Genetic variability for stomatal conductance in Pima cotton and its relation to improvements of heat adaptation

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ABSTRACT Responses of stomata to environment have been intensively studied, but little is known of genetic effects on stomatal conductance or their consequences. In Pima cotton (*Gossypium barbadense* L.), a crop that is bred for irrigated production in very hot environments, stomatal conductance varies genetically over a wide range and has increased with each release of new higher-yielding cultivars. A cross between heat-adapted (high-yielding) and unadapted genotypes produced F₂ progeny cosegregating for stomatal conductance and leaf temperature. Within segregating populations in the field, conductance was negatively correlated with foliar temperature because of evaporative cooling. Plants were selected from the F₂ generation specifically and solely for differing stomatal conductance. Among F₃ and F₄ populations derived from these selections, conductance and leaf cooling were significantly correlated with fruiting prolificacy during the hottest period of the year and with yield. Conductance was not associated with other factors that might have affected yield potential (single-leaf photosynthetic rate, leaf water potential). As breeders have increased the yield of this crop, genetic variability for conductance has allowed inadvertent selection for "heat avoidance" (evaporative cooling) in a hot environment.

Stomata represent a unique adaptation of terrestrial plants that couples leaf gas exchange to water availability. As a result, stomatal function has been a focus of interest for plant physiologists at least since the 17th century. The evolution, development, bioenergetics, biophysics, biochemistry, and environmental responses of stomata have since then been well-documented (1, 2).

There is substantial variation in the stomatal responses of higher plant species to atmospheric humidity, soil water availability, and other environmental factors (3–7). Variation in stomatal response to environment is usually ascribed to processes external to the guard cells, such as osmotic adjustment in leaf mesophyll cells (8, 9). In a very few cases genetic variability for stomatal traits has been demonstrated within the same species, although affected traits were mostly related to stomatal size and density (10). Despite the poor documentation of the genetic control of stomatal properties, the concept is especially important to agriculture, as water availability often limits production, and stomatal traits are major determinants of water use efficiency. Identification of genetic variability for stomatal properties would provide a new tool for plant breeders to improve crop adaptation to stressful environments.

Earlier we began a search for genetic variability for stomatal conductance in crop plants, resulting in the discovery of genetic control of stomatal conductance in American Pima cotton (*Gossypium barbadense* L.) (11). Over the last few decades, breeding this crop for increasing yield under irrigation in a hot arid environment has substantially increased stomatal conductance in the absence of soil water stress (11). As a result, conductance of modern Pima cultivars is very high (midday conductances near 1 mol·m⁻²·s⁻¹). In this respect Pima cultivars resemble those of a closely related species, *Gossypium hirsutum* L. (upland cotton) when they are grown in similar environments (12).

The physiological benefits of very high conductance are unexplained. One hypothesis is that, when ambient daytime temperatures greatly exceed the optimum plant temperature, increasing stomatal conductance promotes evaporative cooling of leaves and thereby reduces thermal stress. For both upland cotton and Pima cotton, the optimum daytime temperature is <30°C (13–15), which is well below commonly occurring air temperatures in most cotton-growing areas. Many studies show substantial cooling of upland cotton foliage (16–18), and a role of stomata in promoting a "heat avoidance" type of heat resistance through evaporative cooling has been proposed (12, 19, 20). Tests of this hypothesis have been equivocal, though, primarily because the lack of identified genetic variability for stomatal conductance in upland cotton precludes comparison to appropriate control plants. We took advantage of genetic variability for conductance in Pima cotton to create populations segregating for conductance level. Here we describe the association between conductance and leaf temperature in these populations, and we report the relationship of these traits to heat resistance (defined as yield potential in a hot environment) and to other traits that have been modified by selection for heat resistance. Our studies show clearly that genetic improvements of heat resistance in this crop depend largely upon increased stomatal conductance and the resultant evaporative cooling of foliage.

