Published online January 22, 2007

Pollen-Based Screening of Soybean Genotypes for High Temperatures

Mohammed A. Salem, Vijaya Gopal Kakani, Sailaja Koti, and K. Raja Reddy*

ABSTRACT

Soybean [*Glycine max* (L.) Merr.] reproduction is sensitive to temperatures > 35°C. Two studies were conducted to determine temperature effects on soybean pollen germination (PG) and to detect genotypic differences. Pollen collected from 44 genotypes (Maturity Groups III to VI) grown outdoors was subjected to in vitro temperatures from 15 to 50°C at 5°C intervals. Genotypes differed significantly for in vitro PG percentage (mean of 81%) and tube length (mean of 437 μm). Mean cardinal temperatures (T_{min}, T_{opt}, and T_{max}) were 13.2, 30.2, and 47.2°C for PG and 12.1, 36.1, and 47.0°C for pollen tube growth. Genotypes differed for leaf cell membrane thermostability (CMTS), but CMTS did not correlate with pollen parameters. Cumulative temperature response index, CTRI (unitless), of each genotype calculated as the sum of eight individual stress responses (ISRs) derived from maximum PG, maximum pollen tube length (PTL), and the maximum (T_{max}), minimum (T_{min}), and optimum (T_{opt}) temperatures for PG and for PTLs was used to group genotypes for temperature tolerance. Heat-tolerant genotype (DG 5630RR) was less sensitive to high temperature (38/30°C) compared with heat-intermediate (PI 471938) and heat-sensitive (Stalwart III) genotypes that had deformed pollen, with reduced apertures and collumellae heads. Hence, pollen can be used as a screening tool for heat tolerance. Most sensitive to temperature was D88-5320 with a CTRI of 6.8, while AG 4403RR was most tolerant with a CTRI of 7.5. Elevated [CO_2] did not modify reproductive parameters or CTRI. The study also revealed that heat tolerance of vegetative tissue had little or no relationship with the heat tolerance of reproductive tissue. Maturity groups lacked a specific trend for tolerance to high temperature. The identified high temperature-tolerant genotypes and temperature-dependent pollen response functions might be useful in soybean breeding and modeling programs, respectively.

First domesticated in northeastern parts of China, conventional and genetically modified cultivars of soybean have been adapted and commercially cultivated in all the climatic zones of the USA and in populated continents of the world. Soybean is an important legume cash crop grown for its oil and protein, with >74.4 million ha worldwide and 29.3 million ha in the USA (FAO, 2006). During 2003, the USA produced 2.37 million Mg of grain (USDA-NASS, 2006). Therefore, soybean production is extremely important to the economic structure of the global food network, especially of the USA.

Soybean is sensitive to changes in daylength and temperature (Allen and Boote, 2000). Breeders and researchers have routinely selected genotypes that are most responsive to a combination of daylength and temperature for a given niche environment (Martineau et al., 1979). However, most U.S. genotypes were derived from a small number of ancestral soybean cultivars brought to the USA in the early 1900s (Carter et al., 1993), indicating a small genetic pool. Lobell and Asner (2003) evaluated the relationship between climate variation and production of corn (*Zea mays* L.) and soybean grown in the USA between 1982 to 1998, and reported that for every 1°C rise in temperature, there was, on average, 17% decrease in yield. Future increases in greenhouse gases are projected to raise earth's surface temperature to anywhere between 1.5 to 11°C by 2100 (Stainforth et al., 2005) that would severely reduce soybean production. Current model simulations indicate more intense, more frequent, and longer-lasting summer heat waves over North America and Europe (Meehl and Tebaldi, 2004). In 2003, Europe experienced a 6°C increase in temperature from the long-term mean and a 50% decrease in precipitation, resulting in crop yield losses of up to 36% (Ciais et al., 2005). It was shown, using field and controlled-environment experiments and modeling exercises, that projected changes in climate will have profound impacts on crop production (Reddy and Hodges, 2000, p. 472). Hence, it is essential to protect crop yields from higher and more frequent episodes of extremely higher temperatures both in current and future climates.

Reproductive growth leading to seed yield is often depressed by the same increases in temperature that enhances vegetative growth and development. Fruit set in cotton (*Gossypium hirsutum* L.; Reddy et al., 1992), bell pepper (*Capsicum annuum* L.; Erickson and Markhart, 2002), corn (Herrero and Johnson, 1980), tomato (*Lycopersicon esculentum* L.; Sato et al., 2002), common bean (*Phaseolus vulgaris* L.), groundnut (*Arachis hypogeae* L.), cowpea [*Vigna unguiculata* (L.) Walp.] (Hall, 2004), and soybean (Ferris et al., 1998) is sensitive to supraoptimal temperatures. Temperature of 30°C was shown to be optimum for growth and development in soybean (Raper and Kramer, 1987), while temperatures between 33 and 40°C reduced growth (Vu et al., 1997). Flower initiation was reduced by temperatures > 32°C (Borthwick and Parker, 1940), and seed formation was delayed at 40–30°C (Thomas et al., 2003). When soybean plants were exposed to temperatures of 35°C for 10 h during the day, yield reductions of about 27% were measured (Gibson and Mullen, 1996).

M.A. Salem, S. Koti, and K.R. Reddy, Dep. of Plant and Soil Sciences, 117 Dorman Hall, Box 9555, Mississippi State Univ., Mississippi State, MS 39762; V.G. Kakani, Dep. of Agronomy, Univ. of Florida, Gainesville, FL 32611. Received July 2006. *Corresponding author (krreddy@pss.msstate.edu).

Published in Crop Sci. 47:219–231 (2007).
Crop Physiology & Metabolism
doi:10.2135/cropsci2006.07.0443

677 S. Segoe Rd., Madison, WI 53711 USA

Abbreviations: CMTS, cell membrane thermostability; CTRI, cumulative temperature response index; DAS, days after sowing; ISR, individual stress response; PAR, photosynthetically active radiation; PG, pollen germination; PTL, pollen tube length; RMSD, root mean square deviation; SEM, scanning electron microscope; SPAR, soil–plant–atmosphere–research.

Recent studies have shown that microsporogensis was more sensitive to high temperature than megasporogenesis, leading to lowered fruit set (Cross et al., 2003; Young et al., 2004). Lower pod and seed set were related to nonviable pollen, unsuccessful anther dehiscence, and pollen shed in corn (Schoper et al., 1987) and tomato (Sato et al., 2000) that reduced pollen tube penetration into the stigma and weakened female performance (Gross and Kigel, 1994). Under humid conditions in the southern USA, soybean anther dehiscence occurs between 0800 and 1000 h, followed by pollination and fertilization. Therefore, high temperatures during flowering or short episodes of high temperature during pollen release and germination may more severely affect male reproductive processes than ovules. High temperatures during flowering have been shown to affect PG, tube growth, and fertilization in several crops (Hall, 2004). Pod yield, the number of flowers produced, and proportion of flowers that are fertilized were reduced by high temperatures in groundnut (Prasad et al., 2001). Pollen abnormalities were observed in soybean (Koti et al., 2005) and common bean (Gross and Kigel, 1994; Suzuki et al., 2001) grown in high temperatures.

In vitro PG and PTL in groundnut and cotton genotypes varied in their responses to T_{min}, T_{opt}, and T_{max} temperatures (Kakani et al., 2002, 2005). Differences in these cardinal temperatures have been used as screening techniques for tolerance or sensitivity of cotton and groundnut genotypes to high temperature (Kakani et al., 2002, 2005). To date, there have been no studies conducted to specifically document in vitro PG and pollen tube growth technique for screening soybean genotypes to high temperatures. Also, studies describing the variation in cardinal temperatures for reproductive parameters in soybean or whether genotypic maturity group variation exists in pollen responses to temperature are lacking. Therefore, the objectives of this research were to (i) quantify the responses to temperature of in vitro PG and PTL of soybean genotypes, (ii) determine cardinal temperatures of each genotype and maturity group and classify them based on their tolerance to temperature, and (iii) determine the basis for tolerance or sensitivity of soybean genotypes by growing sensitive and tolerant genotypes in controlled environment conditions under optimum and high temperatures.

MATERIALS AND METHODS

Two experiments were conducted during the 2003 growing season in an outdoor pot-culture facility (Exp. I) and growth chamber facility known as the soil–plant–atmosphere–research (SPAR) chambers (Exp. II) at the Mississippi Agricultural and Forestry Experiment Station, Mississippi State (38° 28′ N, 88° 47′ W), MS.

Experiment I

Plant Husbandry

Forty-four soybean cultivars, both conventional and genetically modified, belonging to Maturity Groups III, IV, V, and VI and differing in tolerance to abiotic stresses and determinacy, were sown on 3 June 2003 in 12-L white polyvinyl chloride pots filled with fine sand. The pots were 0.65 m tall and 0.15 m in diameter, with a small aperture at the bottom to drain excess water. There were 10 pots for each cultivar, arranged in rows (40 pots per row) with an additional border row maintained on each side and oriented east to west with rows 1 m apart. Emergence was observed 5 d after sowing (DAS), and seedlings were thinned to two per pot at 12 DAS. All pots were irrigated three times a day (0800, 1200, and 1600 h) and for 3 to 9 min each time depending on growth stage, using a computer-controlled drip system with half-strength Hoagland's nutrient solution (Hewitt, 1952) to maintain favorable conditions of water and nutrients (Reddy et al., 2001). The dripper delivery rate was set to 75 mL min^{-1}.

Measurements

Pollen Germination and Pollen Tube Lengths

Soybean flowers (40–50) that showed anthesis on a given day were randomly collected from plants in each genotype between 0900 and 1000 h. Because of differences in flowering dates, in vitro PG tests were conducted during 13 to 20 July, 22 to 29 July, and 28 July to 3 August of 2003 for early, mid-, and late-flowering varieties, respectively. Flowers collected were air-dried for 2 h in a temperature-controlled laboratory (24–25°C) and pollen was dusted onto the germination medium. A uniform distribution of pollen grains was obtained on the surface of the germinating medium by brushing anthers with a nylon hair brush. The medium consisted of 15 g sucrose ($C_{12}H_{22}O_{11}$), 0.03 g calcium nitrate [$Ca(NO_3)_2$ $4H_2O$], and 0.01 g boric acid (H_3BO_3) dissolved in 100 mL of deionized water (Gwata et al., 2003, with modifications; Salem et al., 2004). To this liquid medium, 0.5 g of agar was added and slowly heated on a hot plate. After the agar was completely dissolved, 10 mL of the germinating medium was poured on each of three Petri dishes for each genotype at each temperature and allowed to cool for about 15 min to let the agar solidify. The plates were then covered and incubated at predetermined temperature treatments in incubators (Precision Instruments, New York, USA). The temperature treatments or incubator set points were maintained at 15 to 50 ± 0.2°C at 5°C intervals.

