Published September, 1988

POLLEN SELECTION FOR HEAT TOLERANCE IN COTTON

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Abstract

Pollen from cotton (*Gossypium hirsutum* L.) cultivars, which demonstrate heat tolerance in the field, generally express higher fertility after heat treatment than pollen from heat sensitive cultivars. The objective of this study was to determine, if pollen could be heat treated to eliminate all but those grains with genetic heat tolerance. Genes for heat tolerance could then be selected in the pollen and effectively transferred by the backcross method. A highly heat tolerant breeding line, 7456, of *G. barbadense* L. was used as the donor parent and 'Paymaster 404', a heat sensitive cultivar, as the recurrent parent and all crosses were made with pollen treated 15 h at 35 °C to generate F_1, F_2 and first and second backcross populations. Increased heat tolerance, as measured by fertile pollen after heat treatment, was observed in plants from all populations. Similar heat tolerance patterns were observed in the parents, F_1, F_2, and first backcross populations, when plants were grown to maturity at high temperatures in a growth chamber. Pollen selection through heat treatment allows screening for large numbers of genetic combinations in pollen and may be a valuable method of breeding for heat tolerance and possibly other stress tolerance characteristics.

CONVENTIONAL methods of selecting for desirable traits in crop plants are time proven, effective, and responsible for valuable improvements in essentially every useful trait. However, these methods are often expensive in terms of time, labor and space.

Plant cell and tissue cultures offer novel approaches to screening large numbers of individuals (cells) and can increase selection efficiency for some traits under laboratory conditions (4). Cell selection can be utilized only if desirable genes are identified and isolated in selected cells, which can be regenerated into plants. Pollen selection is an alternative method of selecting for specific traits. It could be used to screen large numbers of haploid cells effectively and efficiently. This method utilizes natural plant systems and eliminates the need to regenerate plants, which would require expensive laboratory equipment. Selected pollen grains will retain viability, germinability, and fertility as they are exposed to environmental stresses.

If genes expressing stress tolerance in the pollen phase were also expressed in the sporophyte, then by pollen selection, it should be possible to transfer these traits into useful cultivars. Such a technique would be useful in transferring heat and/or cold tolerance into parental lines for hybrid development.

Significant research findings support this possibility. Postmeiotic genetic activity was found in the gametophyte when analyzing single pollen grains of several *Cucurbita* species (7,8) by microelectrophoresis. Evidence for genetic control of tolerance to temperature stress was given by Tanksley et al. (11) as they analyzed isozymes of a tomato cultivar, *Lycopersicon esculentum* L., and determined some genes were expressed in both sporophytic and gametophytic phases. They found that 18 of 30 isozymes, isolated in the sporophyte, also were expressed in the gametophyte, and 18 of 19 pollen isozymes were found in one or more stages of sporophytic development. In mouse ear cress (*Arabidopsis thaliana* L.), two recessive embryo-lethal mutants were expressed in the male gametophyte (5).

Zamir et al. (12) demonstrated that pollen from a wild, cold-tolerant species of tomato, *L. hirsutum* L., had higher germination and fertility, and was selectively more functional at low temperatures than pollen from a commercial cultivar. Zamir et al. (13) subsequently transferred genetic cold tolerance expressed in the gametophyte of the wild species to the progeny of the above cross.

To begin this study we evaluated pollen fertility of a wide range of cotton (*Gossypium spp.*) by heat treating their pollen and found that cultivars developed in warmer areas were more fertile than those developed in cooler areas. This indicated that genes allowing the sporophyte to function at high temperatures also allowed the pollen to retain fertility after heat stress.

The transfer of genetic tolerance to high or low temperatures would appear to be the simplest test to evaluate a model of pollen selection. The objective of this study was to determine the potential of transferring genetically controlled heat tolerance by pollen selection into a heat sensitive cotton breeding line.

Materials and Methods

A backcross method was used to test pollen selection for transferring heat tolerance. The donor parent (7456), *G. barbadense* L., was selected from several heat tolerant selections because its pollen remained fertile after heat treatment at 35 °C, and on its performance in subsequent crosses (3,9). This genotype was selected by Dr. Dick Davis, Dep. of Agronomy and Horticulture, New Mexico State Univ., from parental material he originally obtained from Dr. Carl Feaster, Phoenix, AZ. Dr. Feaster reported that the original material expressed high heat tolerance in the field in central Arizona (C.V. Feaster, 1982, personal communication). The recurrent parent, Paymaster 404, a heat-sensitive commercial cultivar adapted to the cooler Texas High Plains, was obtained from Dr. Delbert Hess, currently with Cargill Inc., Minneapolis, MN.

Pollen with assumed genetic heat tolerance was selected by placing flowers, the day before anthesis, in a growth chamber at 100% relative humidity and 35 °C for 15 h. (2). We assumed that pollen with the highest tolerance to heat survived. Flowers emasculated the previous afternoon, were pollinated with treated pollen. The F_1 population, an interspecific cross, was generated by crossing the recurrent *G. hirsutum* parent with treated pollen of 7456, the *G. barbadense* line. The F_1 plants were selfed to give the F_2 generation. Heat treated pollen from the F_1 and first BC populations were used to backcross to the recurrent parent for the first BC and second BC populations, respectively.