MATERIALS AND METHODS

All studies described here were carried out at the Maricopa Agricultural Center of the University of Arizona (33.07°N, 111.98°W, elevation 358 m). This is an experimental farm occupying about 400 ha (1 ha = 10⁴ m²) in the midst of an irrigated agricultural area. The agroecosystem is characterized by fields planted predominantly to cotton and to alfalfa (*Medicago sativa* L.) during the summer, with approximately an equal area of fallow land interspersed. Large uncultivated areas surrounding the agricultural belt carry Sonoran desert vegetation. Rainfall is sparse (<100 mm during the growing season) compared to potential evapotranspiration (about 1000 mm). Much of our work has focused on a heat-sensitive landrace of *G. barbadense* L. of the Sea Island type from the Caribbean island of St. Vincent; on P-70, an elite breeding strain of Pima cotton with excellent heat resistance; and on progeny of crosses between the two. P-70 was the maternal

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parent. The crosses were made in the glasshouse in 1989 and the F_1 generation was grown in the field in 1990 to produce F_2 seeds. Both in glasshouses and in the field, this crop is highly self-pollinated. In 1991 the F_2 generation was grown in the field with ample irrigation. Measurements of photosynthesis were made with a portable steady-state gas-exchange system (Analytical Development, Kent, U.K.), of stomatal conductance with a steady-state porometer (LiCor Instruments, Lincoln, NE; model LI-1600), of leaf temperature in open air using an infrared thermometer with a 4° field of view (Everest Interscience, Los Altos, CA; model 210), and of leaf length and width using a ruler. The dimensions were later converted to leaf areas from a regression relating the product of length and width to actual area. Although the cotton leaf is palmately lobed, all populations had similar leaf shapes. All measurements were on recently expanded young leaves in sunlight near the top of the canopy. Measurements were made repetitively during August and early September in the morning ($\approx$1100 hr), when conductances were near their maximum (11), and in midafternoon ($\approx$1500 hr), when air temperatures were near their maximum. All measurements were taken 3–5 days after an irrigation. The F_2 stand consisted of 45 plants, each of which was characterized. The F_2 plants were highly variable for the visible traits of height and leaf size. All F_2 plants were individually harvested to determine their lint yields and to collect seed.

Leaf temperatures of F_2 plants were predicted for comparison to experimentally determined values by use of a standard energy balance model (21). Leaf temperature was calculated based on an assumed steady state with realistic values assigned to the other terms of the equation. Solar irradiances were recorded at a weather station on the farm.

Within the F_2 population in 1991, 12 individual plants were selected representing the entire range of values of stomatal conductance. Seeds from these F_2 individuals were sown and grown in the field as F_3 populations in 1992. The seed supply was sufficient to allow duplicate single-row stands $\approx$6 m long. The modern heat-resistant cultivar Pima S-7 (22) was also planted for comparison. Stomatal conductances of leaves in all plots were determined on four clear days during July and August. On each day, after completion of the measurements the leaves were shaded and enclosed in plastic bags to protect them from the atmosphere and then immediately detached and placed in a dark humid insulated container for subsequent determination of xylem pressure potentials (Ψ_w) with a pressure chamber (23). Measurements were made only during midafternoon, the hottest part of the day. Six plants from each plot were randomly selected at the end of the season for nondestructive mapping to determine numbers and positions of retained fruits. The fruits produced during the hottest midsummer period (mid-June to mid-August) were identified from the maps, and these data were the basis for ratings of heat resistance. All fruits were then hand-picked for fiber yields and the seeds were saved.

The F_4 generation was grown in 1993 without further selection within populations. Eight of the 12 lines were planted in a randomized study with four replications. Conductance was determined on four days in August, and fruits were harvested at the end of the season for measurement of total yield.

Numerous elite strains and the commercial variety, Pima S-6, were grown in replicated plots in 1990 using standard cultural practices. Stomatal conductances were determined in those plots on several dates as above, and plots were harvested at the end of the season and lint yields were determined.

RESULTS AND DISCUSSION

Leaf temperatures in the field varied widely among genotypes differing in the degree of adaptation to Arizona growing

conditions. For example, in the afternoon of 20 August 1991, when the air temperature was 36°C, leaf temperatures were 33.2°C for the unadapted Sea Island and 29.9°C for the adapted P-70. This difference was significant ($P < 0.05$). Leaf temperature of the obsolete low-yielding cultivar Pima 32 (released in 1949) was 31.9°C, intermediate between the unadapted and the elite strains.

