Plant Cell Reports (1986) 5:332–333

Plant Cell Reports

Short-term storage of cotton pollen*

B. Rodriguez-Garay and J. R. Barrow

New Mexico State University, USDA-ARS, Jornada Experimental Range, Las Cruces, NM 88003, USA

Received June 17, 1986 / Revised version received August 12, 1986 – Communicated by G. C. Phillips

ABSTRACT

Several methods of storing cotton pollen (Gossypium hirsutum L.) were evaluated. A successful pollen storage method that maintains fertility would enhance the crossing of breeding materials. Storing pollen at ultra-low temperatures in liquid nitrogen or at 5°C was not successful. No storage method maintained pollen fertility for more than 72 h. Cotton pollen did maintain adequate fertility up to 24 h at 10 and 15°C, at both low and high humidity when the pollen was stored in the detached flowers. Minimally acceptable pollen fertility was maintained in flowers stored at 15°C at 100% R.H. for 72 h. Use of these methods will allow for better utilization of parental plants when both parents do not flower on the same days.

INTRODUCTION

Pollen storage conditions that maintain fertility increases the efficiency of handling breeding and genetic materials of any plant species. Pollen fertility is affected by many factors. Singh et al. (1961) have shown humidity and light, in addition to temperature, affect pollen fertility in short-term storage. Pollen longevity during storage generally has been increased in several species, by decreasing temperature and humidity. Matthews and Kraus (1981) found that higher moisture and temperature levels reduced pollen quality by increasing metabolic rates and promoting microbial activity. Gramineae pollen, however, was shown by Visser (1955) to respond more favorably to higher humidities. Tomato, Lycopersicon esculentum L., asparagus, Asparagus officinalis L., sunflower, Helianthus annuus L., avacado, Persea americana, Mill., and pine Pinus sp. pollen remained fertile for periods ranging from 3 weeks to more than a year when stored near 0°C and with relative humidity (R.H.) less than 45% (McGuire 1952, Sedgley 1981, Frank et al. 1982 Singh et al 1961, Snope and Elison 1963 and Visser 1955). Pollen of some species was stored successfully for longer periods at ultra low temperatures in liquid nitrogen. Barnabas and Rajki (1976) showed that maize pollen fertility at ultra-low temperatures was influenced more by the water content of the pollen than by the duration of storage. By selecting a suitable water content, they were able to store pollen at -196°C for a year and maintain fertility. Sunflower pollen was used successfully for crossing after 4 years storage at temperatures ranging from -76 to -196°C.

Other methods have also been used in storing pollen. Mishra and Shivanna (1982) compared the efficacy of storing legume pollen at low temperature and humidity using organic solvents including diethylether, cyclohexane, amyl alcohol, and acetone. The maximum period for storing pollen in organic solvents alone was 6 days for Vicia sp. and 50 days for Pisum sp. Under controlled temperature and humidity conditions, fertility was maintained 25 days for Vicia and 75 days for Pisum. In general, pollen storage at low temperature and humidity was more effective than using organic solvents. However, for short-term storage, they recommended using organic solvents. Other compounds such as non-fat powdered milk, egg albumen, and lycopodium were shown by Bullock and Overley (1949) to be effective in maintaining pollen fertility of certain fruit trees.

Difficulties are experienced in making crosses of cotton, Gossypium hirsutum L. when the number of parental plants is small and the plants do not flower simultaneously. Generally, cotton pollen loses its fertility by late afternoon on the day of anthesis. To overcome this problem, it has been a common practice for cotton breeders or geneticists to store cotton flowers in either refrigerators or at room temperatures to maintain fertility for short periods (personal communication, E. L. Turcotte, research geneticist, USDA-ARS, Western Cotton Research Laboratory, 4135 E. Broadway Rd., Phoenix, Arizona 85040). We have been able to maintain pollen fertility in cotton for 48h in some cases by refrigerating the flowers after anthesis, while in others, refrigeration destroyed fertility (unpublished). In our experience, storage temperatures drastically affected cotton pollen fertility. In preliminary observations, we found complete sterility in cotton pollen stored 24h at 5°C and high fertility after 24h at 13°C. Temperatures above 20°C reduced pollen fertility after 24 h of storage. The purpose of this

* USDA-ARS, in cooperation with the New Mexico Agricultural Experimental Station, Journal Article No. 1161, Las Cruces, NM 88003, USA

Offprint requests to: J. R. Barrow

investigation was to evaluate storage conditions that would best extend cotton pollen fertility after anthesis, for better management of available parents in making essential pollinations.