Heat treated pollen from the parents, F_1, F_2, first backcross, and second backcross populations, was used to pollinate emasculated flowers of a tester stock, DHNE, a dou-

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Published in Crop Sci. 28:857–859 (1988).

bled haploid breeding line, to measure heat tolerance in each generation. The styles of doubled haploid nectariless (DHNE) were excised 24 h after pollination and analyzed for the number of pollen tube penetrations, using a technique described by Barrow (2). The individual plant value was the mean number of pollen tube penetrations of five pollinated flowers from each plant.

An F_2 population of 200 plants was grown in plastic pots, 18 cm high, 17 cm diam., and tapering to 11 cm at the bottom, in a medium of 1:1 mixture of sandy loam soil and peat moss. Each pot was fertilized every 30 to 40 d with 1.0 g iron, and 1.3 mL nitrogen zinc solution (NZN), (Agricultural Products Co., AGCO, Artesia, NM) and 1.0 g KH_2PO_4. The heat tolerance value of a plant was determined by counting the pollen tube penetrations in five DHNE styles after being pollinated with heat treated flowers selected at random from the F_2 plant. A broad sense heritability was calculated using the method of Allard (1). Heat tolerance of all populations was compared with the *t*-test described by Steele and Torrie (10).

Heat tolerance was determined in the sporophyte phase of parents and progeny by transferring 10 plants prior to floral bud initiation, of each genotype, into a plant growth chamber at a temperature regime of 37 °C (light) and 27 °C (dark) in a 12 h photoperiod. Heat tolerance was judged on the ability of the genotypes to produce flowers, fertile pollen, and bolls in this environment.

Results and Discussion

Data concerning heat tolerance as measured by mean pollen tube penetrations per style of DHNE is listed in Table 1 for the parents, F_1, F_2, first BC, and second BC populations. Parents and progeny, pollinated with untreated pollen were observed to be fully fertile, with no differences in the mean numbers of pollen tube penetrations. The heat sensitivity of Paymaster 404 was indicated by low pollen fertility, 4.3 holes per stigma. Of the 38 plants tested, only a single style expressed some fertility and was likely a result of pollen escaping treatment or pollen contamination. The donor parent expressed high heat tolerance with 59.3 tube penetrations per style. The F_1 and first backcross populations expressed high levels of heat tolerance with 45.4/30.8 holes per style, respectively. Data in Table 1 suggests a trend showing the reduction of fertility from the 7456 parent (59.3) to the F_1, (45.4) and the first BC (30.8) and second BC (30.3) populations. This reduction may be due to heterozygosity or that the gene(s) for heat tolerance show a greater expression in the *G. barbadense* background than in *G. hirsutum*. There was no reduction of fertility in the second backcross population from the first backcross and possibly the level of fertility would be stable in subsequent backcrosses. The second backcross population retains full fertility with 30.3 penetrations per style. Field testing at high temperatures is necessary to determine the ultimate level of heat tolerance transferred by the pollen selection method.

Table 1. Student *t*-test comparison among the number of pollen tubes of parents, F_1, and BC populations growing in DHNE stylar tissue.

Genotype	Plants	Pollen tubes/style	Variance
	no.		
	Heat-treated pollen		
Paymaster 404	38	4.3a**	21.7
7456	20	59.3b	181.4
F_1	24	45.4c	166.7
First backcross	21	30.8d	278.2
Second backcross	26	30.3d	86.5
F_2	106	20.6e	292.0
	Non-treated pollen		
Paymaster 404	13	74.8a	224.5
7456	7	76.9a	95.0
F_1	7	80.7a	166.2
First backcross	13	80.1a	384.4
Second backcross	11	83.8a	157.0
F_2	26	84.3a	776.1

** Means followed by the same letters are not significantly different at $P < 0.01$.

When the parents and progenies were grown at high temperatures in the growth chamber, differences in the levels of heat tolerance between the parents were distinguished. Paymaster 404 did not flower or yield viable pollen under the high temperature regime. The donor parent, 7456, the F_1, and first backcross plants did flower and produce viable pollen, providing evidence that heat tolerance expressed in 7456 was transmitted to the progeny and subsequent first backcross by pollen selection. These observations indicate that the genes expressing heat tolerance in the gametophyte were dually expressed in the sporophyte.

Of 200 F_2 plants, only 106 survived to flowering. This high mortality rate is characteristic of the interspecific cross. Chromosomal pollen sterility of F_2 plants may have altered the actual numbers of heat tolerant and sensitive plants. Physiological sterility resulting from the heat treatment of pollen may be confounded with chromosomal sterility in the interspecific cross.

A broad sense heritability estimate of 57.8% was calculated using the variances in Table 1. These results suggest a few genes are responsible for the pollen heat tolerance transferred in this study, and indicate that heat tolerance expressed in the gametophyte could be transferred by pollen selection. However, care must be taken to determine that pollen heat tolerance in the material selected as a genetic source will also be expressed in the mature plants of the progeny.