Leaf temperature reflects the energy budget of the leaf. Among the factors determining the energy balance, the two that are most variable genetically are leaf size (affecting the boundary layer conductance) and stomatal conductance (affecting the latent energy component of the energy balance). A third potential factor, leaf heliotropism, is not observed in Pima cotton although it is prominent in upland cotton (24). Both conductance and leaf size were highly variable within the F_2 population, but they segregated independently and the relationship between them was very weak (not shown). This independence allowed the use of regression techniques to determine the role of each trait in cooling. Among F_2 progeny from a cross of Sea Island with P-70, leaf temperature varied over a wide range and was highly correlated with stomatal conductance ($r = -0.76$, Fig. 1). Under the specific conditions of measurement, the slope of the regression of temperature on conductance revealed a decrease of about 1°C for each increase in conductance of 0.2 mol·m^{-2}·s^{-1}. It indicates that the observed genetic variability for conductance, which ranged from approximately 0.4 to 1.2 mol·m^{-2}·s^{-1}, can strongly influence leaf temperature.

Stepwise regression analysis of the data revealed that in midafternoon, the hottest time of the day, leaf temperature was significantly correlated with stomatal conductance ($P < 0.001$, Fig. 1) but the correlation with leaf size was not significant ($P = 0.28$). This result was consistent with the single-leaf energy balance model (21), which indicated that irradiance is a critical factor in determining the influence of leaf size. At absorbed irradiances of $\approx$550 W·m^{-2}, the model predicted no relationship between size and temperature (Fig. 2). Incident solar irradiance at the times of our stomatal measurements (August 1992, midafternoon values) averaged 890 W·m^{-2}. Assuming that 25% of solar irradiance is reflected (25) and 10% is transmitted (26) leads to an estimate that the uppermost leaf absorbed 579 W·m^{-2}, which is near the critical value of 550 W·m^{-2}. Thus the conditions under which we made our measurements effectively removed leaf size as a factor contributing to variability in leaf temperature. Under these conditions, when potential thermal stress is very high,
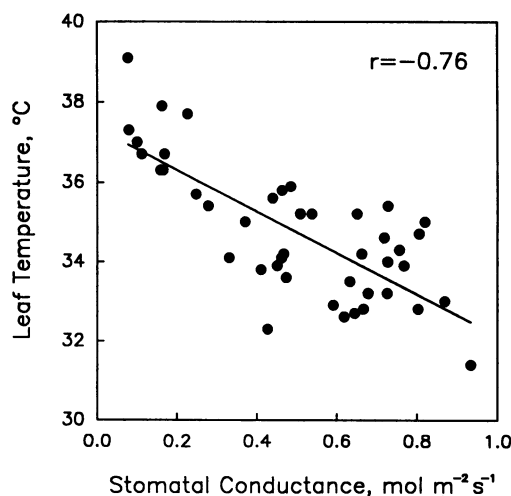

FIG. 1. Dependence of leaf temperature on stomatal conductance in the F_2 generation resulting from a cross of P-70 and Sea Island. All data were taken at midafternoon, when air temperature was approximately constant at 39°C.

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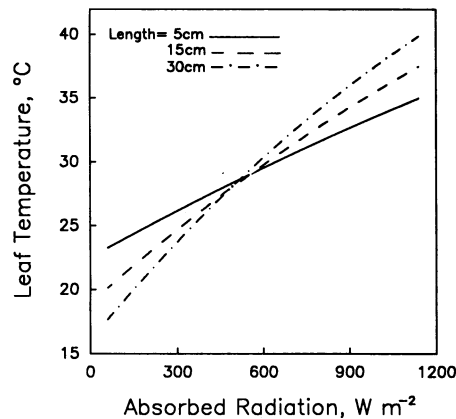*Proc. Natl. Acad. Sci. USA 91 (1994)* 7219

