Differences in in vitro Pollen Germination and Pollen Tube Growth of Cotton Cultivars in Response to High Temperature

V.G. KAKANI1,*, K. R. REDDY1, S. KOTI1, T. P. WALLACE1, P. V. V. PRASAD2, V. R. REDDY3 and D. ZHAO4

1Department of Plant and Soil Sciences, Box 9555, 117 Dorman Hall, Mississippi State University, Mississippi State, MS 39762, USA, 2Agronomy Department, 304 Newell Hall, PO Box 110500, University of Florida, Gainesville, FL 32611, USA, 3USDA-ARS, Alternate Crops and Systems Laboratory, Bldg 001, BARC-W, Baltimore Avenue, Beltsville, MD 20705-2350, USA and 4USDA-ARS, Grazinglands Research Laboratory, El Reno, OK 73036, USA

INTRODUCTION

Global surface temperature has increased by approx. 0.6 °C since the late 19th century and is projected to increase by 1.4–5.8 °C by the end of the current century (Houghton et al., 2001). Further, extreme events such as warmer days with decrease in diurnal temperature range are projected to occur more frequently in the future climates (Dai et al., 2001). Temperature is the important factor controlling plant growth and development. Suitability of a crop to a given location depends not only on the threshold temperatures but also on the length of the growing season. Daily or seasonal temperatures above optimum and temperature extremes, should they coincide with critical stages of plant development, will become a major factor limiting crop production (Hall, 1992). Fruit-set in many agronomic crops is sensitive to high temperature (Reddy et al., 1991, 1992; Peet et al., 1998). Fruit set was reduced on exposure to daytime temperatures of >30 °C for about 13 h in Upland (Gossypium hirsutum) and Pima (G. barbadense) cottons (Reddy et al., 1992), 35 °C for 4 h in Brassica napus (Young et al., 2004), >28 °C for 12 h during flowering in tomato (Lycopersicon esculentum) (Peet et al., 1998; Sato et al., 2002). Seed yield of wheat (Triticum aestivum) (Saini and Aspinall, 1982), corn (Zea mays) (Mitchell and Petolino, 1988) and rice (Oryza sativa) (Matsui et al., 1997) were reduced on exposure to daytime temperatures of 30 °C for 16 h, 38 °C for 16 h and >36 °C for 6 h, respectively. Similarly, pod-set was reduced at day temperatures >28 °C for 12 h in bean (Phaseolus vulgaris) (Prasad et al., 2002) and >28 °C for 12 h in groundnut (Arachis hypogaea) (Prasad et al., 1999a, 2003).

Conventional and transgenic cultivars of cotton are grown across the USA in about 5.3 Mha (Economic Research Service, 2003) and 31.6 Mha around the world under diverse temperature regimes (15–45 °C). Cotton plants aborted most of the squares and flowers when day/night temperatures were >30/20 °C for 13 h (Reddy et al., 1991, 1993). At extremely high day temperatures such as 40 °C for 13 h, all existing squares and flowers were aborted in several Upland cotton cultivars (Reddy et al., 1992, 1995), whereas Pima cotton was highly sensitive and failed to produce fruiting branches (Reddy et al., 1995).

Pollen grains once released from anthers act as independent functional units and are exposed to ambient environment. Therefore, episodes of high temperature during flowering would more severely affect pollen than the deeply seated ovules. In cotton, anther dehiscence occurs during the morning hours of 0700 to 1100 depending on the

* For correspondence. E-mail vgk3@ra.msstate.edu

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prevailing weather conditions while pollen germination occurs within 30 min upon contact of a receptive stigma (Pundir, 1972). Actual fertilization however, occurs anywhere between 12 and 24 h once pollen is released, due to slow growth of the pollen tube (Pundir, 1972). Therefore, high-temperature damage occurring during anthesis is likely to include failure of pollination and/or fertilization, resulting in lower boll-set. Weaver and Timm (1988) suggested that pollen is more sensitive to high temperatures than female reproductive organs, which could account for a lack of fertilization under high-temperature stress. Recent studies have shown that micro- and mega-sporogenesis are injured by high temperature, resulting in reduced fruit set (Cross et al., 2003; Young et al., 2004) but they also suggest that pollen plays a major role in fruit-set under high-temperature conditions. Tomato plants grown at 32/26 °C temperature for 0–15 d before anthesis failed to set fruit due to disruption of anther components (Sato et al., 2002). Young et al. (2004) demonstrated the importance of pollen in fruit-set through reciprocal crossing studies where fruit set was reduced by 88 % when pollen donor plants were treated with high temperature (35 °C for 4 h during day), while fruit set was reduced by 37 % when emasculated receptor plants were treated with high temperature.