Pollen grains were counted for PG (10 fields in each Petri dish) after 24 h of incubation using a microscope (SMZ 800, Nikon Instruments, Kanagawa, Japan). Pollen grain was considered germinated when its tube length equaled the grain diameter (Luza et al., 1987) at a magnification of 6.3×. Percentage PG was calculated by counting the total number of pollen grains and the number of pollen germinated in a microscope field and averaged across 10 fields per Petri dish, resulting in three replications. The PTLs of germinated pollen grains were measured with an ocular micrometer fitted to the eyepiece of the microscope. A total of 90 pollen tubes were measured for each treatment when the PTL was equal or longer than pollen diameter. Earlier studies in our laboratory using the same incubators recorded no differences between the measured incubator and media temperatures (Kakani et al., 2005). Therefore, the average temperature of the incubator during PG was used in the analysis.

Cardinal Temperatures

The maximum PG and PTLs recorded after 24 h were analyzed by linear and nonlinear regression models commonly used to quantify developmental responses to temperatures (Kakani et al., 2002). The fit of each regression equation for the response of PG and PTLs to temperatures was compared for the amount of variation accounted for R^2 and root mean

square deviation (RMSD) for observed and fitted values. The highest R^2 and lowest RMSD for PG and PTLs were with quadratic and modified bilinear models, respectively. Accordingly, the cardinal temperatures were calculated from the fitted equations for all the genotypes.

The nonlinear regression procedure PROC NLIN (SAS Institute, 1998) was used to estimate the parameters of the quadratic and modified bilinear equations. For the quadratic model (Eq. [1]), T_{min}, T_{opt}, and T_{max} were estimated by the following equations (Eq. [2], [3], and [4]):

$$PG = a + bT - cT^2 \quad [1]$$

$$T_{opt} = -b/(2c) \quad [2]$$

$$T_{min} = -b + \frac{\sqrt{b^2 - 4ac}}{2c} \quad [3]$$

$$T_{max} = -b - \frac{\sqrt{b^2 - 4ac}}{2c}, \quad [4]$$

where T is actual treatment temperature, and a, b, and c are genotype-specific constants generated using PROC NLIN in SAS. For the modified bilinear equation, T_{opt} was generated by fitting the bilinear model (Eq. [5]) by SAS, and T_{max} and T_{min} were determined by Eq. [6] and [7] (Kakani et al., 2002):

$$PTL = a + b_1(T - T_{opt}) + b_2 \times ABS(T_{opt} - T) \quad [5]$$

$$T_{min} = \frac{a + (b_2 - b_1) \times T_{opt}}{b_1 - b_2} \quad [6]$$

$$T_{max} = \frac{a - (b_2 + b_1) \times T_{opt}}{b_1 + b_2}, \quad [7]$$

where T is actual treatment temperature and a, b_1, and b_2 are equation constants.

Cell Membrane Thermostability

The technique used to determine membrane thermostability was similar to that developed by Sullivan (1972) and Martineau et al. (1979), with minor modifications. Fully expanded leaves were collected from plants of all genotypes, and two sets [control (C) and treatment (T)] of 2.5-cm^2 leaf discs from five randomly selected leaves were placed in the test tubes containing 10 mL of deionized water. The leaf segments were thoroughly rinsed with three changes of deionized water to remove electrolytes adhering to and released from the cut surface of the segments. After final rinsing, the T set of tubes were drained, capped with aluminum foil to prevent dehydration of tissue during heat treatment, and incubated in a hot water bath at 50°C for 20 min, while the C set of tubes were at 25°C. After incubation, T sets of tubes were brought to 25°C and 10 mL of deionized water was added. Then, both C and T sets of tubes were incubated at 10°C for 18 h. They were brought to 25°C and conductance of control (CEC1) and treatment (TEC1) was measured. Tubes were then placed in an autoclave at 0.1 MPa for 12 min to completely kill tissue, releasing all the electrolytes. Tubes were then cooled to 25°C and final conductance was measured (CEC2 and TEC2). The CMTS was estimated as follows:

$$CMTS\ (\%) = [1 - (TEC1/TEC2)]/[1 - (CEC1/CEC2)] \times 100 \quad [8]$$

Cumulative Temperature Response Index

The ISR of each parameter was calculated as the value of a genotype (P_t) divided by the maximum value (P_h) observed over all the genotypes (Eq. [9]). The CTRI (Eq. [10]) of each genotype was calculated as the sum of eight ISR's derived from maximum PG, maximum PTL, T_{max}, T_{min}, and T_{opt} temperatures of PG and PTLs. Genotypes were classified based on CTRI of all the parameters as tolerant [> minimum CTRI + 3 standard deviation (stdev)], intermediate (> minimum CTRI + 2 stdev and < minimum CTRI + 3 stdev), and sensitive (> minimum CTRI to < minimum CTRI + 2 stdev).

$$ISR = P_t/P_h \quad [9]$$

$$CTRI = \left(\frac{PG\%_t}{PG\%_h} + \frac{PTL_t}{PTL_h} + \frac{PGT_{min_t}}{PGT_{min_h}} + \frac{PG_{opt_t}}{PG_{opt_h}} + \frac{PG_{max_t}}{PG_{max_h}} + \frac{PTLT_{min_t}}{PTLT_{min_h}} + \frac{PTLT_{opt_t}}{PTLT_{opt_h}} + \frac{PTLT_{max_t}}{PTLT_{max_h}} \right) \quad [10]$$

Experiment II

Plant Husbandry

The study was conducted in four sunlit SPAR chambers made up of Plexiglas units that were 2.5 m tall, 2 m long, and 1.5 m wide to accommodate aerial plant parts, and with the soil portion of 1 m deep, 2 m long, and 0.5 m wide. The Plexiglas transmits 96.6 ± 0.5% of incoming photosynthetically active radiation (PAR; wavelength 400–700 nm) (Zhao et al., 2003). Each SPAR unit consists of a heating and cooling system and an environment monitoring and control system. During the experiment, the ambient solar radiation (285–2800 nm) measured with a pyranometer (Model 4-48, The Eppley Laboratory Inc., Newport, RI) was 21.2 ± 0.5 MJ m^{-2} d^{-1}. The SPAR facility has the capability to precisely control temperature at predetermined set points for plant growth studies under near ambient levels of PAR. Details of operation and control of SPAR chambers have been described by Reddy et al. (2001).

On the basis of the CTRI from Exp. I, one genotype from each of the tolerant, intermediate, and sensitive groups—Deltagrow (DG) 5630RR (Maturity Group V, glyphosate-tolerant), PI 471938 (Maturity Group VI), and Stalwart III (Maturity Group III), respectively, were selected for Exp. II. Seeds were sown on 5 Aug. 2003 in pots filled with fine sand. Fifteen 2.5-L pots (five pots for each genotype) were arranged randomly in each SPAR chamber. Plants were watered three times a day with half-strength Hoagland's nutrient solution (Hewitt, 1952) through an automated and computer-controlled drip system. The delivery rate was 75 mL min^{-1} for 3 to 9 min depending on growth stage, at 0800, 1200, and 1600 h to ensure favorable nutrient and water conditions for plant growth (Reddy et al., 2001). Emergence was observed at 5 DAS. Variable-density black shade cloths around the edges of plants were adjusted regularly to match plant height to simulate natural shading in the presence of other plants. The experiment was terminated a few days after flowering, at 51 DAS.

Treatments

The chambers were maintained at temperatures of 30/22°C (day/night) up to 10 DAS. Thereafter, two chambers were

set at each of the two temperature treatments (30/22 and 38/30°C) and two carbon dioxide concentrations ($[CO_2]$) of 360 and 720 μL L^{-1}. In each SPAR chamber, air temperature was monitored and adjusted every 10 s throughout the day and night and maintained within ± 0.5°C, while $[CO_2]$ was monitored and maintained within ± 10 μL L^{-1} of the treatment set points during the day. The daytime temperature was initiated at sunrise and returned to the nighttime temperature 1 h after sunset. Constant humidity was maintained by operating solenoid valves that injected chilled water through the cooling coils located outside the air handler of each chamber. The cooling coils condense excess water vapor from the chamber air to regulate relative humidity at 60%.

Measurements

Flower and Pollen Parameters

Length of flower was measured from tip of the standard petal to the base of the calyx on 20 fresh flowers randomly picked from five plants of each genotype at 51 DAS. Mature anthers were collected 1 d before anthesis from five different plants to determine the number of pollen grains produced per anther. Pollen number was counted by placing a single anther in a water drop on a glass slide and squashed with a needle, and the pollen grains dispersed in the drop of water were counted (Bennett, 1999). Number of pollen from five anthers per genotype under each treatment was counted. Pollen germination and PTLs were measured as described in Exp. I but were germinated at the respective growth temperatures of Exp. II, 2 d before the final harvest.

Pollen Morphology

Pollen morphology was studied in all three genotypes at 50 DAS. Fresh flowers collected between 1900 and 2100 h each day before anthesis and stored in FAA [formaldehyde (2.4 mL): glacial acetic acid (1.6 mL): ethyl alcohol (60 mL)] solution were fixed in 3% glutaraldehyde in 0.1 *M* phosphate buffer, pH 7.2 overnight at 4°C for observation under a scanning electron microscope (SEM). After fixation, specimens were rinsed in buffer, postfixed in 2% osmium tetroxide in 0.1 *M* phosphate buffer for 2 h, then rinsed in distilled water, dehydrated in an ethanolic series, and critical point dried in a Polaron E 3000 Critical Point Dryer (Quorum Technologies, Newhaven, UK). Specimens were mounted on aluminum stubs; sputter coated with gold in a Polaron E 5100 sputter coater (Quorum Technologies), and viewed in a LEO Stereoscan 360 SEM (LEO Electron Microscopy, Thornwood, NY, USA) at an accelerating voltage of 15 kV. Images were recorded on Polaroid Type 55 film (Polaroid, Cambridge, MA, USA).

Data Analysis

To test the significance of temperature and genotypes and their interactive effects on all the pollen parameters, data were statistically analyzed using two-way ANOVA by SAS procedures (SAS Institute, 1998). Five pots per each of the three genotypes were arranged in each SPAR unit following a completely randomized design. The LSD test at $P = 0.05$ was used to distinguish treatment difference means of the parameters measured in this study. Since there were no significant differences ($P = 0.05$) between the two $[CO_2]$ treatments at each of the temperature conditions, the data were averaged before subjecting to statistical analysis.

