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Pollen Staining and High-temperature Tolerance of Bean

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Abstract. **Viability of pollen grains of isogenic sibling bean (*Phaseolus vulgaris* L.) selections of known tolerance of sensitivity to high temperatures (HT), as previously determined by pod retention and seed yield, was compared to that of a common parent bean selection and a cowpea [*Vigna unguiculata* (L.) Walp.] cultivar. Exposure of newly opened flowers to temperatures of 35° or 41°C reduced the viability of pollen grains in all bean selections. Pollen of all sibling selections was less affected by HT than pollen of their common parent suggesting transgressive segregation of factors for HT tolerance. At 41°, most pollen grains were destroyed in the parent bean selection and the 2 HT-sensitive siblings, whereas 44% to 55% of the pollen grains appeared to be viable in the 2 HT-tolerant siblings. Pollen viability of the HT-tolerant cowpea cultivar was not reduced by temperatures to 41°. Pollen staining indicated an interrelationship between pollen viability and tolerance to HT stress among the bean selections. The technique described has the potential for rapid selection of HT-tolerant genotypes in hybrid populations.**

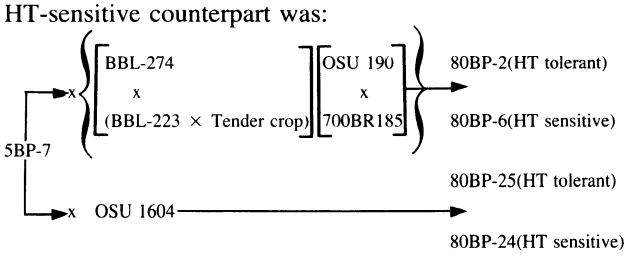
Temperature plays a major role in the growth and productivity of crop plants. Most species show genotypic differences to environmental stresses, and adaptations often are seen in flower development, fruit set, or yield (2, 3, 5). These differences provide incentives for plant breeders to seek available sources of stress tolerance for incorporation into breeding lines. A successful breeding program will depend on the development of rapid and accurate methods of identifying genotypes exhibiting stress tolerance.

Legumes express a wide range of response to high temperature (HT) and water stress. High day, high and low night temperature, and low available soil moisture can affect adversely flower set and pod retention of legumes (3, 5, 13). High temperature is the most critical factor in reduced pod retention on beans (3). Reproductive structures of plants are highly responsive to high temperature (2, 4), and genetic differences in tolerance to temperature have been demonstrated previously (6, 12).

Pollen viability, flower set, and fruit retention are interdependent. Overall assessment of HT tolerance in beans would, therefore, require further information of mutual effects on pollen viability. This study was conducted to examine the use of staining techniques to determine pollen viability and to compare respective differences in pollen viability found in HT-tolerant and HT-sensitive bean selections and a cowpea cultivar.

Materials and Methods

Plant selection. Plant selection was based on prior establishment of traits of HT tolerance and sensitivity found for pod set and seed yield (11). An HT-tolerant snapbean selection, 5BP-7, and 2 pairs of isogenic sibling selections chosen from crosses developed at the Irrigation Experiment Station, Prosser, Wash. (11), using 5BP-7 as a common parent, were used for study. Derivation of the 2 pairs of lines, each with a HT-tolerant and HT-sensitive counterpart was:



A cowpea [*Vigna unguiculata* (L) Walp.] 'California Black Eye #5' (Northrup-King Seed Company) was included as a reference crop, since cowpeas demonstrate a high tolerance to HT relative to common bean (1).

Plant culture. Plants were grown in commercial potting soil (Rod McLellan Co., South San Francisco, CA 94080), in 20-cm clay pots and positioned in 2 environmentally-controlled chambers. Air temperature in one chamber (control) was maintained continuously at 27° ± 2°C during the light period for the duration of the study. In the other chamber (HT stress), air temperature during the light period was maintained at 27° ± 2° until the first flowers opened, at which time it was varied between 27° and 41° ± 2°. In both chambers, aerial temperature during the dark period and soil temperature during both light and dark periods, were maintained at 21° ± 2°. A 14 hr/10 hr (light/dark) regime was used in both chambers. Light intensity at foliage level was about 350 μmol s^{-1} m^{-2}. Relative humidity was 75% to 80% during the dark period and 50% to 55% during the light period. Similar replicated studies were conducted during the winters of 1981 and 1982.

Conditioning temperature treatments. After the first few flowers had opened fully, the air temperature of the stress chamber during the light period was increased to 35°C for 2 days, returned to 27° for 4 days, increased to 41° for 2 days, and lowered to 27° for 4 days. In addition to evaluating the influence of temperature on pollen viability, sequential heating identified the stage of flower development when pollen grains appeared to be most sensitive to heat.