MATERIALS AND METHODS

Cotton flowers used for these studies were obtained from a doubled haploid, nectarless breeding stock designated as DHNE. Both long- and short-term pollen storage conditions were evaluated. For long-term storage the pollen was collected at anthesis, immediately sealed in glass vials, and placed in liquid nitrogen (-196°C). Pretreatments included dehydrating pollen for 4 and 6 h over $CaCl_2$ in a dessicator at 15°C, prior to sealing and placing the vials in liquid nitrogen.

For short-term storage in a refrigerated atmosphere, pollen was kept intact in detached flowers rather than vials. Flowers were collected at anthesis and kept at the ambient R.H., which was less than 30% (L), or at 100% R.H. (H). Flowers were placed in 100 ml beakers in unlighted growth cabinets at specified temperatures of 10, 15, and 20°C, for periods of 24, 48, and 72 h at the ambient R.H. (L). Other flowers were exposed to 100% R.H. (H) by placing them in 100 ml beakers inside 200 ml beakers each with 25 ml of distilled water and sealed with petri dish covers. Flowers in these double beakers or humidity chambers were placed in growth cabinets at the same temperatures, time periods, and in the dark as listed above.

After treatment, the flowers were used to pollinate mature flowers that had been emasculated carefully the previous afternoon. Twenty-four h after pollination the styles were removed at the ovary and placed in Carnoy's solution and analyzed as described by Barrow (1983). Sections of the style adjacent to the ovary were examined microscopically to determine pollen tube penetration into the ovary. Controls were represented by pollinating stigmas with untreated, fully fertile pollen shortly after anthesis. The experimental design was a split-split plot with 4 replications. Temperatures represented the main plots and humidity and hours the sub and sub-sub plots, respectively. A mean of four or five stigmas represented each observation.

RESULTS AND DISCUSSION

Pollen fertility was reduced by 19 and 37% after dehydration pretreatments of 4 and 6 h, respectively. No pollen retained fertility after being stored in liquid nitrogen. Therefore, no long-term storage methods for cotton pollen were found.

Short-term storage methods maintained full fertility for 24 h, reduced fertility for 48 h and minimally acceptable fertility for 72 h (Table 1) with treatments of 10°C L, RH.

Pollen fertility was higher when stored at high humidity at 20°C than at low humidity at 20°C. No significant effects of humidity at 10 and 15°C were observed. Cotton pollen fertility appears to be more sensitive to temperature effects than humidity. In contrast to pollen of other species, that stores well at temperatures near 0°C, we found that cotton pollen does not survive 24 h at 5°C or lower. Storage conditions were similar for several breeding stocks and cultivars at Las Cruces, New Mexico. We have noted some genetic lines that tolerate higher temperatures and possibly might have a different storage temperature (unpublished cotton flowers should be stored at temperatures ranging from 10 to 15°C. Caution should be exercised not to store pollen in laboratory refrigerators, where temperatures drop below 10°C, or at room temperatures above 20°C since these temperatures would be expected to reduce fertility.

LIST OF TABLES

Table 1. Mean number of cotton pollen tube penetrations through the style at the ovary, 24, 48, and 72 h of storage after anthesis at 10, 15, and 20°C at ambient 30% (L) and 100% (H) R.H., compared to untreated fresh pollen (control).

Treatment	24 h	48 h	72 h
Control	82.8 A**	82.8 A	82.8 A
10L	60.4 B	16.3 BC	5.1 B
10H	58.7 B	6.8 CD	1.2 B
15L	61.1 B	9.9 BCD	0.6 B
15H	61.6 B	18.1 B	0.7 B
20L	7.6 D	0.0 D	0.0 B
20H	36.3 C	0.0 D	0.0 B

**Means followed by different letters in the same column are significantly different at the 0.01 probability level.

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