RESULTS

Experiment I

Response of Pollen Germination to Temperature

During the pollen collection and measurement period, the average ambient air temperature corresponding to the three sets of dates were 27.2 ± 0.4, 25.9 ± 0.7, and 26.1 ± 0.2°C, respectively. Before determining percentage PG, pollen grains of 44 soybean cultivars were incubated for 24 h on the in vitro germination medium. For clarity, only data and the response functions of four different soybean genotypes that showed variability for PG in response to the temperature treatments are shown in Fig. 1A. Of the various linear and nonlinear models tested, the quadratic model described the response of PG well due to its high coefficient of determination (R^2) and least root mean square error.

There was significant variation ($P = 0.001$) among genotypes in the maximum percentage of germination, with a low of 70% (P 93B67RR) to a high of 93% (HBK 4920RR), and with an average of 81% (Table 1). The

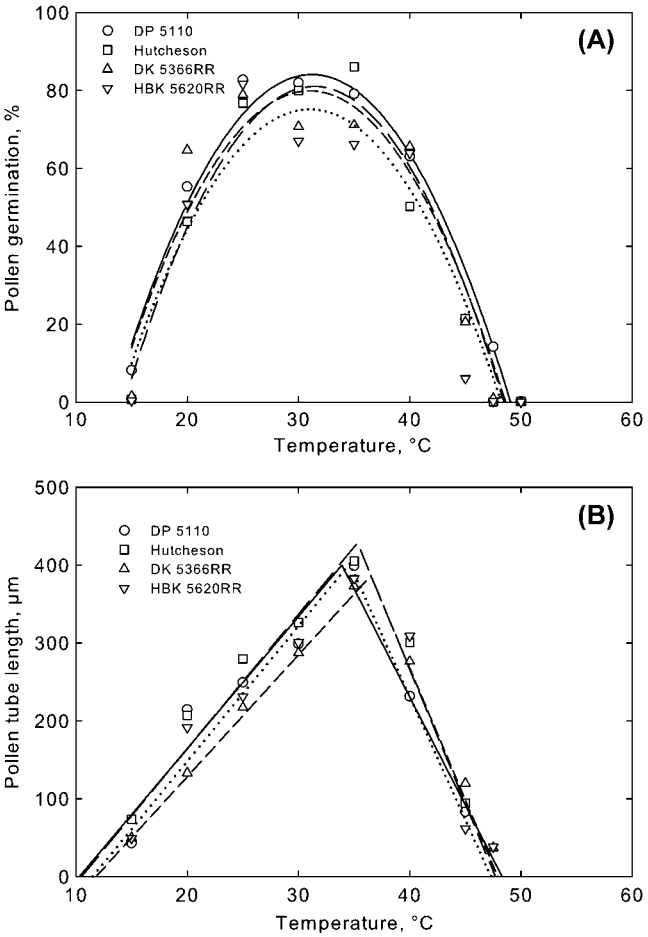

Fig. 1. Typical in vitro (A) pollen germination and (B) pollen tube length responses to temperature along with the fitted quadratic and modified bilinear lines, respectively, of four soybean genotypes (DP 5110, Hutcheson, DK5366RR, and HBK 5620RR). The symbols are recorded germination percentages and pollen tube lengths after 24 h and the lines (solid, DP 5110; long dash, Hutcheson; short dash, DK 5366RR; dots, HBK 5620RR) are predicted values by the respective fitted equations.

Table 1. Cell membrane thermostability (CMTS), maximum pollen germination (PG), cardinal temperatures (minimum temperature, T_{min}; optimum temperature, T_{opt}; and maximum temperature, T_{max}), and model goodness of fit (R^2) of PG response to temperature of 44 soybean genotypes along with SE.

Genotypes	Maturity group	CMTS	Maximum PG	Cardinal temperature			R^2
				T_{min}	T_{opt}	T_{max}	
		%		°C			
Williams 82	III	74.0 ± 4.3	79.5	13.1 ± 0.1	29.9 ± 0.4	46.7 ± 0.6	0.94
P 93B67RR	III	60.0 ± 5.7	70.1	14.0 ± 0.1	30.2 ± 0.0	46.5 ± 0.0	0.91
Stalwart III	III	74.9 ± 2.1	75.7	11.8 ± 0.4	28.9 ± 0.2	46.1 ± 0.0	0.97
DK 3964RR	III	77.4 ± 4.0	84.8	13.9 ± 0.0	30.8 ± 0.1	47.6 ± 0.2	0.96
DP 3861RR	III	66.5 ± 4.6	84.6	14.2 ± 0.1	30.5 ± 0.0	46.9 ± 0.1	0.94
Maverick	III	67.4 ± 3.0	85.1	14.4 ± 0.1	30.8 ± 0.1	47.2 ± 0.0	0.96
DP 4748S	IV	53.8 ± 5.8	84.7	14.6 ± 0.0	31.2 ± 0.0	47.8 ± 0.1	0.95
Progeny 4910	IV	65.0 ± 6.7	87.5	14.4 ± 0.1	30.7 ± 0.1	47.1 ± 0.1	0.95
DK 4868RR	IV	67.4 ± 6.3	88.9	13.9 ± 0.2	30.3 ± 0.1	46.6 ± 0.1	0.98
HBK 4920RR	IV	57.8 ± 6.5	92.9	14.2 ± 0.1	30.8 ± 0.1	47.4 ± 0.1	0.91
DP 4690RR	IV	60.4 ± 2.3	89.2	14.2 ± 0.0	30.8 ± 0.1	47.5 ± 0.2	0.97
RT 4809RR	IV	69.4 ± 4.7	82.1	13.2 ± 0.1	30.6 ± 0.5	48.0 ± 0.3	0.98
AG 4403RR	IV	78.0 ± 3.8	90.9	14.5 ± 0.0	31.0 ± 0.0	47.5 ± 0.0	0.94
DT 97-4290	IV	67.5 ± 5.7	84.3	14.4 ± 0.0	31.0 ± 0.0	47.6 ± 0.1	0.94
DP 3478RR	IV	74.2 ± 3.1	82.0	14.0 ± 0.0	30.7 ± 0.0	47.5 ± 0.1	0.93
SN93-6181	IV	77.0 ± 5.4	73.0	14.4 ± 0.1	31.3 ± 0.1	48.3 ± 0.1	0.96
Stressland	IV	77.7 ± 4.0	80.4	15.2 ± 0.0	31.6 ± 0.0	48.1 ± 0.1	0.96
AG 4902RR	IV	73.4 ± 2.1	75.3	13.7 ± 0.1	30.6 ± 0.1	47.5 ± 0.1	0.94
DK 4965RR	IV	67.6 ± 5.0	76.5	14.8 ± 0.1	31.7 ± 0.1	48.7 ± 0.2	0.96
DG 4950RR	IV	77.0 ± 4.1	84.8	14.8 ± 0.1	30.9 ± 0.0	47.1 ± 0.0	0.96
DP 4331RR	IV	70.8 ± 4.2	79.4	14.2 ± 0.1	30.9 ± 0.1	47.6 ± 0.1	0.94
DP 4933RR	IV	58.7 ± 8.7	82.8	12.3 ± 0.2	30.1 ± 0.0	48.0 ± 0.1	0.92
SG 498RR	IV	70.4 ± 2.4	84.4	14.3 ± 0.3	30.7 ± 0.1	47.1 ± 0.1	0.94
P 9594	V	55.5 ± 5.8	79.6	13.4 ± 0.2	30.0 ± 0.2	46.6 ± 0.2	0.98
DP 5110	V	77.4 ± 5.1	83.6	13.4 ± 0.2	30.4 ± 0.1	47.5 ± 0.1	0.87
Hutcheson	V	73.4 ± 2.6	83.3	13.0 ± 0.2	30.5 ± 0.3	47.9 ± 0.3	0.94
DK5366RR	V	85.7 ± 2.5	83.6	13.9 ± 0.3	30.8 ± 0.2	47.7 ± 0.6	0.96
HBK 5620RR	V	82.4 ± 8.2	80.1	14.4 ± 0.2	30.5 ± 0.2	46.7 ± 0.1	0.92
DP 5915RR	V	78.3 ± 2.4	82.5	14.3 ± 0.3	30.4 ± 0.1	46.6 ± 0.1	0.92
D 95B43RR	V	74.7 ± 4.3	75.2	14.6 ± 0.5	30.7 ± 0.2	46.8 ± 0.1	0.95
D68-0099	V	76.0 ± 2.8	76.9	9.8 ± 0.3	28.2 ± 0.2	46.5 ± 0.1	0.90
DG 5630RR	V	74.0 ± 8.4	85.2	11.4 ± 0.2	29.2 ± 0.1	47.0 ± 0.0	0.95
DP 5414RR	V	75.2 ± 3.5	82.4	14.2 ± 0.2	30.3 ± 0.1	46.5 ± 0.0	0.96
DP 5644RR	V	77.3 ± 3.8	87.3	11.7 ± 0.1	29.3 ± 0.1	46.9 ± 0.1	0.94
DP 5806RR	V	77.2 ± 4.3	81.2	12.7 ± 0.1	29.9 ± 0.0	47.1 ± 0.1	0.98
D68-0102	VI	69.0 ± 1.9	80.4	12.4 ± 0.1	29.5 ± 0.1	46.6 ± 0.1	0.91
D88-5320	VI	81.4 ± 3.9	74.3	8.7 ± 0.1	27.7 ± 0.0	46.7 ± 0.1	0.96
PI416937	VI	74.4 ± 2.0	79.1	12.0 ± 0.1	30.3 ± 0.1	48.7 ± 0.1	0.97
PI471938	VI	65.2 ± 1.4	83.2	11.9 ± 0.3	29.4 ± 0.1	46.9 ± 0.0	0.98
NTCPR 94	VI	77.0 ± 2.8	78.2	9.5 ± 0.1	28.0 ± 0.1	46.6 ± 0.0	0.97
N94-7784	VI	70.6 ± 3.7	84.0	14.2 ± 0.1	30.4 ± 0.1	46.6 ± 0.0	0.93
Arksoy	VI	74.0 ± 4.4	78.0	9.2 ± 0.5	28.2 ± 0.3	47.2 ± 0.1	0.95
Centennial	VI	85.7 ± 2.7	83.2	13.7 ± 0.1	30.2 ± 0.1	46.6 ± 0.1	0.96
Dare	VI	83.6 ± 3.2	79.1	12.7 ± 0.2	30.7 ± 0.1	48.6 ± 0.0	0.90
Mean		72.0	80.8	13.2	30.2	47.2	0.95
LSD		6.88***	5.8***	0.82***	0.43***	0.44***	