FIG. 2. Predicted leaf temperature as a function of absorbed irradiance and leaf size. The energy balance for calculation of leaf temperature was from Gates (21). Assumptions for the analysis were as follows: air temperature, $35°C$; wind speed, 1 $m·s^{-1}$; relative humidity, 40%; and stomatal conductance, 0.85 $mol·m^{-2}·s^{-1}$.
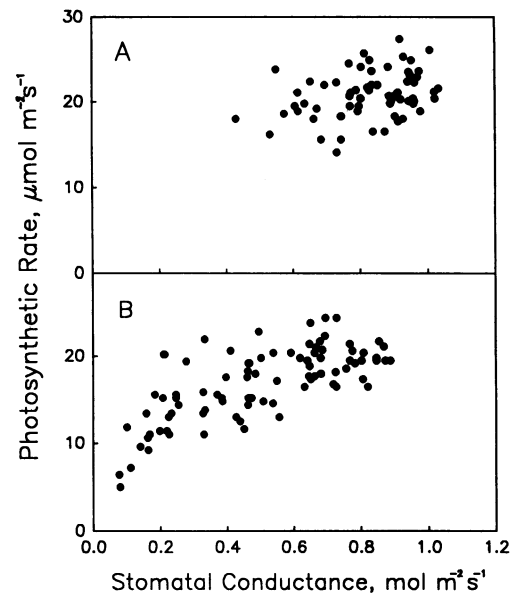

FIG. 3. Relationship between photosynthetic rate and stomatal conductance of Pima cotton leaves. (A) Measurements in late morning. (B) Measurements in midafternoon. Determinations of photosynthetic rate and conductance were virtually concurrent on the same leaves.

stomatal conductance is the major genetically variable trait affecting leaf temperature. Nonetheless, we note that leaf size affects leaf temperature under conditions other than those described above. For example, in the late mornings of the measurement days, when absorbed irradiance was <500 $W·m^{-2}$, leaf size exerted a significant negative influence on leaf temperature ($P = 0.02$). This observation is also consistent with the model (Fig. 2).

Stomata can also restrict the diffusion of CO_2 into the leaf. When interpreting the role of stomata in heat resistance, one must consider this restriction, which might limit photosynthetic gas exchange. Rigorous determination of the stomatal limitation to photosynthesis requires an $A:C_i$ curve (photosynthetic rate as a function of intercellular [CO_2]) for each leaf (27). We could not acquire so much data within the time window available. Instead, we studied whether variation in conductance within the F_2 population was associated with variation in photosynthetic rate. A strong association would indicate the possibility of (but not prove) stomatal restrictions. A very weak association would demonstrate the absence of a stomatal restriction. In the late morning, a response of photosynthetic CO_2 uptake to stomatal conductance was undetectable, indicating little or no role of stomata in limiting CO_2 assimilation (Fig. 3). In the afternoon, however, the range of conductances within the F_2 population was shifted to lower values. At this time of day, in some leaves conductance was low enough to be associated with photosynthetic limitations, although over most of the conductance range there was no relationship between the two variables (Fig. 3). The threshold conductance for relief of stomatal limitations to photosynthesis was $0.3–0.4$ $mol·m^{-2}·s^{-1}$. For comparison, in the afternoon when conductances were below their daily maximum, the conductances of both the heat-resistant and the heat-sensitive parental populations (mean ± SE of $0.81 ± 0.02$ and $0.50 ± 0.04$ $mol·m^{-2}·s^{-1}$, respectively) were well above the threshold. Even in the latter case, the mean was several standard errors above the threshold conductance, implying that occurrence of a significant stomatal limitation to photosynthesis was rare in the parental lines. Thus it is unlikely that the stomatal association with heat resistance in Pima cotton is mediated by direct limitations to leaf photosynthetic rates.

Our studies demonstrate a strong association between stomatal conductance and heat adaptation, and they suggest a linkage mediated by evaporative cooling rather than photosynthetic gas exchange, but they do not prove a cause-and-effect relationship. To resolve this issue of cause and effect, in 1991 we selected 12 plants from the F_2 generation

representing the entire range of genetic variation for conductance and harvested seeds for planting the next year. Mean conductances of the F_3 populations in 1992 were highly correlated with conductances in 1991 of the individual F_2 plants from which they had originated ($r = 0.92$, $n = 12$). Among the F_3 lines in 1992, stomatal conductance was closely related to leaf temperature ($r = -0.89$) and also to the number of fruits produced by plants during the summer and retained until the fall harvest ($r = 0.68$) (Table 1). The plant mapping technique targeted fruits produced during the hottest period and thus excluded fruits produced before or after that period. As a result, the increased retention detected in the more productive F_3 lines specifically reflects their ability to set fruits under heat stress. All F_3 strains set fruits copiously at the end of the summer, when temperatures were lower than in midsummer. Nonetheless, rankings of strains for yield at the end of the season were positively correlated with rankings for stomatal conductance ($r = 0.63$, $n = 12$) because of differences in fruit set during the summer. Conductance was measured only during the hot months of July and August, not during October when the late fruits were maturing.