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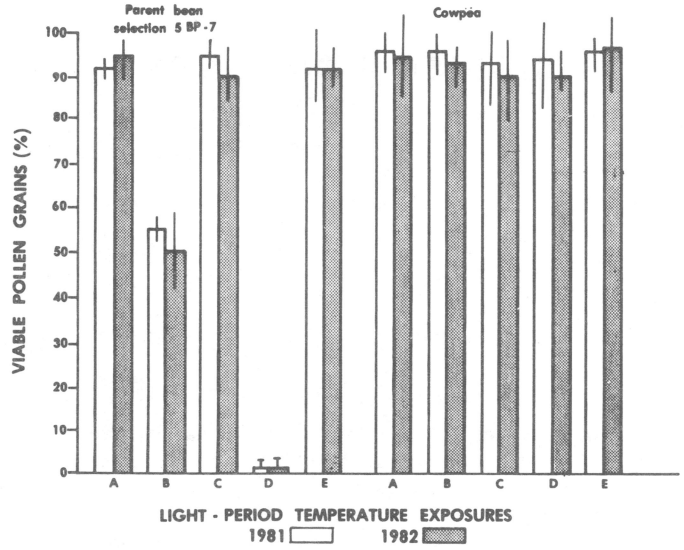

Fig. 1. Pollen viability of common bean vs. cowpea after exposure to 35° and 41°C. Plant root and dark-period air temperatures were maintained at 21° ± 2°. A 14 hr/10 hr (light/dark) photoperiod was used. Pollen viability was determined by phyloxin-methyl green staining (10) immediately after the last day of each light-period temperature exposure. Light-period, air-temperature exposures: **A** = 27° ± 2° (continuous); **B** = 2 days at 35° ± 2°; **C** = 4 days at 27° ± 2° after 2 days at 35° ± 2° exposure; **D** = 2 days at 41° ± 2°; and **E** = 4 days at 27° ± 2° after 2 days at 41° ± 2° exposure. Vertical bars represent ± one SD.

Pollen viability test. Although the staining technique used in this study (10) was developed to distinguish viable from nonviable pollen grains, it does not evaluate actual pollen germination or pollen tube growth. Hence, it should be considered as a measure of apparent viability only.

Just prior to imposing altered temperature regimes to simulate HT stress, pollen collected from newly opened flowers in both chambers was tested for viability by microscopic straining (10). Pollen grains were shaken onto a 25 × 75 mm glass slide, a drop of methyl green-phyloxin stain was added and the grains were covered with a 22 mm cover glass. Methyl green stains the cellulose of cell walls a bluish-green color. Phyloxin preferentially stains cytoplasm a deep-red color. Aborted and severely damaged pollen grains stained bluish-green, although some showed residual traces of red-stained cytoplasm. In contrast, pollen grains considered viable were swollen, unbroken, and stained red with a bluish-green cell wall. Viable pollen grains were determined microscopically at x125. A minimum of 500 pollen grains was counted from each newly opened flower taken from plants given different temperature treatments: a) immediately after the 2nd light period at 35°C; b) after the 4th light period at 27° following the 35° treatment; c) after the 2nd light period at 41°; and d) after the 4th light period at 27° following the 41° treatment. Pollen viability of flower buds at the white-petal stage (unopened) was determined also for each temperature treatment. Equal numbers of newly opened flowers and flowers at the white-petal stage from unstressed plants in the control chamber were collected and evaluated for pollen viability at the same time.

Results and Discussion

Pollen viability in newly opened flowers of the HT-sensitive parent bean selection, 5BP-7, was reduced nearly 50% at 35°C and 100% at 41° (Fig. 1). In contrast, none of the pollen of the HT-tolerant cowpea appeared to be affected adversely by HT exposure of flowers.

Based on pollen staining, all isogenic sibling lines were more tolerant of 35°C (Fig. 2) than their parent, 5BP-7 (Fig. 1). After 2 days at 35° (Fig. 2), about 30% to 50% more pollen grains were viable in flowers of HT-sensitive siblings, 80BP-6 and 80BP-24, than in flowers of 5BP-7 (Fig. 1). Pollen viability of the more HT-tolerant sibling lines, 80BP-2 and 80BP-25, however, was reduced by less than 10% after 2 days at 35°. After 35° exposure (Fig. 2), 80BP-2 had 35% and 43% more viable pollen in 1981 and 1982, respectively, than its parent 5BP-7 (Fig. 1), and 20% and 14% more viable pollen than the more HT-sensitive sibling line, 80BP-6 (Fig. 2), in 1981 and 1982, respectively. Line 80BP-25 (Fig. 2), had 30% and 38% more viable pollen after exposure to 35° than its parent, 5BP-7 (Fig. 1), in 1981 and 1982, respectively, and 5% and 9% more than its HT-sensitive sister, 80BP-24 (Fig. 2), in 1981 and 1982, respectively. Pollen viability in other bean cultivars also was reduced by exposure to 35° (6).

