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## Viability of Celery Pollen After Collection and Storage

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*Additional index words.* *Apium graveolens*, hybridization, fertilization

**Abstract.** A technique for collection and storage of viable celery (*Apium graveolens* L.) pollen is reported for the cv. Tall Utah 52-70R and the annual strain A143. Umbels at anthesis were dried in an incubator for 14 hr at 31°C, crushed by hand, and passed through a sieve. The pollen released by this operation was collected on a sheet of paper and stored in gelatin capsules. High drying temperatures were detrimental to pollen viability. Pollen collection at different times of the day did not affect viability. After 6 days at 24°, a significant percentage of pollen grains was still viable. The viability declined close to 0% in 9 to 12 days. Pollen of A143 survived better than that of 'T.U. 52-70R'. Pollen stored for 9 months at 4° maintained a viability of 45% to 50%, but declined close to 0% by the 18th month. Pollen stored at -10° maintained a viability of 10% to 30% by the 18th month. Storage of celery pollen at -10° for 6 to 9 months will keep enough viability for pollinations. Use of stored viable pollen will help with the crossing of celery, as it is often difficult to synchronize the flowering of different plants.

Celery requires vernalization to flower. While a cold period usually enables celery to enter the reproductive phase, cultivars differ in their low temperatures requirements (4). Some will flower shortly after vernalization, while others might take more than 6 months (1, 2), making it difficult to coordinate crossings of cultivars that flower at different times. In potato and in several other crops, viable pollen of early flowering plants is stored for later use (5). A series of experiments was initiated to determine the longevity of celery pollen under storage. Two celery phenotypes were used, the annual strain A143 (PI 1257228) and the cultivar Tall-Utah 52-70R ('T.U. 52-70R'). Plants were grown in a greenhouse at 24°C during the day (14 hr) and 18° during the night. The pollen collection was as follows: umbels were sampled 3 days after the outer whorl of florets on the umbels had opened and anthers were visibly shedding pollen. Three umbels were collected from three plants of each phenotype. The umbels were separated in umbels, placed in petri dishes, and dried in an incubator. The dried umbels were placed on a USA standard testing sieve No. 100 (opening 150 mm). They were then crushed by hand and the pollen collected on a sheet of paper. About 1 to 10 mg of pollen was introduced into gelatin capsules for storage

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at various temperatures.

Pollen viability was tested by fluorochromacia following the technique reported by Heslop-Harrison (3). For pollen viability counts (PVCs), pollen grains were dusted on a drop of 10<sup>-6</sup> M fluorescein diacetate in 0.5 M sucrose on a microscope slide. Three slides were prepared for each pollen sample. For each slide, five counts were obtained from five different fields of vision using a ×10 objective mounted on a universal Zeiss microscope equipped with epifluorescence. The percentage of fluorescent (viable) pollen grains obtained was transformed to arcsin values and subjected to analysis of variance.

*Optimum temperature for pollen drying.* Umbels from A143 and 'T.U. 52-70R' were dried at 31°, 33°, or 38°C for 14 hr in an incubator. No significant loss of pollen viability was observed for either of the two varieties at 31° (Table 1). Subjecting the pollen to 38° was detrimental to the pollen of both phenotypes, while 33° was detrimental only to the pollen of A143. Thus, the remaining experiments described hereafter were done with pollen dried at 31°.

The percentage of viable pollen taken directly from umbels before drying (fresh pollen) tended to be higher in A143 (40% to 82%) than in 'T.U. 52-70R' (40% to 59%). This difference probably is due to the presence of older pollen in the anthers of 'T.U.

Table 1. Percent viability of fresh pollen and of pollen obtained from umbels dried for 14 hr at three temperatures for two celery phenotypes.

Phenotype	Viability (%)			
	Fresh	31°C	33°C	38°C
A143	82.6	82.0	61.1	36.9
T.U. 52-70R	54.3	41.7	41.6	33.7

LSD 0.05 = 14.7.