Table 1. Simple correlations among physiological traits across Pima cotton populations selected solely for stomatal conductance

| | Correlation coefficient | | | |
	Stomatal conductance	Leaf size	Leaf Ψ_w	Leaf temp.
Leaf size	NS	—		
Leaf Ψ_w	NS	-0.75*	—	
Leaf temp.	-0.89*	NS	NS	—
Fruit set	0.68*	-0.66*	0.62*	-0.75*

The traits monitored included conductance, leaf temperature (temp.), leaf size, leaf water potential (Ψ_w), and number of fruits set during the hottest part of the summer. The ranges of the genotype means were as follows: conductance, $0.89–1.27$ $mol·m^{-2}·s^{-1}$; leaf size, $0.95–1.70$ g of dry weight; leaf water potential, -1.22 to -1.41 MPa; leaf temperature, $34.0–35.6°C$; fruits set during summer, $7.6–17.2$ per plant. NS, not significant.
*Significant at $P = 0.05$.

For the F_4 generation in 1993, the relationship between conductance and yield was stronger than for the F_3 in 1992 (r = 0.86, n = 8). These correlations show that direct selection solely for the stomatal conductance trait enhances heat resistance.

Leaf temperature of the F_3 lines was not significantly related to leaf size or leaf Ψ_W in our measurements (Table 1). Nonetheless, these latter characteristics were significantly correlated with fruit retention in their own right (r = 0.66 and 0.62, respectively), and with each other (r = -0.75), implying the existence of a second important heat resistance mechanism independent of leaf cooling (Table 1). The correlation between leaf size and leaf Ψ_W was negative, as was that between leaf size and fruit number, indicating that small leaves are associated with high Ψ_W and greater fruit retention. The data imply that leaf size may affect the plant water balance; this physiologically distinct mechanism of heat resistance could also explain in part the selection pressure for small leaves in modern Pima cotton lines.

The high-yielding cultivar Pima S-7 was included in these tests for comparison to the 12 F_3 strains in 1992 (but was excluded from the correlation analyses in Table 1). Rankings of S-7 for fruit set during the summer, high conductance, low leaf temperature, small leaf size, and high leaf Ψ_W placed it 1, 3, 1, 1, and 2, respectively. The relationships among these traits across the F_3 strains (Table 1) did not change when Pima S-7 was included in the data base. This implies that the traits associated with heat resistance in S-7 are similar to those monitored in the F_3 strains and shown to be associated with heat resistance there (stomatal conductance, leaf temperature, leaf size, and leaf Ψ_W).

We consistently find that the temperature differential between unadapted and adapted lines in the field is more pronounced in the afternoon than in the morning. This may occur in part because the large leaf trait of unadapted lines promotes cooler foliage than small leaves at irradiances typical of the morning (Fig. 2). More importantly, though, stomatal conductance follows a diurnal cycle, with the less-adapted lines closing their stomata more than the adapted lines during the afternoon (11). Among several elite strains, conductance of all strains was very high in the morning (Fig. 4). In the afternoon, however, the conductance of all strains had declined, with the conductance of the lowest-yielding strains declining the most. Yield was more closely related to conductance determined in the afternoon than to that deter-

mined in the morning (Fig. 4). These data are consistent with other evidence reported here that emphasizes the importance of midafternoon conductance to adaptation. Taken together, all of the data indicate that stomatal conductance during the afternoon is very closely related to heat resistance.