*** Significant at $P = 0.001$.

cardinal temperatures derived from the quadratic fit for PG differed significantly among cultivars. The magnitude of T_{min} ranged from 9.8°C (D68-0099) to 15.2°C (Stressland), with an average of 13.2°C. The T_{opt} ranged from 27.7°C (D88-5320) to 31.2°C (DP 4748S), with an average of 30.2°C. The T_{max} ranged from 46.1°C (Stalwart III) to 48.7°C (DK 4965RR), with an average of 47.2°C. The mean values for T_{min}, T_{opt}, and T_{max} for PG were 13.2, 30.2, and 47.2°C, respectively, with a mean R^2 of 0.94. The results showed that Maturity Group IV had the highest means for all three cardinal temperatures (T_{min}, T_{opt}, and T_{max}) in PG. Maturity group V had the highest mean for PG (83%). The three cardinal temperatures for PG in Maturity Groups III, IV, V, and VI were 13.5, 30.1, and 46.8°C; 14.1, 30.8, and 47.6°C; 13.0, 30.0, and 46.9°C; and 11.5, 29.3 and 47.1°C, respectively (Table 2). A significant correlation was observed between T_{min} and T_{opt} ($R^2 = 0.88$; Fig. 2A). No significant correlation was found between T_{max} and T_{min} values for PG. However, T_{opt} was slightly correlated with T_{max} ($R^2 = 0.31$; Fig. 2B).

Response of Pollen Tube Length to Temperature

Similar to PG, genotypes significantly differed for PTL at different temperatures. The variability for PTL in response to temperature of four genotypes is illustrated in Fig. 1B. The modified bilinear model best described (mean R^2 of 0.94) the response of PTL to temperature in all genotypes. The maximum PTL ranged from 380 μm (P 416937) to 524 μm (DP 5644RR), with a mean of 437 μm (Table 3).

Cardinal temperatures for PTL differed significantly ($P = 0.001$) among genotypes and maturity groups (Fig. 3; Table 2 and 3). Values of optimum temperature ranged from 33.3°C (Progeny 4910) to 39.5°C (DP 5806RR), with an average of 36.0°C. The minimum temperature ranged from 10.2°C (Stalwart III) to 14.3°C

Table 2. Cell membrane thermostability (CMTS), pollen germination (PG) percentage, and pollen tube length (PTL) and cardinal temperatures (minimum temperature, T_{min}; optimum temperature, T_{opt}; and maximum temperature, T_{max}) averaged across maturity groups of soybean genotypes, along with their statistical significance.

Parameter	Maturity Group III	IV	V	VI	LSD
CMTS, %	69.5 ± 2.4	68.8 ± 1.8	75.4 ± 1.3	74.9 ± 2.4	4.5*
PG, %	82.3 ± 2.4	83.2 ± 1.2	83.4 ± 1.3	83.1 ± 0.7	2.5ns†
T_{min}, °C	13.6 ± 0.4	14.2 ± 0.2	13.1 ± 0.4	11.6 ± 0.7	0.7*
T_{opt}, °C	30.2 ± 0.3	30.9 ± 0.1	30.0 ± 0.2	29.4 ± 0.4	0.4*
T_{max}, °C	46.8 ± 0.2	47.6 ± 0.1	47.0 ± 0.1	47.2 ± 0.3	0.3*
PTL, µm	444 ± 17.2	447 ± 7.6	420 ± 7.7	436 ± 12.8	17.3*
T_{min}, °C	12.1 ± 0.6	12.2 ± 0.3	11.7 ± 0.4	12.4 ± 0.4	0.8ns
T_{opt}, °C	35.2 ± 0.7	35.2 ± 0.3	36.8 ± 0.7	37.1 ± 0.6	0.9*
T_{max}, °C	46.5 ± 0.30	47.7 ± 0.1	47.1 ± 0.3	46.6 ± 0.2	0.4*

* Significant at $P = 0.05$.
† ns = not significant.

(DP 4933RR), with an average of 12.1°C. Maximum temperature ranged from 45.3°C (Williams) to 48.5°C (DK 4868RR), with an average of 47.0°C. The mean values for T_{min}, T_{opt}, and T_{max} for PTL were 12.1, 36.0, and 47.0°C, respectively. Meanwhile, PTL of Maturity Group VI showed the highest means in T_{min}, and T_{opt}, whereas Maturity Group IV had the uppermost means of T_{max} and mean amount for PTL (Table 2). Average PTL was similar in Maturity Groups III, V, and VI, but had a higher mean value in Maturity Group IV. The three cardinal temperatures for PTL for Maturity Group III, IV, V, and VI were 12.1, 35.2, and 46.4°C; 12.1, 35.1, and 47.7°C; 11.6, 36.8, and 47.1°C; and 12.3, 37.0, and 46.6°C, respectively (Table 2). There were no significant correlations between cardinal temperatures of PTL.

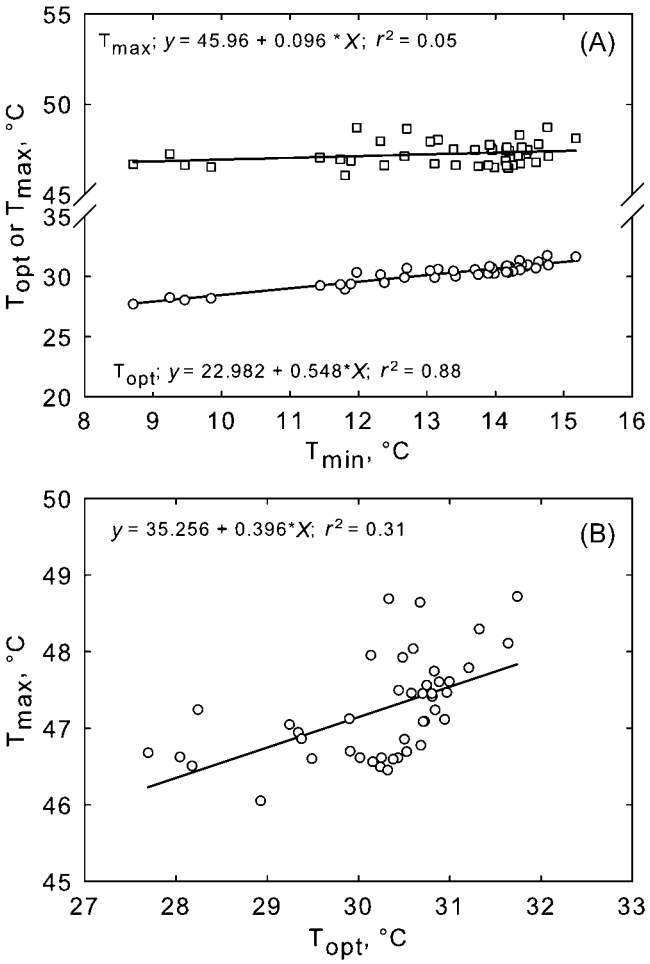

Fig. 2. Relationship between (A) minimum (T_{min}) and optimum (T_{opt}) and maximum (T_{max}) temperatures, and (B) T_{opt} and T_{max} for pollen germination of 44 soybean genotypes.

Cell Membrane Thermostability

The leaf CMTS differed significantly among genotypes and ranged from 54% in DP 4748S to 86% in Centennial and DK 5366RR, with an average of 72% (Table 1). However, the CMTS had a poor correlation with all the pollen parameters (data not shown). Genotypes with low and high CMTS were randomly distributed in all maturity groups. On average, Maturity Groups III and IV showed significantly ($P = 0.05$) lower CMTS values compared with Groups V and VI.

Cumulative Temperature Response Index

The cumulative of individual temperature response showed that each of the PG and pollen tube parameters contributed to the variability of responses to temperature among 44 soybean genotypes (Table 4). Among the 44 soybean genotypes, 13 belonged to tolerant, nine to sensitive, and 22 to intermediate group. Genotypes which had CTRI < 7.120 were classified as sensitive to high temperature due to lower ability or smaller impact on whole response compared with intermediate genotypes that had a score > 7.13 and < 7.31. Tolerant genotypes were those that had a high CTRI value > 7.31 (Table 4). Genotype D88-5320 was most sensitive to temperature with the lowest CTRI of 6.762, while genotype AG 4403RR was the most tolerant with a CTRI of 7.5. Figure 3A and 3B shows the response of mean PG and PTL to temperature of sensitive, intermediate, and tolerant groups based on CTRI. The mean maximum PG differed significantly ($P = 0.05$) among groups and the values were 77.2 for sensitive, 82.0 for intermediate, and 84.9 for the tolerant group (data not presented). The mean maximum PTLs, however, were not significantly different among the groups: 426.5 for the sensitive, 439.5 for the intermediate, and 442.6 for the tolerant group. The mean cardinal temperatures of T_{min} and T_{opt} for PG and T_{min} and T_{max} for PTLs differed significantly ($P = 0.01$ to 0.05) among groups, while T_{max} for PG and T_{opt}

Table 3. Maximum pollen tube length (PTL), cardinal temperatures (minimum temperature, T_{min}, optimum temperature, T_{opt}, and maximum temperature, T_{max}), and model goodness of fit (R^2) of pollen tube response to temperature of 44 soybean genotypes along with SE.