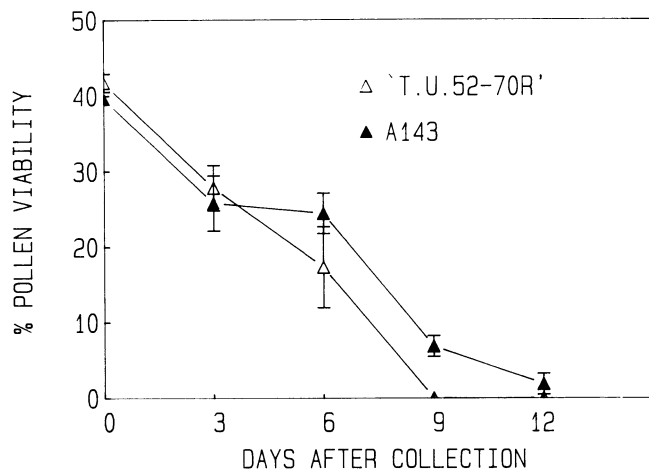


Fig. 1. Viability of pollen stored at 24°C for two celery phenotypes. Error bars indicate  $\pm$  SE.

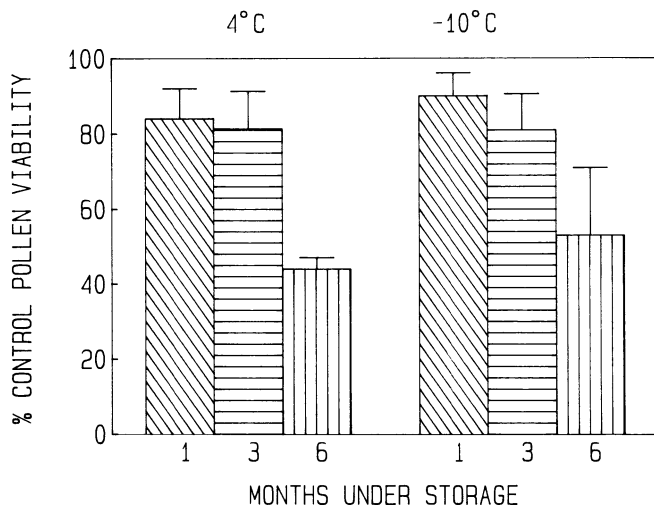


Fig. 2. Viability of celery pollen accession A143 stored at two temperatures for 6 months, expressed as percentage of control (100% control = 60% actual viability). Error bars indicate  $\pm$  SE.

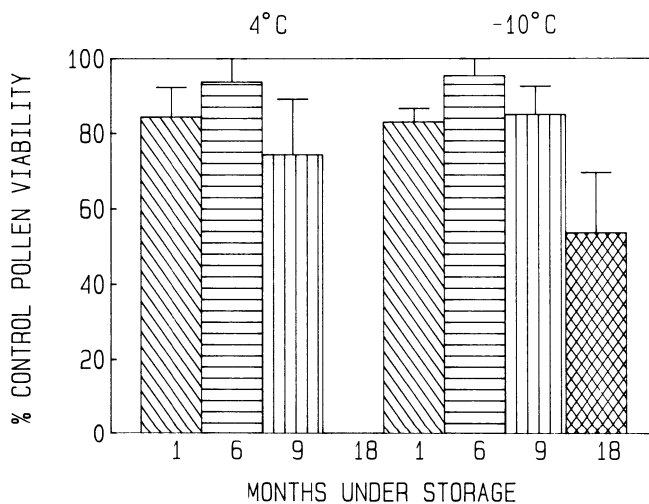


Fig. 3. Viability of celery pollen accession A143 stored at two temperatures for 18 months expressed as percentage of the control. (100% control = 55.5% actual viability). Error bars indicate  $\pm$  SE.

52-70R', which might have dehisced a few days prior to the collection.

*Time of the day and pollen viability.* Um-

bels collected at 8:00 AM, 12:00 PM, and 4:00 PM from plants of 'T.U. 52-70R' gave PVCs of 53.8%, 49.2%, and 55.9%, re-

spectively. These differences were not statistically significant ( $F = 1.23$ ; 2 and 6 df), suggesting that time of collection during the day does not affect pollen viability.