Results from in vitro studies with peanuts showed that genotypes varied in response to temperature for cardinal temperatures ($T_{\text{min}}$, $T_{\text{opt}}$ and $T_{\text{max}}$), pollen germination percentage and maximum pollen tube length (Kakani et al., 2002). The differences in cardinal temperature were mainly responsible for tolerance/susceptibility of peanut genotypes to high temperature (Kakani et al., 2002; Craufurd et al., 2003). Upland cotton genotypes were bred for heat tolerance by selecting progenies developed from surviving pollen grains when exposed to 35 °C for 15 min (Rodriguez-Garay and Barrow, 1988), suggesting that pollen could be used to screen cotton cultivars for high-temperature tolerance. Recently Burke et al. (2004) reported an optimum temperature of 28 °C for in vitro pollen germination with greenhouse-grown cotton cultivar Gregg 65. However, variation in cardinal temperatures for pollen germination and pollen tube growth in cotton cultivars have not been studied. Therefore, identification of cardinal temperatures for pollen germination and pollen tube growth and developing response functions will be useful for understanding mechanisms of high-temperature tolerance.

A vegetative physiological parameter widely used to study plant tolerance to temperature is cell membrane thermostability. It was successfully used to screen cotton cultivars for high-temperature tolerance (Ashraf et al., 1994; ur Rahman et al., 2004). Cultivars showing high membrane thermostability gave higher seed cotton yield under high-temperature conditions during flowering and boll-filling period (Malik et al., 1999; ur Rahman et al., 2004). Recent studies in peanuts showed that cell membrane thermostability was not highly correlated with yield loss or pollen germination under high-temperature conditions (Kakani et al., 2002; Craufurd et al., 2003). Thus, it is essential to understand the relationships between responses of pollen to high temperature and leaf membrane thermostability in cotton. The objectives of this study were to (a) quantify the effect of temperature on pollen germination and pollen tube growth of different cotton cultivars, (b) determine cardinal temperatures for pollen germination and pollen tube growth, and (c) compare pollen (total germination and maximum pollen tube growth) response to temperature with leaf membrane thermostability.

**MATERIALS AND METHODS**

**Plant growth**

Twelve cotton cultivars representing traditional, improved and transgenics expressing variable tolerance to drought, high temperature and other biotic stresses were evaluated in the present study (Table 1). The plants were grown during the summer of 2002 in an experimental field at the R. R. Foil Plant Science Research Center, Mississippi State University (33°28′N, 88°47′W). Plants were grown under recommended cultural practices for commercial production. The growing temperatures were optimum during the squaring and flower collection period. The mean temperatures during the period of 3 weeks prior to flower collection

**Table 1. Trade name, maturity group, leaf-type and specific traits of 12 cotton cultivars evaluated for tolerance to high temperature (Cotton Farming, 2002)**