Genotypes	Maturity group	Maximum PTL	Cardinal temperature (°C)			
			T_{min}	T_{opt}	T_{max}	R^2
		μm				
Williams 82	III	416	13.4 ± 0.4	34.2 ± 0.2	45.3 ± 0.1	0.93
P 93B67RR	III	431	10.5 ± 0.5	34.3 ± 0.4	47.2 ± 0.3	0.90
Stalwart III	III	486	10.2 ± 0.3	34.6 ± 0.0	46.3 ± 0.1	0.96
DK 3964RR	III	421	11.7 ± 2.3	34.6 ± 0.1	47.1 ± 0.8	0.94
DP 3861RR	III	408	14.2 ± 0.0	38.5 ± 0.0	47.0 ± 0.0	0.95
Maverick	III	407	12.4 ± 1.3	34.8 ± 0.0	45.8 ± 0.2	0.96
DP 4748S	IV	427	11.4 ± 0.1	34.7 ± 0.0	47.0 ± 0.0	0.96
Progeny 4910	IV	413	10.9 ± 0.0	33.3 ± 0.0	48.2 ± 0.0	0.92
DK 4868RR	IV	454	13.3 ± 2.0	34.9 ± 0.0	48.5 ± 0.4	0.92
HBK 4920RR	IV	415	13.3 ± 1.1	35.9 ± 0.1	48.3 ± 1.0	0.91
DP 4690RR	IV	411	12.2 ± 0.0	34.5 ± 0.1	47.2 ± 0.0	0.92
RT 4809RR	IV	413	10.5 ± 0.3	34.8 ± 0.1	47.2 ± 0.1	0.91
AG 4403RR	IV	399	13.9 ± 0.0	36.0 ± 0.2	47.8 ± 0.0	0.97
DT 97-4290	IV	423	10.7 ± 0.0	35.9 ± 0.0	48.4 ± 0.0	0.94
DP 3478RR	IV	415	11.5 ± 0.0	35.6 ± 0.0	48.4 ± 0.0	0.96
SN93-6181	IV	464	11.4 ± 0.0	34.3 ± 0.1	47.7 ± 0.0	0.93
Stressland	IV	455	11.9 ± 0.3	33.4 ± 0.3	47.8 ± 0.3	0.92
AG 4902RR	IV	444	13.0 ± 0.1	34.6 ± 0.0	47.7 ± 0.0	0.95
DK 4965RR	IV	392	14.2 ± 0.0	36.9 ± 0.0	47.7 ± 0.0	0.91
DG 4950RR	IV	438	10.4 ± 0.1	38.9 ± 0.0	47.5 ± 0.1	0.95
DP 4331RR	IV	440	12.7 ± 0.0	34.5 ± 0.0	47.0 ± 0.0	0.96
DP 4933RR	IV	416	14.3 ± 0.4	34.1 ± 0.1	47.0 ± 0.2	0.89
SG 498RR	IV	461	10.7 ± 0.2	34.8 ± 0.0	47.4 ± 0.0	0.95
P 9594	V	493	10.8 ± 0.3	35.9 ± 0.0	47.1 ± 0.0	0.98
DP 5110	V	478	10.3 ± 0.3	33.9 ± 0.1	48.3 ± 0.0	0.95
Hutcheson	V	458	10.5 ± 0.4	35.3 ± 0.0	47.7 ± 0.3	0.97
DK5366RR	V	414	11.8 ± 0.0	36.5 ± 0.1	47.9 ± 0.0	0.99
HBK 5620RR	V	406	11.5 ± 0.2	34.6 ± 0.1	47.3 ± 0.0	0.99
DP 5915RR	V	481	11.1 ± 0.1	39.5 ± 0.1	47.2 ± 0.0	0.95
D 95B43RR	V	422	14.1 ± 1.5	33.8 ± 0.0	43.7 ± 0.1	0.94
D68-0099	V	445	12.9 ± 1.0	39.3 ± 0.0	46.5 ± 0.0	0.96
DG 5630RR	V	499	13.9 ± 0.0	36.1 ± 0.0	47.6 ± 0.0	0.93
DP 5414RR	V	422	10.3 ± 0.3	39.5 ± 0.1	47.2 ± 0.0	0.97
DP 5644RR	V	524	12.4 ± 1.8	39.9 ± 0.0	47.8 ± 0.4	0.99
DP 5806RR	VI	488	11.7 ± 0.7	39.6 ± 0.2	47.6 ± 0.4	0.98
D68-0102	VI	452	12.4 ± 1.2	39.8 ± 0.0	46.5 ± 0.1	0.97
D88-5320	VI	382	10.2 ± 0.0	34.6 ± 0.0	47.1 ± 0.0	0.97
PI416937	VI	380	12.9 ± 0.0	38.1 ± 0.0	46.9 ± 0.0	0.94
PI471938	VI	457	14.0 ± 0.2	36.4 ± 0.1	46.2 ± 0.0	0.94
NTCPR 94	VI	439	12.2 ± 0.3	37.3 ± 0.0	46.4 ± 0.0	0.95
N94-7784	VI	385	11.4 ± 1.6	35.7 ± 0.2	46.4 ± 0.3	0.95
Arksoy	VI	457	14.2 ± 0.6	34.5 ± 0.1	45.6 ± 0.7	0.98
Centennial	VI	482	11.9 ± 0.8	37.5 ± 0.0	46.4 ± 0.0	0.99
Dare	VI	441				
Mean		437	12.1	36.1	47.0	0.95
LSD		11.44***	1.34***	0.313***	0.48***	

*** Significant at $P = 0.001$.

for PTLs were different among groups based on CTRI (Tables 1 and 3).

Experiment II

Flower Morphology, Pollen Production, and Pollen Viability

To understand the mechanisms of high temperature injury on reproductive processes of soybean, one genotype was selected from each CTRI group based on Exp. I. They were Stalwart III (sensitive), PI 471938 (intermediate), and DG 5630RR (tolerant). The supraoptimal temperature (38/30°C) in comparison with the optimum temperature (30/22°C) modified the reproductive parameters of three soybean genotypes grown in SPAR units. Flower length (mm) increased on exposure to supraoptimal temperature in Stalwart III (sensitive) and DG 5630RR (tolerant), while a slight decrease was recorded in the intermediate genotype (Table 5). Supraoptimal temperature reduced pollen grain number by 57% in the sensitive genotype, Stalwart III; by 19% in the intermediate genotype, PI 471938; and by 27% in the tolerant genotype, DG 5630RR (Table 5). Similarly, PG was reduced at supraoptimal temperatures. Pollen germination percentage of 82, 86, and 82% at optimum temperature were reduced to 22, 46, and 43% at supraoptimum temperature in Stalwart III, PI 471938, and DG 5630RR, respectively. On exposure to supraoptimal temperature, PTL was reduced by 34% in the sensitive genotype, Stalwart III, while a 17% reduction was recorded in the intermediate genotype, PI 471938. In contrast, a 4% increase in final tube length was recorded for the tolerant genotype, DG 5630RR.

Pollen Grain Morphology

Scanning electron microscopic images of pollen produced for plants grown in optimum and high tempera-

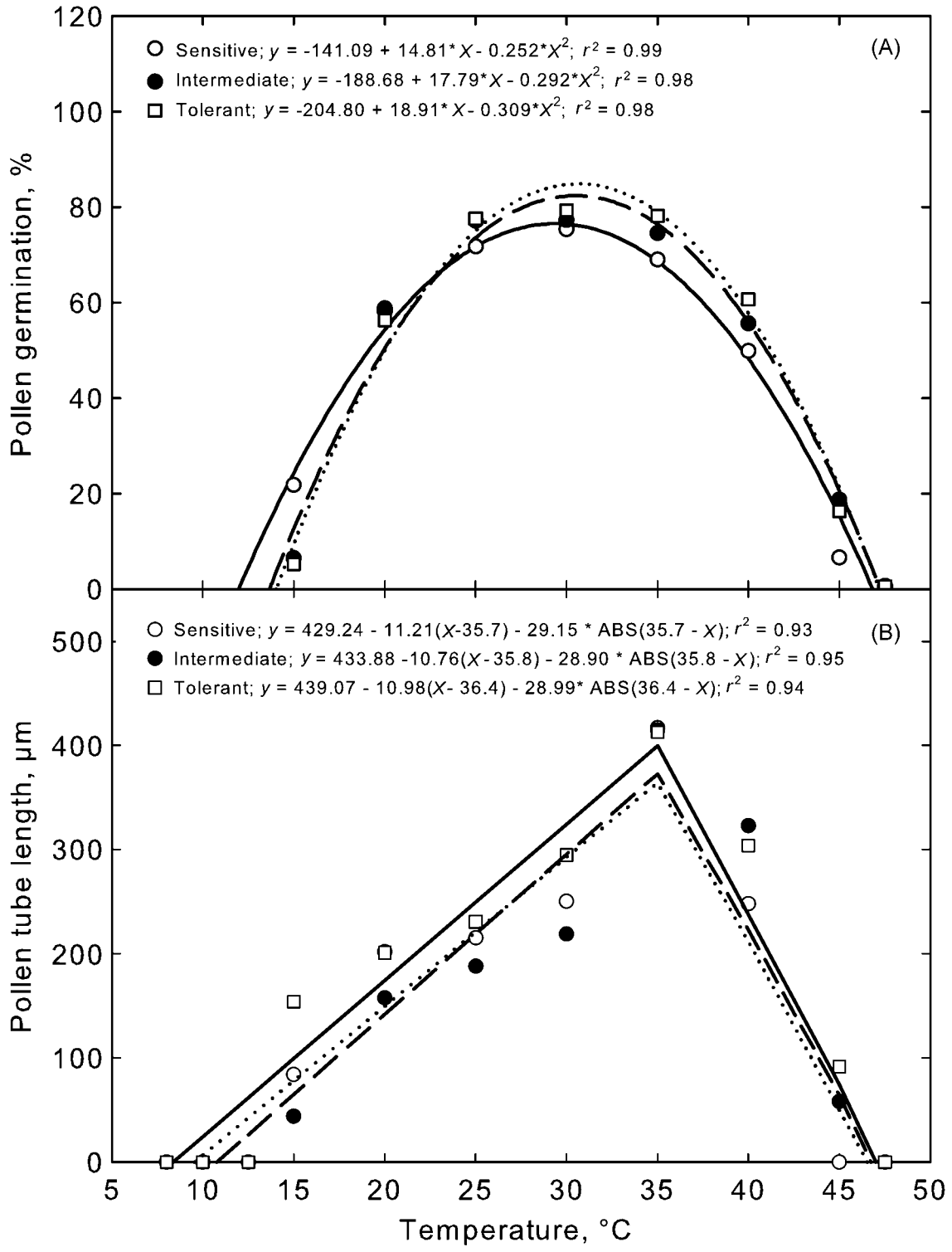

Fig. 3. Response of mean (A) pollen germination and (B) pollen tube length to temperature of sensitive, intermediate, and tolerant groups of soybean genotypes based on cumulative temperature response index (CTRI).

ture and 720 µL L^{-1} [CO_2] conditions are presented in Fig. 4 and 5. Pollen produced from the plants grown at the optimum temperature conditions showed no visible morphological differences between the sensitive, intermediate, and tolerant genotypes (Fig. 4A, 4C, 4E, 5A, 5C, and 5E). When plants are exposed to high temperature, pollen abnormalities were observed in all three genotypes (Fig. 4B, 4D, 4F, 5B, 5D, and 5F). The magnitude of the pollen morphological abnormalities, however, was more evident in the sensitive (Stalwart III) and intermediate (PI 471938) genotypes than in the tolerant (DG 5630RR) genotype. In general, fewer numbers of pollen grains were shriveled in the tolerant genotype grown at high temperature compared with the

Table 4. Classification of soybean genotypes into tolerant, intermediate, and sensitive groups based on cumulative temperature response index (CTRI; unitless), along with individual score of CTRI values in parenthesis. The CTRI is the sum of individual component responses of maximum pollen germination (PG), maximum pollen tube length (PTL), and cardinal temperatures for PG and PTL.