The stomatal component of heat resistance is apparently dependent upon evaporative cooling. This represents a heat avoidance strategy, in that leaf temperature is decreased without necessarily increasing tissue tolerance of thermal stress. Heat resistance mechanisms are known in cotton and numerous other species (28, 29), but, to our knowledge, genetic variability for an avoidance type of heat resistance has not been reported previously. Although the range of mean leaf temperatures of the F_3 strains was slightly less than 2°C (Table 1), at the extreme temperatures encountered in the field, these are significant differentials. Increasing daytime air temperature from 37°C to 40°C [a change estimated to increase leaf temperature 2°C at constant conductance (21)] decreased the number of retained reproductive structures on Pima cotton from about 15 to almost zero (20). Clearly the relative effect of small temperature changes is magnified near the edge of the window of adaptation. We conclude that under the extreme field conditions of midsummer in Arizona, the observed leaf temperature differences could be important to adaptation. The mechanism(s) by which foliar temperature might be transduced into fruit retention is not yet known.

It should be noted that many simple crop models relate biomass accumulation to transpiration, based upon the presumably similar physical limitations to exchange of CO_2 and water vapor between leaf and air (30). These models may fail for a heat-avoider like Pima cotton, as under most conditions stomatal conductance exceeds the threshold for diffusive limitations to photosynthesis (Fig. 3). The "excess" transpiration promotes partitioning of biomass to fruits. The existence of separate mechanisms linking transpiration to biomass accumulation and to partitioning adds another level of complexity to the relationship between water use and crop yield.

Finally, what factors might limit the effectiveness of a heat avoidance mechanism of thermal adaptation? With the emphasis shifted to plant temperature rather than air temperature, all factors that limit evaporative cooling must be considered important. One such limitation is the supply of available water. Evaporative cooling consumes very large amounts of water. If breeders continue to enhance heat avoidance, full irrigation to supply water may be an increasingly critical input into Pima cotton production systems. Another factor limiting effectiveness of a heat avoidance strategy is atmospheric humidity. For many years American Pima cottons have been bred in a desert agroecosystem. The heterogeneity, aridity, and advective conditions of the irrigated desert agroecosystem maximize the effectiveness of stomata in controlling the rate of evaporation of water from the leaves (31). In a more humid environment, stomata might have much less influence upon leaf cooling. Our results imply that extending the range of modern heat-avoiding Pima cottons to humid or nonirrigated environments may be unlikely.

In conclusion, we report the existence of significant genetic variability for stomatal conductance in Pima cotton. Selection for improved heat resistance (fruit set during heat stress) of irrigated Pima cotton has been accompanied by increasing stomatal conductance and decreasing leaf temperature, especially during the afternoon. Conversely, one cycle of selection solely for conductance from a segregating population led to decreasing leaf temperature and enhanced yield. Evaporative cooling was largely stomatally controlled, especially during the hot afternoon, and its magnitude was sufficient to account for large differences in crop productivity. Other putative consequences of increasing stomatal conduc-
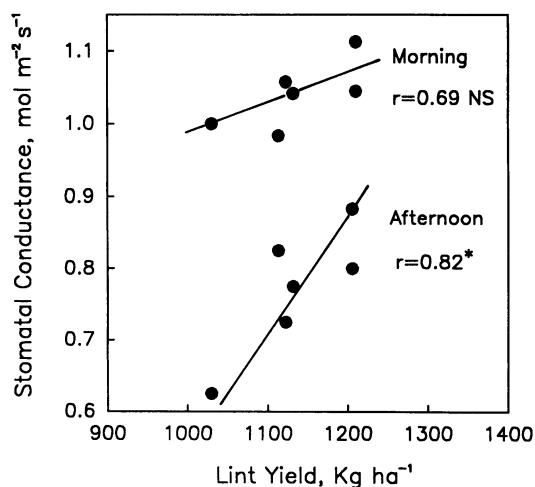

FIG. 4. Relationship of yields of high-yielding Pima cotton lines to their stomatal conductances in the late morning and in the afternoon. The correlation with afternoon conductance is significant (P < 0.05), whereas the correlation with morning conductance is not significant (NS).

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tance (relief of limitation to photosynthetic rate) were shown to be minor. Taken together, these findings provide strong evidence that stomatal conductance is the basis of much of the improved heat resistance of modern Pima cottons.

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