<table>
<thead>
<tr>
<th>Cultivar*</th>
<th>Maturity group</th>
<th>Leaf type</th>
<th>Special traits</th>
</tr>
</thead>
<tbody>
<tr>
<td>(1) Acala 1517–99</td>
<td>Full</td>
<td>Hairy</td>
<td>Verticillium tolerant</td>
</tr>
<tr>
<td>(2) BXN 49 B</td>
<td>Early–mid</td>
<td>Hairy</td>
<td>Contains BXN and BG genes</td>
</tr>
<tr>
<td>(3) DP 458 B/RR</td>
<td>Mid–full</td>
<td>Smooth</td>
<td>Good heat tolerance</td>
</tr>
<tr>
<td>(4) DP 5415 RR</td>
<td>Mid–full</td>
<td>Smooth</td>
<td>Outstanding yield potential</td>
</tr>
<tr>
<td>(5) FM 832</td>
<td>Med–full</td>
<td>Okra/smooth</td>
<td>Adaptable to drought</td>
</tr>
<tr>
<td>(6) FM 832 B</td>
<td>Med–full</td>
<td>Okra/smooth</td>
<td>Good water use efficiency</td>
</tr>
<tr>
<td>(7) NuCOTN 33 B</td>
<td>Mid–full</td>
<td>Smooth</td>
<td>Widely adapted</td>
</tr>
<tr>
<td>(8) NuCOTN 35 B</td>
<td>Mid–full</td>
<td>Smooth</td>
<td>Good fibre quality</td>
</tr>
<tr>
<td>(9) ST 457</td>
<td>Early–mid</td>
<td>Hairy</td>
<td>Conventional cultivar</td>
</tr>
<tr>
<td>(10) ST 4793 R</td>
<td>Early–mid</td>
<td>Hairy</td>
<td>Contains RR genes</td>
</tr>
<tr>
<td>(11) ST 4892 BR</td>
<td>Early–mid</td>
<td>Hairy</td>
<td>Contains BG and RR genes</td>
</tr>
<tr>
<td>(12) STV 825</td>
<td>Full</td>
<td>Smooth</td>
<td>Conventional cultivar</td>
</tr>
</tbody>
</table>

*1(1), New Mexico State University, Las Cruces, NM; (2), (9), (10) and (11), Stoneville Pedigree Seed Company, Memphis TN; (3), (4), (7) and (8), Delta and Pine Land Company, Scott, MS; (5) and (6), Bayer CropScience US, Kansas City, MO; (12), conventional.

1, B, Bollgard®; R, Roundup Ready® (both trademarks of Monsanto Technology, LLC).
were 27 ± 0.24 °C and during the flower collection period were 28 ± 0.31 °C.

Pollen collection and growth medium

The flowers for this study were collected from the first fruiting position between 55 and 60 d after emergence. Plant to plant variation can also be a significant source of variation in pollen germination measurements (Sari-Gorla et al., 1994). To minimize the effect of this variation without having to perform individual determinations on many plants of single cultivars, pollen from flowers on different plants was taken as a sample in the present study. Fresh cotton flowers were collected at the time of anther dehiscence, between 0730 and 0830 h, from ten plants per each cultivar, and immediately placed in plastic bags and carried to the laboratory. The improved pollen growth medium of Taylor (1972), consisting of 2 g agar, 30 g C12H22O11, 5.3 mg KNO3, 51.7 mg MnSO4, 10.3 mg H2BO3, 10.3 mg MgSO4.7H2O made up to 100 mL with deionized water, was used in this study. The medium was placed in Petri dishes and temperature equilibrated before sprinkling the pollen on the medium. Pollen was sprinkled on the media by gently tapping a set of three flowers directly above the surface of the medium in each Petri dish. Approximately, 800–1000 pollen grains were sprinkled on each Petri dish. Three Petri dishes of each genotype at each temperature treatment were used as replications. The whole procedure was completed within 30 min to avoid temperature treatment were used as replications. The Petri dish. Three Petri dishes of each genotype at each approximately, 800–1000 pollen grains were sprinkled on each Petri dish. Approxim-ately, 800–1000 pollen grains were sprinkled on each Petri dish. Three Petri dishes of each genotype at each temperature treatment were used as replications. The whole procedure was completed within 30 min to avoid pollen desiccation. Partial opening of the Petri dish lids allowed a relative humidity of about 50% to be maintained and also prevented moisture accumulation on germinating pollen grains and avoided pollen rupture as cotton pollen is highly sensitive to moisture (Burke et al., 2003).

Temperature treatments

Petri dishes with media containing pollen were incubated in the dark at temperatures between 10 and 45 °C at 5 °C intervals in growth cabinets (Percival Scientific, Inc., Perry, IA, USA) and observed for germination. As pollen did not germinate at temperatures of 10 and 45 °C, additional incubation temperatures of 12.5 and 42.5 °C were included. On a given day, all 12 cultivars were tested at a given temperature. Growth cabinets were maintained at predetermined temperature and temperature of cabinet and media were recorded at 1-h intervals using a Campbell CR10X data logger. No differences were observed between measured cabinet and media temperatures. The average temperature of the growth cabinet during pollen germination was used in the analysis.