*Pollen viability declines at room temperature.* This experiment was designed to determine the longevity of celery pollen at room temperature (24°C). Pollen was stored in gelatin capsules and sampled for PVCs at 0, 3, 6, 9, and 12 days. Viability declined steadily during this period (Fig. 1). A significant percentage of pollen grains was still viable in both phenotypes after 6 days at room temperature, 17.3% for 'T.U. 52-70R', and 24.4% for A143. By the 9th day, A143 still had 7% viable pollen, while in 'T.U. 52-70R' the viability was completely lost. Thus, celery pollen can be stored for a few days at 24°. The results suggest that pollen of A143 has a better survival than that of 'T.U. 52-70R'.

*Long-term storage of celery pollen.* To monitor the decline of pollen viability for A143 in storage, pollen bulked from three plants was placed into 18 gelatin capsules. One-half of the capsules was stored at 4°C and the other half to -10°. Three capsules per storage regime were sampled for PVCs after 1, 3, and 6 months under storage. After the counts, the capsules were discarded. Fig. 2 shows the steady decline in pollen viability through time, expressed as percentage of the control (viability of pollen immediately after drying). After 6 months, about half of the viability was still present in the pollen stored at either temperature. Since the viability percentage of the pollen at collection approximated 60%, the actual pollen viability after 6 months in storage was around 30%.

To determine the decline in pollen viability during prolonged storage, another experiment was performed, including both 'T.U. 52-70R' and A143. Samples for PVCs were taken after 1, 6, 9, and 18 months in storage at 4° and -10°C. The decline in pollen viability, expressed as percentage of the control (Figs. 3 and 4), followed a trend similar to that observed for the first experiment (Fig. 2). Pollen of the cultivar T.U. 52-70R lost viability more rapidly than that of A143 after 1 month in storage at either temperature. No significant differences between storage regimes were observed for up to 6 months for both varieties. After 18 months at 4° in storage, no viable pollen was found for either of the two phenotypes. At -10°, however, 5.5% of 'T.U. 52-70R' pollen and 30% of A143 pollen was still viable (Figs. 3 and 4).

Pollinations with stored pollen resulted in viable seed, making possible for us to use this technique routinely in our breeding program. Although celery pollen under storage maintains a useful level of viability for at least 18 months at -10°C, it varies among cultivars. Reducing the storage period to 6 to 9 months at -10° will assure a high level of viability reducing the risk of differential pollen survival favoring the selection of certain genotypes. The use of stored viable pollen will help the breeder with the crossing of celery, as it is difficult to synchronize the flowering of different plants.

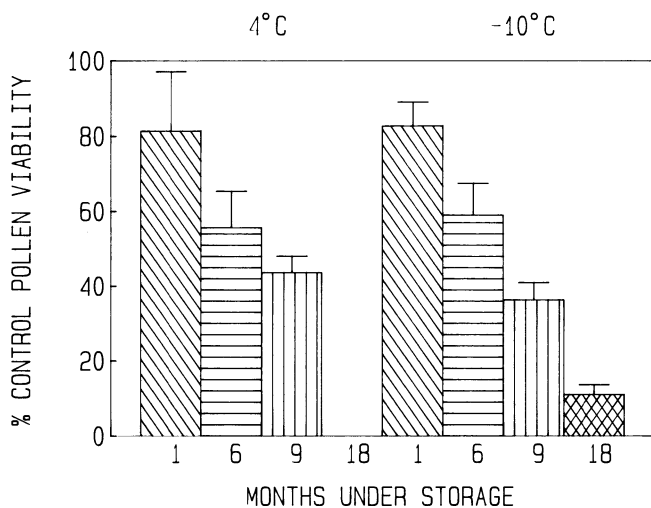


Fig. 4. Viability of celery pollen cv. T.U. 52-70R stored at two temperatures for 18 months expressed as percentage of the control (100% control = 50.9% actual viability). Error bars indicate  $\pm$  SE.