Genotype classification based on CTRI		
Tolerant (CTRI = 7.50–7.31)	**Intermediate (CTRI = 7.12–7.30)**	**Sensitive (CTRI = 6.76–7.11)**
DP 5915RR (7.310)	Williams 82 (7.144)	D88-5320 (6.762)
DP 4690RR (7.314)	Hutcheson (7.175)	Arksoy (6.825)
DG 4950RR (7.347)	DP 5110 (7.193)	PI416937 (6.870)
DG 5630RR (7.355)	Progeny 4910 (7.206)	D68-0099 (6.902)
Stressland (7.342)	P 9594 (7.211)	Stalwart III (6.912)
DP 5414RR (7.395)	D68-0102 (7.220)	P 93B67 (6.976)
DP 5806RR (7.401)	DP 3478RR (7.228)	NTCPR 94 (6.988)
Centennial (7.447)	DARE (7.229)	RT 4809 (7.072)
DK 4965RR (7.451)	N94-7784 (7.232)	HBK 5620RR (7.111)
DK 4868RR (7.453)	SN93-6181 (7.235)	
DP 3861RR (7.472)	DK 3964RR (7.237)	
HBK 4920RR (7.497)	P 95B43RR (7.247)	
AG 4403RR (7.500)	DP 4933RR (7.256)	
	AG 4902RR (7.258)	
	PI 471938 (7.263)	
	DT 97-4290 (7.264)	
	Maverick (7.269)	
	SG 498RR (7.270)	
	DP 5644RR (7.284)	
	DK5366RR (7.287)	
	DP 4748S (7.295)	
	DP 4331RR (7.30)	

same genotype grown under optimum temperature conditions. Also, pollen produced from plants grown in optimum temperature conditions were triporate, that is, having three protruding apertures (Fig. 4A, 4C, and 4E), while the pollen produced from plants grown at the high temperature had fewer apertures in all genotypes (Fig. 4B, 4D, and 4F). The effect was greater in the sensitive and intermediate genotypes than in the tolerant genotype. When observed under higher magnification, differences in pollen exine structure were observed among the three genotypes and treatments. The columellae heads were well defined on the pollen grains of plants grown at optimum temperature, but severe reductions were observed under high-temperature grown plants in the sensitive genotype, Stalwart III. In general, no such decrease in columellae heads was visible in the intermediate and tolerant genotypes under supraoptimum temperature compared with optimum temperature-grown plants (Fig. 5).

DISCUSSION

Plant growth and development, particularly reproductive processes such as PG, pollen tube growth, and fruit set are affected by temperature more than by any other environmental factor when water is not a limiting factor. This is the first study in soybean that targeted a large number of genotypes from Maturity Groups III to VI for intraspecific variability of PG and tube growth responses to temperature. Even though genotypes differed in their flowering dates, similar temperature conditions prevalent during the flowering period and fertilization and irrigation conditions avoided confounding effects of ambient environment on PG. In the present study, in vitro PG and pollen tube growth were affected by temperature. Even though all 44 genotypes responded to temperature similarly, significant intraspecific differences were observed for all PG and pollen tube parameters. This is the first report to show the intraspecific variability in soybean to a wide range of temperatures even though several others reported the influence of temperature on fruit set and seed yield of soybean (Baker et al., 1989; Allen and Boote, 2000).

In the present in vitro study, significant differences in PG and PTL were observed between genotypes, rendering in vitro-based studies of relevance to the in vivo situation. Pollen germination percentage observed in the present study was higher, with a mean value of 81%, compared with several other published studies; 44% in cotton (Kakani et al., 2005), 56% in groundnut (Kakani et al., 2002), 68% in pigeon pea [*Cajanus cajan* (L.) Millsp.] (Jayaprakash and Sarla, 2001), and 64 to 76% in soybean (Gwata et al., 2003; Koti et al., 2005). Similar to PG, genotypic differences were also recorded for PTL. Maximum PTLs, with a mean of 437 μm, were comparable with those recorded in earlier studies with

Table 5. Influence of temperature and its interaction on soybean flower morphology, pollen production, pollen germination (PG), and tube length measured 60 d after seed emergence. On the basis of the cumulative temperature response index from Exp. I, one genotype from each of the tolerant, intermediate, and sensitive groups—Deltagrow (DG) 5630RR (Maturity Group V, glyphosate-tolerant), PI 471938 (Maturity Group VI), and Stalwart III (Maturity Group III), respectively, were selected. The response index is calculated as percentage between the values at optimum and high temperature treatment for each parameter.

Parameter	Genotype	Day/night temperature treatments 30/22°C	38/30°C	Response index
		Pollen production, no. anther^{-1}		
	Stalwart III	537 ± 4.4	243.0 ± 5.9	−54.7
	PI 471938	285 ± 9.5	229.5 ± 7.4	−19.5
	DG 5630RR	264 ± 13.3	192.4 ± 2.2	−27.1
LSD G	16.05***			
LSD T	13.10***			
LSD G × T	22.70***			
		PG, %		
	Stalwart III	81.8 ± 2.7	22.2 ± 1.8	−72.8
	PI 471938	86.4 ± 3.6	46.2 ± 1.7	−46.5
	DG 5630RR	82.3 ± 2.9	43.0 ± 6.2	−47.7
LSD G	7.06***			
LSD T	5.07***			
LSD G × T	9.98***			
		PTL, μm		
	Stalwart III	303.0 ± 13.0	200.1 ± 10.0	−33.7
	PI 471938	310.7 ± 10.0	260.1 ± 9.0	−17.7
	DG 5630RR	220.8 ± 10.6	230.6 ± 10.0	3.3
LSD G	1.59***			
LSD T	1.95***			
LSD G × T	2.76***			
		Flower length, mm		
	Stalwart III	8.7 ± 0.1	9.2 ± 0.1	6.4
	PI 471938	9.8 ± 0.1	9.4 ± 0.1	−4.1
	DG 5630RR	9.5 ± 0.2	9.7 ± 0.3	1.6
LSD G	0.43*			
LSD T	0.35ns†			
LSD G × T	0.61ns			

* Significant at $P = 0.05$.
*** Significant at $P = 0.001$.
† ns = not significant.

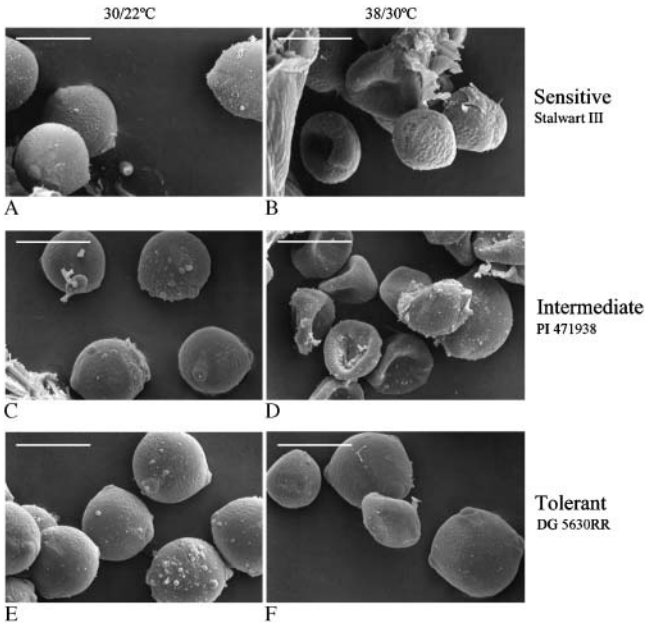

Fig. 4. Scanning electron microscopic images of pollen grains from pollen of three soybean genotypes, sensitive (Stalwart III, A and B), intermediate (PI 471938, C and D), and tolerant (DG 5630RR, E and F) at optimal temperature of 30/22°C (A, C, and E) and supraoptimal temperature of 38/30°C (B, D, and F). The white line in each picture is 20.00 μm in length.

artificial germination media for several other crops [1000–1800 μm for corn (Binelli et al., 1985); 450–1400 μm for groundnut (Kakani et al., 2002); 20–60 μm for muskmelon (*Cucumis melo* L.) (Maestro and Alvarez, 1988); and 410–1400 μm for cotton (Kakani et al., 2005)]. Therefore, differences in percentage PG and PTLs observed at optimum temperature and in response to temperature in the current study provide evidence for soybean genotypic variability that can be used to screen genotypes for high temperature tolerance.

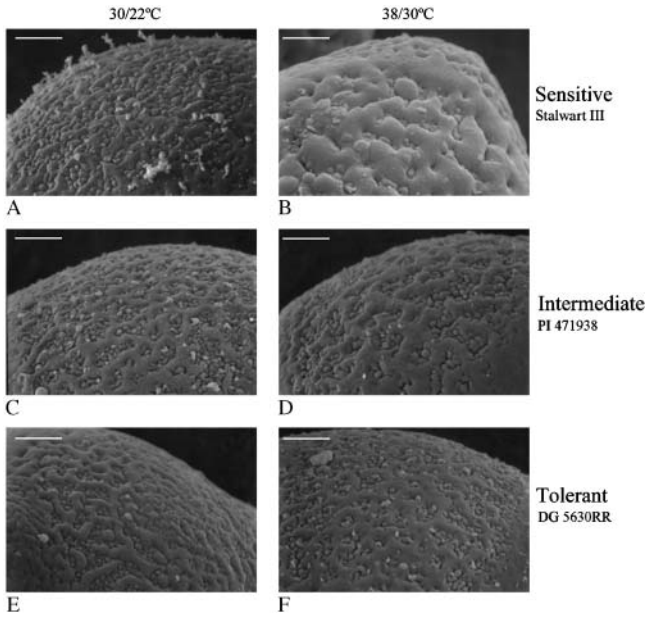

Fig. 5. Scanning electron microscopic images of pollen grains surface from pollen of three soybean genotypes, sensitive- Stalwart III (A and B), intermediate- PI 471938 (C and D) and tolerant- DG 5630RR (E and F) at optimal temperature of 30/22°C (A, C and E) and supraoptimal temperature of 38/30°C (B, D and F). The white line in each picture is 2.00 μm in length.