Pollen germination and pollen tube measurements

Pollen germination (PG) was determined by direct microscopic observation (Nikon Scientific, Kanagawa, Japan). A pollen grain was considered germinated when pollen tube length (PTL) was at least equal to or greater than the grain diameter (Kakani et al., 2002). Germination percentage was determined by dividing the number of germinated pollen grains per field of view by the total number of pollen per field of view and expressed as percentage. Measurements of pollen tube length were recorded directly by an ocular micrometer fitted to the eyepiece of the microscope. Mean pollen tube length was calculated as the average length of 20 pollen tubes measured from each Petri dish after 24 h. The replicated values on maximum pollen germination and tube length were analysed using the one-way ANOVA procedure (SAS Institute, 1997).

Curve fitting and analysis

Maximum pollen germination percentage and pollen tube length recorded after 24 h of incubation, at each temperature, were analysed using linear and nonlinear regression techniques to quantify developmental responses to temperature. Quadratic (Yan and Wallace, 1998), cubic or higher order polynomial (Tollenaar et al., 1979) and modified broken-stick or bilinear (Omanga et al., 1995) equations were applied to data and examined to determine the best-fit model. The modified bilinear equation (eqn 1) provided the greatest R² value and smallest root mean squared deviation (r.m.s.d.) for both pollen germination and pollen tube length and was used to estimate cardinal temperatures, minimum (Tmin), optimum (Topt) and maximum (Tmax), for pollen germination and pollen tube length of all cultivars (Kakani et al., 2002). The PROC NLIN procedure in SAS (SAS Institute, 1997) was used to estimate parameters in the modified bilinear equation. A modified Newton–Gauss iterative method was used to determine Topt based on the lowest r.m.s.d. values between observed and predicted values. Values of Tmin and Tmax were estimated using parameters derived from the modified bilinear equations (eqns 2 and 3). Replicated values of cardinal temperatures were then analysed using the one-way ANOVA procedure in SAS (SAS Institute, 1997).

\[
Pollen germination (\%) or pollen tube length = a + [b_1 (T - T_{opt})] + [b_2 (ABS(T_{opt} - T))] \quad (1)
\]

\[
T_{min} = \frac{[a + T_{opt}(b_2 - b_1)]/(b_1 - b_2)}{\quad (2)}
\]

\[
T_{max} = \frac{[a - T_{opt}(b_2 + b_1)]/(b_1 + b_2)}{\quad (3)}
\]

where a, b1 and b2 are equation constants, T the various temperatures at which germination and tube growth were studied, and T_{opt} the optimum temperature for germination or pollen tube growth.

Cell membrane thermostability measurements

At the time of pollen sampling, cell membrane thermostability of leaves was measured using the procedure described by Martineau et al. (1979). Each sample assay consisted of two sets of five leaf discs cut with a 1-2-cm-diameter punch from five fully expanded leaves
on the main stem. Samples were replicated three times each. Before each assay, the two paired sets of leaf discs were placed into two separate test tubes with 20 mL of deionized water, after washing them thoroughly with at least four changes of deionized water to remove electrolytes released from cut cells at the periphery of the discs. To avoid evaporation and leakage of contents, test tubes were sealed with aluminum foil. One set of test tubes was incubated for 20 min at 55 °C in a temperature-controlled water bath, whilst the other set was left at room temperature of approx. 25 °C. Test tubes were then immediately incubated at 10 °C for 12 h and inverted several times to mix the contents. After incubation, the initial measurement of conductance was measured by an electrical conductivity meter (Corning Checkmate II; Corning Inc., New York, USA), after which tubes were sealed with aluminum foil and autoclaved at 120 °C and 0-15 MPa for 20 min to kill leaf tissues. Autoclaved tubes were cooled to 25 °C, contents mixed thoroughly and final conductance was recorded. Relative injury (RI) to cell membranes resulting from the temperature treatments was calculated using eqn (4)

\[
\text{RI\%} = \left\{1 - \left[1 - \frac{(T_i/T_f)}{(C_i/C_f)}\right]\right\} \times 100 \quad (4)
\]

where \( T \) and \( C \) refer to the conductance of the treatment (55 °C) and control (25 °C) solution, respectively, and the subscripts \( i \) and \( f \) indicate initial and final conductance, respectively. The ratio of the initial to the final conductance \( (T_i/T_f) \) is a relative measure of electrolyte leakage caused by elevated temperature and consequently a measure of the extent of damage to cellular membranes. One-way ANOVA in SAS (SAS Institute, 1997) was carried out to identify cultivar differences.