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NON-NODULATING MUTANTS IN COMMON BEAN

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Abstract

Estimates of nitrogen fixation by common bean (*Phaseolus vulgaris* L.) have been made using non-nodulating soybean or other non-fixing plant species to measure soil nitrogen because non-nodulating bean lines are not available. Such lines are also needed for studies on the genetic control of nodulation. The objective of this study was to obtain (using induced matagenesis) nodulation mutants of the bean lines 'RIZ 30' and 'RIZ 36'. One hundred and twelve non-nodulated plants were identified in the M2 generation following treatment of imbibed seeds with 0.08 M ethyl methane sulfonate for 4 h. Screening was carried out in perlite with a nitrogen-free nutrient solution, and seeds were inoculated with a mixture of *Rhizobium* strains CIAT 632 and CIAT 899 before emergence. Plants were uprooted 3 wk after planting, and those without nodules were transferred to soil for seed production. The absence of nodulation by eight lines was confirmed in the M3 and M4 generations following inoculation with several other strains of *Rhizobium*, and in the M5 generation two mutant lines were evaluated in soil. Seed set in these lines is poor, though improved by applying nitrogen.

COMMON bean is the most important grain legume worldwide, grown mainly by small farmers in the Third World, and often under conditions of low soil fertility. In most conditions bean plants are unable to satisfy their N requirements through symbiotic nitrogen fixation. Work has been in progress for a number of years at CIAT (1987) to select plant genotypes with enhanced symbiotic potential. Progress would be accelerated by obtaining non-nodulating genotypes which could be used as checks for monitoring soil nitrogen availability in evaluation nurseries using either the difference method or ^{15}N isotope dilution method to estimate N_2 fixation. They would also serve for more fundamental studies on physiological processes, such as the effects of nitrogen stress during different phases of growth (Selamet and Gardner, 1985) and the genetic control of nodule development and function.

Induced mutation, using ethyl methane sulfonate (EMS) or gamma irradiation, has been used to modify nodulation characteristics of a number of grain legumes including pea (*Pisum sativum* L.) (Jacobsen, 1984; Kneen and LaRue, 1984), soybean [*Glycine max* (L.) Merr.] (Carroll et al., 1985a) and chickpea (*Cicer arietinum* L.) (Davis et al., 1985). To our knowledge neither induced nor naturally occurring non-nodulating bean lines are available. The objective of this study was to identify such lines using mutagenesis methods employed successfully with other legumes.

Although natural variability for sensitivity of nodulation to mineral N has been observed (CIAT, 1988), no bean genotypes have been identified that nodulate well in the presence of high levels of nitrate. Such mutants have been induced in soybean (Carroll et al., 1985b) and pea (Jacobsen, 1984). Bean in the tropics are most frequently intercropped with maize, banana, or other non-legume plants. Therefore, the advantage of having varieties able to nodulate and fix nitrogen in the presence of fertilizer applied to the associated crop is clear, and motivated the search for nitrate insensitive mutants, along with the search for non-nodulating mutants.

Materials and Methods

Two cultivars of bean were used, RIZ 30 and RIZ 36, both of which had been selected for their nodulation capacity and symbiotic effectiveness. Seeds of each cultivar were soaked in tap water for 16 h and then 2000 seeds of each were treated with 0.04 M and 0.08 M EMS for 2 and 4 h (500 seeds per treatment), and sown in the glasshouse. Harvest of the M1 generation was completed in January 1986, and seeds from each treatment were bulked. The M1 plants were grown at high density, making the harvest of individual plants impossible.

Screening of the M2 generation was carried out in sterilized seed trays containing perlite and inoculated with a mixture of *Rhizobium leguminosarum* biovar *phaseoli* strains CIAT 632 and CIAT 899. RIZ 30 and RIZ 36 nodulate abundantly with these physiologically and serologically distinct strains. Plants were watered daily with an N-free nutrient solution. A total of 1680 M2 plants of each cultivar were screened between January and May 1986. Plants were uprooted 3 wk after planting, and non-nodulated plants were selected and transplanted to soil for seed production. Another 1680 plants of each cultivar were screened over the same period in a +N nutrient solution (150 mg KNO_3 L^{-1}) to look for mutants capable of forming nodules in the presence of mineral N. Counts of chlorophyll and other apparent mutations (dwarf plants, tall plants, leathery leaves, and pale or variegated leaves) were made 2 wk after planting in both −N and +N treatments. The albino chlorophyll mutants died within 2 to 3 wk.

Seeds from the selected M2 plants were screened under the same conditions with two replications of three seeds per line. Each tray had a central row of the appropriate parental cultivar, flanked by three rows of test lines. Plants were scored for nodulation 3 wk after planting. Lines that were uniformly non-nodulating were selected and grown on to produce M4 seed. This seed was then tested in the glasshouse at Wye College, against several other isolates of *R. phaseoli* at doses of greater than 10^6 cells per seedling, and at CIAT, Colombia where backcrosses to the original parental material were also made. The M5 seed of nod 109, and nod 125 together with seed of the parent lines were tested in high N soil conditions to compare plant growth and in low N con-

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Published in Crop Sci. 28:859–860 (1988).