For pollen response to temperature use of linear and nonlinear regression models such as quadratic (Yan and Wallace, 1998), cubic, or higher-order polynomials (Tollenaar et al., 1979); beta distribution (Yin et al., 1995) and bilinear models (Omanga, 1994; Kakani et al., 2002, 2005) are not uncommon. In the current study, a quadratic model best described PG and a modified bilinear model was best for pollen tube growth in response to temperature for all soybean genotypes. The average cardinal temperatures for PG, namely T_{min} = 13.2°C, T_{opt} = 30.2°C, and T_{max} = 47.2°C, and cardinal temperatures for PTL, namely T_{min} = 12.1°C, T_{opt} = 36.1°C, and T_{max} = 47.0°C, are similar to those cardinal temperatures recorded in several other crops such as groundnut (Kakani et al., 2002), cotton (Kakani et al., 2005), and snake melon [*Cucumis melo flexuosus* (L.) Naudin.; Matlob and Kelly, 1973]. The identified response functions and parameters can be used in soybean crop models to improve their sensitivity under high-temperature conditions.

Significant differences were recorded between the maturity groups for maximum PG, pollen tube growth, and the cardinal temperatures for PG and PTL. The trends, however, were not consistent among the maturity groups; that is, no increasing or decreasing order with increase or decrease in maturity groups. This is not surprising since most of genotypes grown in USA were derived from a small number of ancestral soybeans brought into the country during the early 20th Century (Carter et al., 1993). This forbids breeders to select genotypes for heat tolerance. Therefore, changes projected in climate including extreme temperature events (Mearns et al., 2001) would severely damage soybean and other crop reproductive processes (Hall, 1992; Reddy et al., 1999).

Several physiological parameters have been used to identify genetic diversity for heat tolerance in crops such as wheat (*Triticum aestivum* L.; Reynolds et al., 1994), Pima cotton (*Gossypium barbadense* L.; Lu et al., 1998), and cowpea (Hall, 2004). In the present study, genotypic differences existed for the physiological parameter, CMTS, but no significant ($P > 0.05$) correlations were observed with pollen parameters. Recent studies in groundnut and cotton (Kakani et al., 2002, 2005) also support our findings that physiological parameters such as CMTS may not be a useful parameter for discriminating genotypes for high temperature tolerance during the reproductive phase.

The CTRI derived as the sum of all reproductive parameters varied significantly among genotypes. Physiologically, higher PG would permit more pollen tubes to reach the ovaries for successful fertilization and longer pollen tube elongation would favor early fertilization and avoid peak temperatures during the day. The high cardinal temperatures would mainly favor pollen survival and promote fertilization under current and projected high temperature climate. Hot climates are

characterized by warm mornings and high midday temperatures; hence, higher cardinal temperatures would favor survival and PG once released from anthers in hot climates. Therefore, the cumulative effect of pollen parameters provides reproductive advantage under high-temperature conditions. Among the 44 soybean genotypes, based on CTRI, 13 belonged to tolerant, 22 to intermediate, and nine to sensitive group. The CTRI values, averaged across tolerant, intermediate, and sensitive soybean genotypes based on reproductive parameters, were significantly different among groups except for maximum PTL and T_{max} for PTL. The strong negative linear relationship between day temperatures of 28 to 48°C and fruit number, fruit set, pollen production, and PG in groundnut (Prasad et al., 1999) and tomato (Sato et al., 2000) also suggests that pollen parameters are good indicators of a genotype's survivability in high temperature environments. Recently, Domínguez et al. (2005) demonstrated in tomatoes that PG and tube growth tolerance to cold temperature can be used to breed genotypes that can withstand cold temperatures. Hence, gametophytic selection can be utilized to screen genotypes for high temperature tolerance.

To understand pollen mechanisms and to test the hypotheses that CTRI is a useful tool to find soybean genotypic diversity, one sensitive (Stalwart III), one intermediate (PI 471938), and one tolerant (DG 5630RR) genotype from each CTRI-based group were selected and grown in controlled environments at optimum (30/22°C) and high (38/30°C) temperatures. All three genotypes showed no significant differences in pollen and flower parameters at optimum temperature, but the magnitude of differences were more clear and significant when plants were grown at high temperature. Sensitive genotype, Stalwart III, produced fewer pollen grains per anther at high temperature than at optimum, with significantly greater inhibition of PG and shorter pollen tubes compared with tolerant (DG 5630RR) and intermediate (PI 471938) genotypes. This confirms that CTRI using pollen parameters can be used to discriminate soybean genotypes response to high temperatures. However, the intermediate genotype (PI 471938) did not show intermediate characteristics for pollen number, PG, and flower length probably due to selection of a typical genotype from the pool of genotypes. These genotypes were classified differently in an earlier study by Koti et al. (2005) under multistress conditions. The differences in tolerance classification from the current study could be due to difference in parameters used in these two studies. Koti et al. (2005) used flower length, pollen number along with PG, and PTL used in the current study. Flower parts such as petals are not good indicators of heat tolerance (Crone et al., 2001) and the percentages of viable pollen or germination determine successful fertilization under high-temperature conditions (Schoper et al., 1987). Since pollen development during various phases of microsporogenesis is more sensitive to abiotic stresses such as high temperature (Peet et al., 1998; Prasad et al., 1999; Cross et al., 2003; Young et al., 2004), any factor that affects processes leading to pollen formation may result in loss of pollen viability. Therefore, pollen parameters would be good indicators in determining reproductive tolerance to high temperature.

In this study, the morphology of pollen was affected more severely and significantly in the heat-sensitive and heat-intermediate genotypes than in the heat-tolerant genotype at the high temperature. Compared with heat-tolerant genotypes, heat-intermediate and heat-sensitive genotypes showed more flattened and collapsed pollen in the high temperature conditions, resulting in poor PG at these conditions. High temperature (Cross et al., 2003; Koti et al., 2005) and water deficit stress (Shen and Webster, 1986) during reproductive development have been reported to cause similar abnormal exine with deeply pitted and smooth regions in soybean and flax (*Linum usitatissimum* L.). Since exine originates from the tapetum (Dickinson and Potter, 1976), any factor that disrupts normal pollen development will result in abnormal pollen because of the close association between tapetal layer and pollen development. High-temperature induced early degeneration of the tapetal layer (Ahmed and Hall, 1993; Suzuki et al., 2001) may contribute to more abnormal pollen production in the heat-sensitive and heat-intermediate genotypes than in the heat-tolerant genotype. The exact physiological mechanisms affecting poor PG at high temperatures need further investigation.

In conclusion, the sensitive nature of genotype tolerance to high temperature under in vitro conditions was partially confirmed by the in vivo study. Thus, pollen parameters would be more useful than those based on vegetative organs for screening soybean genotype tolerance to high temperature. There were no specific trends for heat tolerance among maturity groups. The narrow range of cardinal temperatures, and lack of ameliorating effects of elevated [CO_2] on pollen parameters, however, suggests that more exploration of soybean germplasm and its wild relatives is essential for developing soybean cultivars tolerant to current extreme climatic events and to projected future warmer climates. In addition, the identified cardinal temperatures and mean response functions could be incorporated into crop models to increase the accuracy of soybean simulation models under current extreme and projected future climates.

ACKNOWLEDGMENTS

The project was funded in part by the USDA UV-B Monitoring Program and the RSTC-NASA. We thank D. Brand for technical support and Bill Monroe, Electron Microscopy Center for his help during SEM studies. We acknowledge Drs. T. Carter, R. Smith and G. Williams for providing the seed of genotypes used in this study and Dr. Larry Heatherly for helpful discussions and suggestions. Contribution from the Department of Plant and Soil Sciences, Mississippi State University, Mississippi Agricultural and Forestry Experiment Station paper no. J-10836.

REFERENCES

Ahmed, F.E., and A.E. Hall. 1993. Heat injury during early floral bud development in cowpea. Crop Sci. 33:764–767.

Allen, L.H. Jr., and K.J. Boote. 2000. Crop ecosystem responses to climate change: Soybean. p. 133–160. *In* K.R. Reddy and H.F. Hodges (ed.) Climate change and global crop productivity. CAB International, Wallingford, UK.

Baker, J.T., L.H. Allen, Jr., K.J. Boote, P. Jones, and J.W. Jones. 1989. Response of soybean to air temperature and carbon dioxide concentration. Crop Sci. 29:98–105.

Bennett, S.J. 1999. Pollen–ovule ratios as a method of estimating breeding systems in *Trifolium* pasture species. Aust. J. Agric. Res. 50:1443–1450.

Binelli, G.D., E.V. Manincor, and E. Ottaviano. 1985. Temperature effects on pollen germination and pollen tube growth in maize. Gen. Agric. 39:269–281.

Borthwick, H.A., and M.W. Parker. 1940. Floral initiation in Biloxi soybeans as influenced by age and position of leaf receiving photoperiodic treatment. Bot. Gaz. (Chicago) 101:806–812.

Carter, T.E., Z. Gizlie, and J.W. Burton. 1993. Coefficient-of-parentage and genetic similarity estimates for 258 North American soybean cultivars released by public agencies during 1945–1988. USDA Tech. Bull. No. 1814. U.S. Gov. Print. Office, Washington, DC.

Ciais, P., M. Reichstein, N. Viovy, A. Granier, J. Ogée, V. Allard, M. Aubinet, N. Buchmann, et al. 2005. Europe-wide reduction in primary productivity caused by the heat and drought in 2003. Nature (London) 437:529–533.

Crone, D., J. Rueda, K.L. Martin, D.A. Hamilton, and J.P. Mascarenhas. 2001. The differential expression of a heat shock promoter in floral and reproductive tissues. Plant Cell Environ. 24:869–874.

Cross, R.H., S.A. McKay, A.G. McHughen, and P.C. Bonham-Smith. 2003. Heat-stress effects on reproduction and seed set in *Linum usitatissimum* L. (flax). Plant Cell Environ. 26:1013–1020.

Dickinson, H.G., and U. Potter. 1976. The development of patterning in the alveolar exine of *Cosmos bipinnatus*. New Phytol. 76: 543–550.

Domínguez, E., J. Cuartero, and F. Fernández-Muñoz. 2005. Breeding tomato for pollen tolerance to low temperatures by gametophytic selection. Euphytica 143:253–263.

Erickson, A.N., and A.H. Markhart. 2002. Flower developmental stage and organ sensitivity of bell pepper (*Capsicum annuum* L.) to elevated temperature. Plant Cell Environ. 25:123–130.

FAO. 2006. Food and Agriculture Organization of the United Nations [Online]. Available at http://www.fao.org (verified 30 Nov. 2006). FAO, Rome.