**Principal component analysis (PCA)**

A PCA using PROC PRINCOMP of SAS (SAS Institute, 1997) was applied to pollen germination and pollen tube growth parameters to identify the parameters that best describe cultivar tolerance to temperature. Values of maximum pollen germination percentage (PG\%\text{max}) and pollen tube length (PTL\text{max}) cardinal temperatures \( (T_{\text{min}}, \ T_{\text{opt}} \text{and} T_{\text{max}}) \) for pollen germination and pollen tube length and RI of 12 cultivars were included in the PCA. Eigenvectors generated by PCA were used to identify parameters that best differentiated cultivars for temperature tolerance. The first two PC scores, PC1 and PC2 that accounted for maximum variability of the parameters tested, were used to group the cultivars. The cultivars which had +PC1 and +PC2 scores were classified as tolerant, +PC1 and -PC2 scores as moderately tolerant, -PC1 and +PC2 as moderately susceptible and finally -PC1 and -PC2 as susceptible.

**RESULTS**

**Pollen germination**

Pollen grains started germinating in about 10 min on contact with the in vitro medium. Figure 1 shows the variation for pollen germination in response to temperature of two cultivars for clarity. Cultivar differences for both germination percentage and cardinal temperatures were observed (Table 2). Maximum percentage of germination ranged from 33 (NuCOTN 35 B) to 60 % (DP 458 B/RR), with a mean of 44 %. The modified bilinear equation provided best-fit to predict the cultivars pollen germination response to temperature (Fig. 1). The average \( R^2 \) value for all cultivars tested was 88 % (Table 2). Cardinal temperatures for pollen germination differed greatly among cultivars. Values of \( T_{\text{min}} \) ranged from 11-1 °C (BXN 49B) to 20-2 °C (ST 457) with an average of 15-1 °C. Optimum temperature \( (T_{\text{opt}}) \) ranged from 28-4 °C for ST 4793 R to 35-4 °C for ST 4892 BR with an average \( T_{\text{opt}} \) of 31-4 °C. The \( T_{\text{max}} \) values ranged from 40-8 °C for ST 4892 BR to 46-2 °C for STV 825 with an average \( T_{\text{max}} \) of 43-3 °C (Table 2).

**Pollen tube growth**

Cultivars differed significantly in pollen tube length at optimum temperatures (Fig. 2). Pollen tubes remained stable without rupturing for 24 h after germination on the in vitro medium. Pollen tube length ranged from 605 μm for Acala 1517–99 to 903 μm for BXN 49B, with an average of 778 μm (Table 3). Similar to pollen germination, the modified bilinear function described the response of pollen tube length to temperature. The modified bilinear model fit is shown for two cultivars that had high variation in pollen tube length and cardinal temperatures for pollen tube growth (Fig. 2). The \( T_{\text{min}} \) ranged from 9-8 °C for Acala 1517–99 to 13-4 °C for NuCOTN 35 B with an average \( T_{\text{min}} \) of 12-1 °C. The \( T_{\text{opt}} \) ranged from 25-9 °C for STV 825 to 33-3 °C for Acala 1517–99 with an average \( T_{\text{opt}} \) of 28-3 °C. Values of \( T_{\text{max}} \) ranged from 42-1 °C for Acala 1517–99, FM 832 and NuCOTN 33 B to 44-3 °C for ST 457 with an average of 42-8 °C (Table 3).
The leaf cell membrane thermostability expressed as percentage relative injury (RI%) differed significantly among cultivars and ranged from 41% for DP 458 B/RR to 76% for NuCOTN 35 B with an average of 62% (Table 3). Relative injury had poor correlation with pollen germination and pollen tube length (Fig. 3).