The improved tolerance to HT of the progeny, above that of their parent, 5BP-7, may have resulted from transgressive segregation of HT factors from both parents. The 80BP-2 and -6 sibling lines were derived from a cross of 5BP-7 and other bean breeding lines developed at Prosser, Wash., where day and night temperatures during the summer bloom period are typically high. The 80BP-24 and-25 sibling lines were derived from crosses of 5BP-7 and OSU lines developed at Corvallis, Ore., where bloom-period temperatures generally are cooler and days are more overcast than at Prosser. The differences in response of progeny to HT suggested the possibility that yield selection under arid and HT conditions at Prosser favored the accumulation of genetic factors for HT tolerance.

At 41°C, genotypic differences in tolerance of pollen grains to HT were pronounced (Fig. 3). Most pollen grains from flowers of the 2 HT-sensitive sibling lines, 80BP-6 and 80BP-24,

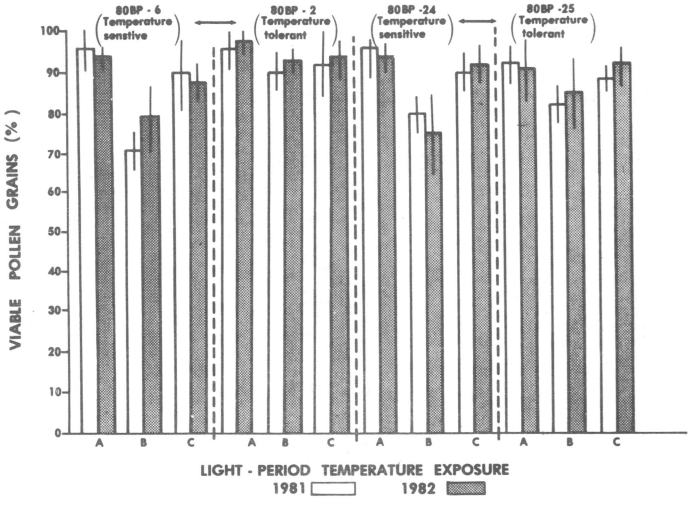

Fig. 2. Pollen viability of paired bean sibling selections after exposure to 35°C. Plant root and dark-period air temperatures were maintained at 21° ± 2°. A 14 hr/10 hr (light/dark) photoperiod was used. Pollen viability was determined by phyloxin-methyl green staining (10) immediately after the last day of each light-period temperature exposure. Light-period, air-temperature exposures: **A** = 27° ± 2° (continuous); **B** = 2 days at 35° ± 2°; and **C** = 4 days at 27° ± 2° after 2 days at 35° ± 2° treatment. Vertical bars represent ± one SD.

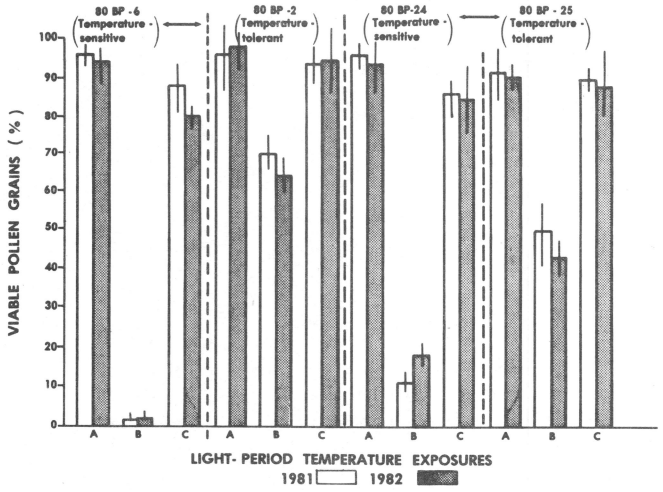

Fig. 3. Pollen viability of paired bean sibling selections after exposure to 41°C. Plant root and dark-period air temperatures were maintained at 21° ± 2°. A 14 hr/10 hr (light/dark) photoperiod was used. Pollen viability was determined by phyloxin-methyl green staining (10) immediately after the last day of each light-period temperature exposure. Light-period, air-temperature exposures: **A** = 27° ± 2° (continuous); **B** = 2 days at 41° ± 2°; and **C** = 4 days at 27° ± 2° after 2 days at 41° ± 2° treatment. Vertical bars represent ± one SD.

had aborted after 2 light-period exposures to 41°. Anthers of some flowers were devoid almost completely of pollen grains. Reduced pollen production also has been reported in flowers of tomatoes (4) and on tassels of corn (7) exposed to HT. Our results differ from those of Halterlein et al. (6), however, who found that more bean pollen was produced at 35° than at 25°.