Ferris, R., T.R. Wheeler, P. Hadley, and R.H. Ellis. 1998. Recovery of photosynthesis after environmental stress in soybean grown under elevated CO_2. Crop Sci. 38:948–955.

Gibson, L.R., and R.E. Mullen. 1996. Soybean seed quality reductions by high day and night temperature. Crop Sci. 36:1615–1619.

Gross, Y., and J. Kigel. 1994. Differential sensitivity to high temperature of stages in the reproductive development of common bean (*Phaseolus vulgaris* L.). Field Crops Res. 36:201–212.

Gwata, D., P. Wofford, P. Pfahler, and K.J. Boote. 2003. Pollen morphology and *in vitro* germination characteristics of nodulating and nonnodulating soybean (*Glycine max* L.) genotypes. Theor. Appl. Gen. 106:837–839.

Hall, A.E. 1992. Breeding for heat tolerance. Plant Breed. Rev. 10:129–168.

Hall, A.E. 2004. Comparative ecophysiology of cowpea, common bean and peanut. p. 271–325. *In* H.T. Nguyen and A. Blum (ed.) Physiology and biotechnology integration for plant breeding, Marcel Dekker, New York.

Herrero, M.P., and R.R. Johnson. 1980. High temperature stress and pollen viability of maize. Crop Sci. 20:796–800.

Hewitt, E.J. 1952. Sand and water culture methods used in the study of plant nutrition. Tech. Comm. 22. Farnham Royal Commonwealth Agricultural Bureau, Bucks, UK.

Jayaprakash, P., and N. Sarla. 2001. Development of an improved medium for germination of *Cajanus cajan* (L.) Millsp. pollen *in vitro*. J. Exp. Bot. 52:851–855.

Kakani, V.G., P.V.V. Prasad, P.Q. Craufurd, and T.R. Wheeler. 2002. Response of *in vitro* pollen germination and pollen tube growth of groundnut (*Arachis hypogaea* L.) genotypes to temperature. Plant Cell Environ. 25:1651–1661.

Kakani, V.G., K.R. Reddy, S. Koti, T.P. Wallace, P.V.V. Prasad, V.R. Reddy, and D. Zhao. 2005. Differences in *in vitro* pollen germination and pollen tube growth of cotton cultivars in response to high temperature. Ann. Bot. (London) 96:59–67.

Koti, S., K.R. Reddy, V.R. Reddy, V.G. Kakani, and D. Zhao. 2005. Interactive effects of carbon dioxide, temperature, and ultraviolet-B radiation on soybean (*Glycine max* L.) flower and pollen morphology, pollen production, germination, and tube lengths. J. Exp. Bot. 56:725–736.

Lobell, D.B., and G.P. Asner. 2003. Climate and management contributions to recent trends in U.S. agricultural yields. Science (Washington, DC) 299:1032.

Lu, Z., R.G. Percy, C.O. Qualset, and E. Zeiger. 1998. Stomatal conductance predicts yields in irrigated Pima cotton and bread wheat grown at high temperatures. J. Exp. Bot. 49:453–460.

Luza, J.G., V.S. Polito, and S.A. Weinbaum. 1987. Staminate bloom date and temperature responses of pollen germination and tube growth in two walnut (*Juglans regia* L.) species. Am. J. Bot. 74:1898–1903.

Maestro, M.C., and J. Alvarez. 1988. The effects of temperature on pollination and pollen tube growth in muskmelon *Cucumis melo* L. Sci. Hortic. (Canterbury, UK) 36:173–181.

Martineau, J.R., J.E. Specht, J.H. Williams, and C.Y. Sullivan. 1979. Temperature tolerance in soybeans. I. Evaluation of a technique for assessing cellular membrane thermostability. Crop Sci. 19:75–78.

Matlob, A.N., and W.C. Kelly. 1973. The effect of high temperature on pollen tube growth of snake-melon and cucumber. J. Am. Soc. Hortic. Sci. 98:296–300.

Mearns, L.O., W. Easterling, C. Hays, and D. Marx. 2001. Comparison of agricultural impacts of climate change calculated from high and low resolution climate change scenarios: Part I. The uncertainty due to spatial scale. Clim. Change 51:131–172.

Meehl, T.G.A., and C. Tebaldi. 2004. More intense, more frequent, and longer lasting heat waves in the 21st century. Science (Washington, DC) 305:994–997.

Omanga, P.A. 1994. Measurement and prediction of flowering in pigeon pea [*Cajanus cajan* (L.) Millsp.]. Thesis, Univ. of Reading, Reading, UK.

Peet, M.M., S. Sato, and R.G. Gardner. 1998. Comparing heat stress effects on male-fertile and male-sterile tomatoes. Plant Cell Environ. 21:225–231.

Prasad, P.V.V., P.Q. Craufurd, and V.G. Kakani. 2001. Influence of high temperature during pre- and postanthesis stages of floral development on fruit-set and pollen germination in peanut. Aust. J. Plant Physiol. 28:233–240.

Prasad, P.V.V., P.Q. Craufurd, and R.J. Summerfield. 1999. Fruit number in relation to pollen production and viability in groundnut exposed to short episodes of heat stress. Ann. Bot. (London) 84: 381–386.

Raper, C.D., and P.J. Kramer. 1987. Stress physiology. p. 589–641. *In* J.R. Wilcox (ed.) Soybeans: Improvement, production, and uses. ASA Monogr. 16. 2nd ed. ASA, CSSA, and SSSA, Madison, WI.

Reddy, K.R., G.H. Davidonis, A.S. Johnson, and B.T. Vinyard. 1999. Temperature regime and carbon dioxide enrichment alter cotton boll development and fiber properties. Agron. J. 91:851–858.

Reddy, K.R., and H.F. Hodges (ed.). 2000. Climate change and global crop productivity. CAB International, Wallingford, UK.

Reddy, K.R., H.F. Hodges, J.J. Read, J.M. McKinion, J.T. Baker, L. Tarpley, and V.R. Reddy. 2001. Soil–plant–atmosphere–research (SPAR) facility: A tool for plant research and modeling. Biotronics 30:27–50.

Reddy, K.R., V.R. Reddy, and H.F. Hodges. 1992. Temperature effects on cotton fruit retention. Agron. J. 84:26–30.

Reynolds, M.P., M. Balota, M.I.B. Delgado, I. Amani, and R.A. Fischer. 1994. Physiological and morphological traits associated with spring wheat yield under hot, irrigated conditions. Aust. J. Plant Physiol. 21:717–730.

Salem, M.A., V.G. Kakani, and K.R. Reddy. 2004. Temperature effects on *in vitro* pollen germination and pollen tube growth of soybean genotypes [CD-ROM]. Ann. Meetings Southern Br. ASA 27–29 June 2004, Biloxi, MS.

SAS Institute. 1998. SAS guide to macro processing. v. 8, 2nd ed. SAS Inst., Cary, NC.

Sato, S., M.M. Peet, and J.F. Thomas. 2000. Physiological factors limit fruit set of tomato (*Lycopersicon esculentum* Mill.) under chronic high temperature stress. Plant Cell Environ. 23:719–726.

Sato, S., M.M. Peet, and J.F. Thomas. 2002. Determining critical pre- and postanthesis periods and physiological processes in *Lycopersicon esculentum* Mill. exposed to moderately elevated temperatures. J. Exp. Bot. 53:1187–1195.

Schoper, J.B., R.J. Lambert, and B.L. Vasilas. 1987. Pollen viability, pollen shedding, and combining ability for tassel heat tolerance in maize. Crop Sci. 27:27–31.

Shen, X.Y., and B.D. Webster. 1986. Effects of water stress on pollen of *Phaseolus vulgaris* L. J. Am. Soc. Hortic. Sci. 111:807–810.

Stainforth, D., T. Aina, C. Christensen, M. Collins, N. Faull, D. Frame, J. Kettleborough, S. Knight, A. Martin, J.M. Murphy, C. Piani, D. Sexton, L.A. Smith, S. Spicer, A. Thorpe, and M. Allen. 2005. Uncertainty in predictions of the climate response to rising levels of greenhouse gases. Nature (London) 433:403–406.

Sullivan, C.Y. 1972. Mechanisms of heat and drought resistance in grain sorghum and methods of measurement. p. 247–264. *In* N.G.P. Rao and L.R. House (ed.) Sorghum in the seventies. Oxford & IBH Publishing Co., New Delhi, India.

Suzuki, K., H. Takeda, T. Tsukaguchi, and Y. Egawa. 2001. Ultrastructural study of degeneration of tapetum in anther of snap bean (*Phaseolus vulgaris* L.) under heat-stress. Sex. Plant Rep. 13:293–299.

Thomas, J.M.G., K.J. Boote, L.H. Allen, Jr., M. Gallo-Meagher, and J.M. Davis. 2003. Elevated temperature and carbon dioxide effects on soybean seed germination and transcript abundance. Crop Sci. 43:1548–1557.

Tollenaar, M., T.B. Daynard, and R.B. Hunter. 1979. Effect of temperature on rate of leaf appearance and flowering date in maize. Crop Sci. 19:363–366.

USDA-NASS. 2006. NASS—National Agricultural Satistics Service [Online]. Available at: http://www.nass.usda.gov/index.asp (cited 2004; modified 1 Dec. 2006; verified 1 Dec. 2006). USDA, Washington, DC.

Vu, J.C.V., L.H. Allen, Jr., K.J. Boote, and G. Bowes. 1997. Effects of elevated CO_2 and temperature on photosynthesis and rubisco in rice and soybean. Plant Cell Environ. 20:68–76.

Yan, W., and D.H. Wallace. 1998. Simulation and prediction of plant phenology for five crops based on photoperiod by temperature interaction. Ann. Bot. (London) 81:705–716.

Yin, X., M.J. Kropff, G. McLaren, and R.M. Visperas. 1995. A nonlinear model for crop development as a function of temperature. Agric. For. Meteorol. 77:1–16.

Young, L.W., R.W. Wilen, and P.C. Bonham-Smith. 2004. High temperature stress of *Brassica napus* during flowering reduces micro- and megagametophyte fertility, induces fruit abortion, and disrupts seed production. J. Exp. Bot. 55:485–495.

Zhao, D., K.R. Reddy, V.G. Kakani, J.J. Read, and J.H. Sullivan. 2003. Growth and physiological responses of cotton (*Gossypium hirsutum* L.) to elevated carbon dioxide and ultraviolet-B radiation under controlled environmental conditions. Plant Cell Environ. 26:771–782.