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## Inheritance of Low Temperature Tolerance in Beans at Several Growth Stages

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Additional index words. *Phaseolus vulgaris*, genetics, cold tolerance

**Abstract.** Narrow sense heritabilities were 28%, 56%, 45%, and 74%, respectively, imbibition at 5°C and, at 16°, for seedling vigor, plant vigor, and days to bloom in a cross of NY 590 x BBL 92. Cold tolerance at these stages was inherited independently. Pod set at 16° behaved as a recessive, compared to only setting at warmer temperatures. Selections made under 16° generally did very well in an unusually cool season in New York. Double setting was absent in lines that showed set at 16°, and present in many cultivars.

Beans (*Phaseolus vulgaris* L.) are generally susceptible to low temperature injury at all stages of growth. Temperatures of 10°C or below during imbibition and germination may result in permanent injury and vigor reduction, and prolonged temperatures at or below 15° to 16° can result in stunted plants with no crop.

'Comtesse de Chambord' and 'Widusa' germinate well at 9° to 9.5°C on a germination board, but both lacked vigor at low (10°) growing temperatures (7). Likewise, cultivars such as 'BBL 92' (2) will germinate at low temperatures (8° to 9°) on a germination blotter, but, in soil or greenhouse mix, emerge slowly and have very poor vigor un-

der continued low temperature conditions. Kemp (6) identified bean lines with good leaf growth at 10° (6). NY 5-161 and 'NY 590' (2) germinate at 9.5° to 10° and have good plant vigor at low temperatures.

Although several selection procedures for cold tolerance have been developed (1, 5), they are plant-destructive or not adapted to selection among large numbers of plants. Dickson (2) found that germination of beans at 5°C for 5 days, followed by growth of beans at 16° correlated well with field performance under cool conditions. Farlow et al. (4) observed that low day temperatures reduced seed number per pod due to slow pollen growth. Dickson et al. (3) showed that low night temperatures had less effect on pod set than did low day temperatures.

This paper reports on the use of several screening procedures for evaluating the inheritance of low temperature tolerance in beans at imbibition, seedling and mature vegetative plant growth stages, days to bloom, and at pod set.

Lines NY 590, NY 23, and BBL 92 were used to create two families to study the inheritance of low temperature tolerance during germination, days to bloom, seedling vigor, vegetative vigor, and pod set. Both NY 590 and NY 23 exhibit good low temperature germination (emergence), bloom in 61 days at 16°C and set moderately well under cool nights, although line NY 590 has better seedling vigor than NY 23. Under low temperatures, BBL 92 germinates poorly, exhibits poor seedling vigor, takes 73 days to bloom at 16°, and has poor pod set.

Backcross and F<sub>2</sub> seed were developed from crosses of NY 590 x BBL 92 and NY 23 x BBL 92. The F<sub>2</sub> seed and BC seed were planted in the field and seed harvested on an individual plant basis. The F<sub>3</sub> and BC F<sub>2</sub> lines from individual BC and F<sub>2</sub> plants were planted in late November in 15-cm pots in Cornell mix prewetted and cooled to 5°C in a growth chamber. After 5 days, the pots were moved to a greenhouse at 16° where, from December to March in Geneva, N.Y., it was possible to keep the temperature of the greenhouse at 16° with about  $\pm$  1° fluctuation. Good air turbulence resulted in uniform temperatures throughout the greenhouse.

Three pots, each with five seeds from each individual F<sub>2</sub> and BC plant, were planted. The mean performance of the beans in the three pots was used as the value for each F<sub>2</sub> or BC<sub>1</sub> plant. Line means were analyzed rather than the performance of the individual F<sub>2</sub> seed, since the performance of a single seed is unsatisfactory for evaluating factors related to germination. Seedling emergence and vigor were recorded 3 to 4 weeks after planting, and each pot then was thinned to the two most vigorous seedlings. Seedling vigor was rated on a 1 to 5 scale to represent the minimum to maximum expression of the character. Plant vigor was recorded at 60 days after planting when the earliest plants were starting to bloom. The plant height at 60 days was measured; plants <15 cm were rated 1,

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