**Principal component analysis**

PCA is a multivariate technique for examining relationships among several quantitative variables and is especially a valuable analytical technique in exploratory data analysis (Johnson, 1998). The PCA identified the pollen parameters that best separated the cultivars for their tolerance to temperature. The first three principal component vectors (PC1, PC2 and PC3) accounted for 78% of the total variation (Table 4). The PC1 eigenvector contrasted cultivars with high positive loadings for variables PTL_{max}, PG_{%max}, PTL_{max} and PG_{T_{max}} (Table 4; Fig. 4). Cultivars with higher PG_{%max} and PTL_{max} were placed on the right of the biplot while cultivars with low values were placed on the left of the biplot (Fig. 4). The PC2 had high positive loadings for PG_{T_{opt}} indicating the role of optimum temperature in separating sensitive from tolerant cultivars. The cultivars were divided into four groups based on the scores of the first two principal components (Fig. 4): group 1 cultivars as tolerant with positive scores for PC1 and PC2, group 2 as moderately tolerant with positive PC1 and negative PC2 scores, group 3 as moderately susceptible with negative PC1 and positive PC2 and finally group 4 as susceptible with negative PC1 and PC2 scores (Table 5).

**DISCUSSION**

Temperature is among the most important environmental factors affecting plant reproductive processes such as pollen germination, pollen tube growth and fruit-set. In the present study, *in vitro* pollen germination and pollen tube growth of all cultivars were severely reduced under both high and low temperature conditions. Earlier studies on cotton pollen by Suy (1979) and Barrow (1983) have shown that high temperatures (>30 °C) inhibit *in vivo* pollen germination and pollen tube penetration, but neither cultivar differences nor response to temperature were studied. In the current study, all 12 cultivars had defined temperature optima, above and below the point of which pollen germination and pollen tube growth were reduced. The modified bilinear model best described the response of pollen germination and pollen tube growth to temperature (Figs 1 and 2).

**Table 2. Maximum pollen germination percentage, modified bilinear equation constants, and cardinal temperatures for pollen germination of 12 cotton cultivars in response to temperature**

<table>
<thead>
<tr>
<th>Cultivar</th>
<th>Maximum pollen germination (%)</th>
<th>Equation constants</th>
<th>Cardinal temperatures (°C)</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td></td>
<td>a</td>
<td>b₁</td>
</tr>
<tr>
<td>DP 458 B/RR</td>
<td>59.8</td>
<td>66.0</td>
<td>-0.11</td>
</tr>
<tr>
<td>STV 825</td>
<td>52.8</td>
<td>53.4</td>
<td>0.09</td>
</tr>
<tr>
<td>FM 832 B</td>
<td>49.8</td>
<td>50.4</td>
<td>-0.06</td>
</tr>
<tr>
<td>NuCOTN 33 B</td>
<td>46.8</td>
<td>50.4</td>
<td>0.10</td>
</tr>
<tr>
<td>ST 4892 BR</td>
<td>45.9</td>
<td>48.7</td>
<td>-3.35</td>
</tr>
<tr>
<td>Acala 1517–99</td>
<td>45.5</td>
<td>51.0</td>
<td>-1.87</td>
</tr>
<tr>
<td>DP 5415 RR</td>
<td>44.3</td>
<td>56.6</td>
<td>-1.40</td>
</tr>
<tr>
<td>FM 832</td>
<td>42.5</td>
<td>44.7</td>
<td>0.25</td>
</tr>
<tr>
<td>ST 4793 R</td>
<td>36.4</td>
<td>38.3</td>
<td>0.25</td>
</tr>
<tr>
<td>ST 457</td>
<td>35.6</td>
<td>41.8</td>
<td>-1.48</td>
</tr>
<tr>
<td>BXN 49B</td>
<td>35.5</td>
<td>42.7</td>
<td>-1.46</td>
</tr>
<tr>
<td>NuCOTN 35 B</td>
<td>33.0</td>
<td>38.2</td>
<td>-0.68</td>
</tr>
<tr>
<td>Mean</td>
<td>43.99</td>
<td>–</td>
<td>–</td>
</tr>
<tr>
<td>s.e.d.</td>
<td>4.03***</td>
<td>–</td>
<td>–</td>
</tr>
</tbody>
</table>

***Significant at \( P = 0.001 \) level.

–, Data not analysed statistically.

