisture[a]	Container[b]	Storage time at last test (days)	
P27	−73	23DB	5.7	P	2,911[c]	100
P4		23DB	3.0	P	2,892[c]	100
P24		18DB	7.2	P	2,590[c]	100
P24		18DB	7.2	V	2,590[c]	100
P15		B39	7.0	P	2,590[c]	100
P28		23DBE	5.0	P	2,589[c]	100
P29	−18	23DB	ND	V	2,615[c]	100
P30		23DB	5.7	P	2,192	0
P8		23BE	6.2	P	2,234	0
P31		23BE	ND	V	1,840	1
P32		23DB	4.0	V	1,839[c]	100
P33		23DBE	4.4	V	1,519	0
P34		23BE	6.0	V	1,520	0
P35		23DB	7.9	V	1,496[c]	33
P36		23DB	5.0	V	1,486[c]	100
P37		186	12.5	V	1,486	0
P38		186	8.2	V	1,487[c]	33
P39		23DB	6.9	V	1,481[c]	100
P40		23DB	7.0	P	1,483	0
P41		186	6.2	V	1,120[c]	3
P42		23BE	6.9	V	790	0
P43		bmr	ND	GP	390[c]	100
P44		bmr	ND	GP	379	0
P45		bmr	ND	GP	375[c]	11
P46		81B	ND	GP	370[c]	41
P16	5	B39	8.0	P	2,590	0
P47		23DB	7.9	V	1,840	0
P48		23DB	6.8	V	1,839	0
LSD (0.05)						33

[a] Pollen moisture content on September 2, 1989. ND – not determined
[b] GP – Glassine bag placed inside zip-lock plastic bags; P – Zip-lock plastic bag; V – 4-ml glass vial
[c] Pollen continues to be viable

tions at collection but more probably by beginning and ending moisture contents, which is more difficult to control with glassine bags. It is important that the pollen and bag (which can hold substantial moisture) are thoroughly dried. Storage in glassine bags is faster and more convenient but takes more space and can be risky, due to the moisture absorbed by the bag.

Although more data are needed, there appears to be an inbred or genotype response for maintaining pollen storage viability at −18 °C. The treatments at −18 °C that set 100% seed (P29, P32, P36, and P36) after 4 or more years of storage were all inbred 23DB at 4.0%–6.9% (and one undetermined) pollen moisture and stored in glass vials. Pollen from other inbreds with a similar range of pollen moisture and stored in glass vials (P33 and P34) was not viable.

Viability of pearl millet pollen has been maintained for 7 (2,615 days) and almost 8 (2,911 days) years stored at −18 °C and −73 °C, respectively. Data shows that less than 7% pollen moisture content (Hanna et al. 1986) is still a safe level for pollen at −73 °C. Pearl millet pollen at 7% moisture can be stored for about a year at −18 °C and 5 °C (Hanna et al. 1986), but it appears that pollen moisture content will need to be lower at −18 °C than at −73 °C to maintain pollen viability for extended periods.

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