The fact that pollen viability of the 2 HT-tolerant sibling lines, 80BP-2 and 80BP-25, at 41°C, was reduced only to about 70% and 57%, respectively, may be significant, since Johri and Vasil (9) reported that normal fruit set in plants can occur when less than 50% of the pollen grains are viable.

Pollen from flowers of 5BP-7, which opened 4 days after each HT exposure, appeared not to be affected adversely by HT (Fig. 1). Sensitivity of bean pollen to HT appeared to be greatest prior to, or as flowers were first opening. Iwahori (8) showed that pollen sensitivity to HT in tomatoes was greatest 5 to 9 days prior to anthesis. Pollen grains from bean flowers at the early white-petal stage (unopened) were not adversely affected by HT, since nearly 90% of the grains appeared to be viable. Resistance of immature pollen grains to HT damage would provide an escape mechanism by which high fruit or seed yields are maintained, even when short periods of HT may prevail daily under field conditions.

This study demonstrated that a significant difference existed between HT-sensitive and HT-tolerant beans in the percentage of mature pollen grains that survived HT exposure when measured by cytoplasmic staining. Although cytoplasmic staining readily distinguishes badly damaged pollen grains, it does not indicate whether pollen will germinate and pollen tubes grow. The fact that apparent viable pollen grains were significantly more abundant in flowers of HT-tolerant plants in comparison to those of HT sensitive plants suggested that HT tolerance in beans may be at least partially based on maintaining viable pollen grains. Apparent insensitivity of pollen grains of the HT-tolerant cowpea to heat damage up to 41°C also would suggest the use of staining of pollen grains of flowers exposed to HT as a means of screening to find HT-tolerant plants.

Literature Cited

1. Aykroyd, W.R. and J. Doughty. 1964. Legumes in human nutrition. Food and Agr. Org. of the U.N. Rome, Italy.
2. Chang, W.N. and B.E. Struckmeyer. 1976. Influence of temperature, time of day, and flower age on pollen germination, stigma receptivity, pollen tube growth, and fruit set of *Allium cepa* L. J. Amer. Soc. Hort. Sci. 101(1):81–83.
3. Davis, J.F. 1945. The effect of some environmental factors on the set of pods and yield of white pea beans. J. Agr. Res. 70:237–249.
4. El Ahmadi, A.B. and M.A. Stevens. 1979. Reproductive responses of heat-tolerant tomatoes to high temperatures. J. Amer. Soc. Hort. Sci. 104(5):686–691.
5. Fisher, V.J. and C.K. Weaver. 1974. Flowering, pod set, and pot retention of lima bean in response to night temperature, humidity, and soil moisture. J. Amer. Soc. Hort. Sci. 99(5):448–450.
6. Halterlein, A.J., C.D. Clayberg, and I.D. Teare. 1980. Influence of high temperature on pollen grain viability and pollen tube growth in the styles of *Phaseolus vulgaris* L. J. Amer. Soc. Hort. Sci. 105(1):12–14.
7. Herrero, M.P. and R.R. Johnson. 1980. High temperature stress and pollen viability of maize. Crop Sci. 20:796–800.
8. Iwahori, S. 1965. High temperature injuries in tomato. IV Development of normal flower buds and morphological abnormalities of flowers treated with high temperature. J. Japan Soc. Hort Sci. 34:33–41.
9. Johri, B.M. and I.K. Vasil. 1961. Physiology of Pollen. Bot. Rev. 27:325–381.
10. Owczarzak, A. 1952. A rapid method for mounting pollen grains. Stain Technology 27:249–251.
11. Silbernagel, M.J. 1979. Release of multiple disease resistant germplasm. Ann. Rpt. Bean Impr. Coop. 22:37–41.
12. Smith, F.L. and R.H. Pryor. 1962. Effects of maximum temperature and age on flowering and seed set in three bean varieties. Hilgardia 33:669–688.
13. Stobbe, E.H., D.P. Ormrod, and C.J. Woolley. 1966. Blossoming and fruit set patterns in *Phaseolus vulgaris* L. as influenced by temperature. Can. J. Bot. 44:813–819.