**Fig. 2.** Pollen tube length in response to temperature (symbols) and their fitted lines based on modified bilinear equation of two cotton cultivars (BXN 49B and Acala 1517-99). Cultivars with variation for maximum pollen tube length are presented for clarity. Error bars indicate ± s.e.
and pollen tube length (PTLmax) recorded at optimum temperature of germination of 71% at 37°C (1972). Recently, Burke mean of 44%, much higher than that observed by Taylor et al. (Herrero and Johnson, 1980; Kuo et al., 1981; Kakani et al., 1985), 450–1400 µm for peanuts (Kakani et al., 2002) and 20–60 µm for muskmelon (Maestro and Alvarez, 1988). Therefore, the observed differences in pollen germination and pollen tube length in the present study were a reflection of cultivar variability.

Cultivar differences for cardinal temperatures were recorded in the current study (Tables 2 and 3). Cultivar DP 458 B/RR had an average pollen germination of about 60% and had a Tmax of 46°C. The conventional cultivar, STV 825, also had a high Tmax of 46°C and the average pollen germination was 53% (Table 2). The average cardinal temperatures for pollen germination and pollen tube growth were 14°C (Tmin), 31°C (Topt) and 43°C (Tmax). Values obtained for cotton were similar to those reported for peanut (Tmin = 14, Topt = 30–34, and Tmax = 43°C; Kakani et al., 2002) and snake melon (Cucumis melo)
melanin (Matlob and Kelly, 1973), corn (Binelli et al., 1985) and peanuts (Kakani et al., 2002). Recent studies with Brassica napus have suggested that reduced pollen germination rather than pollen viability under high temperature is the major cause of low pollen fertility (Young et al., 2004). Prasad et al. (1999b) in peanuts and Aloni et al. (2001) in bell pepper established a high correlation between in vitro pollen germination and fruit-set/seed-set under high-temperature conditions; this suggests that pollen germination could be a useful tool for testing cultivar tolerance to high temperature. Therefore, the ability of pollen to germinate and grow well at temperatures above 30 °C could be used as a tool to identify high-temperature tolerance in cotton cultivars. Further studies will be required to determine the minimum number of germinated pollen grains required to have effective fertilization.

In the current study, the membrane thermostability expressed as relative injury ranged between 35 and 73 %, but had a poor correlation with pollen parameters (Fig. 3). Recently, ur Rahman et al. (2004) also concluded that membrane thermostability is not a useful parameter for discriminating high-temperature tolerance of cotton cultivars under ambient temperatures. In cotton, heat tolerance does not correlate with degree of cell membrane lipid saturation (Rikin et al., 1993), suggesting factors other than membrane stability may be limiting reproductive growth and development at high temperature. However, the genotypic differences for pollen germination and pollen tube growth identified in this study could be due to the variation in their pollen carbohydrate concentration. Studies have shown that carbohydrates are responsible for pollen development and, especially, pollen cytoplasmic carbohydrates and sucrose are involved in protecting pollen viability during exposure and dispersal (Pacini et al., 1996) and for pollen germination, simple sugars are the primary substrates (Stanley, 1971). In pepper plants, exposure to high temperature (32/26 °C) for 8 d resulted in pollen germination of 6 % and shorter pollen tubes compared with maximum pollen germination of 25 % obtained at normal temperature (28/22 °C) (Aloni et al., 2001). This was attributed to a decrease in sucrose utilization by pollen grains under high temperature, even though the pollen grains accumulated more starch and sugars than under normal temperature conditions. In contrast, a decrease in starch and sugar concentration was recorded in tomato pollen grown under high temperature (32/26 °C) conditions (Pressman et al., 2002). Therefore, under-utilization or unavailability of carbohydrates hinders pollen germination on exposure to high temperatures. Future studies need to study the genotypic differences or pollen carbohydrate concentration and its role in determining the temperature tolerance of cotton pollen.

The PCA is perhaps the most useful statistical tool for screening multivariate data with significantly high correlations (Johnson, 1998). The first three principal components, PC1, PC2 and PC3 from PCA, explained about 72 % of the total cultivar pollen variability in response to temperature. The cluster analysis applied to the principal components divided the cultivars into four distinct groups (Fig. 4; Table 5). The PC1 eigenvectors for variables PG%max

![Fig. 4. First and second principal component scores (PC1 and PC2) for the identification of cotton cultivar response to temperature. The eigenvectors for variables are indicated by thick lines radiating from the centre showing the direction (angle) and magnitude (length) for maximum pollen germination (PG%; 2) and maximum pollen tube length (PTL; 1), cardinal temperatures (Tmin, Ttop and Tmax) for pollen germination percentage (PG%max) (3, 4, 5) and pollen tube length (PTLmax) (6, 7, 8) and cell membrane thermostability as RI (9).](image)

<table>
<thead>
<tr>
<th>Tolerant (+PC1, +PC2)</th>
<th>Moderately tolerant (+PC1, −PC2)</th>
<th>Moderately susceptible (−PC1, +PC2)</th>
<th>Susceptible (−PC1, −PC2)</th>
</tr>
</thead>
<tbody>
<tr>
<td>DP 458 B/RR (2.54, 0.57)</td>
<td>STV 825 (−0.64, −0.69)</td>
<td>ST 457 (−0.46, 1.35)</td>
<td>FM 832 B (−0.97, −0.49)</td>
</tr>
<tr>
<td>ST 4793 R (2.05, 1.41)</td>
<td>ST 4892 BR (0.99, −0.75)</td>
<td>Acala 1517−99 (−2.36, 3.26)</td>
<td>BNX 49B (−1.28, −0.96)</td>
</tr>
<tr>
<td>DP 5415 RR (0.89, 0.06)</td>
<td>NuCOTN 33 B (−1.45, −2.05)</td>
<td>NuCOTN 35 B (−1.40, −1.03)</td>
<td>FM 832 (−1.48, −0.67)</td>
</tr>
</tbody>
</table>

The principal component scores were obtained from the principal component analysis. The PC1 had highest positive loadings for PG, PTL, PG Ttop and PTL Tmax and the PC2 vector had highest positive loading for PG Ttop. The cultivars which had +ve scores for PC1 and PC2 were classified as tolerant, +PC1 and +PC2 scores as moderately tolerant, −PC1 and +PC2 as moderately susceptible and finally −PC1 and −PC2 as susceptible. Values in parenthesis are the PC1 and PC2 scores of the cultivar.

(Tmin = 10, Ttop = 30 and Tmax = 48 °C; Matlob and Kelly, 1973). However, the differences in cardinal temperatures did not reflect the tolerance or susceptibility of a cultivar to high temperatures because the cultivars which had a higher optimum temperature did not always have a higher temperature maximum or vice versa. Cultivars that had higher Ttop also had a higher pollen germination percentage and maintained a higher pollen germination even at high temperatures. Similar pollen behaviour was observed in snake
and PTLmax have high positive loadings, while variables PTL Topt and PG% Ttop have high negative loadings. The PC1 vectors indicated that cultivars with high optimum temperature do not necessarily have high pollen germination or long pollen tubes. But, tolerance to high temperatures will result only from successful fertilization of the megagametophyte that requires both pollen germination and pollen tube elongation. Cultivars that had higher PG%max maintained higher germination percentage at above optimum temperatures compared with those that had lower PG%max. Cultivars ST 4793 R, DP 458 B/RR and DP 5415 RR, with higher pollen germination and longer pollen tubes and with high T Ttop, were classified as tolerant, and cultivars FM 832, FM 832 B, NuCOTN 33 B were classified as susceptible to high temperature.

In conclusion, the cultivars with higher PG%max, PTLmax and an optimum temperature >32 °C for maximum pollen germination in vitro on a simple defined medium can be used for screening cultivars to high-temperature tolerance. However, for accurate yield predictions, future studies should quantify boll retention under high temperature and investigate the relationship between pollen germination, boll number and air temperatures under controlled conditions with high levels of solar radiation. Studies will also be required to validate the performance of high-temperature-tolerant cultivars identified by these in vitro methods in high-temperature environments.

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LITERATURE CITED


