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The effects of high-temperature stress on the germination of pollen grains of upland cotton during square development

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Abstract The reproductive stage of flowering plants is sensitive to high-temperature stresses. High temperature is a major factor influencing pollen grain viability in upland cotton (*Gossypium hirsutum*). The objective of this study was to identify the relationship between cotton pollen germination percentage and temperature by assaying the pollen germination of four upland cotton cultivars in vitro at different temperatures during the blooming period. The results showed that in vitro pollen germination percentage was related to the culture temperature of pollen germination and the temperature of the square development process. High temperature affected pollen development and germination, and high-temperature tolerance differed among the cotton cultivars. The pollen germination percentage decreased rapidly with changes in the culture temperature from 30 to 39 °C. A culture temperature of 35 °C might be a critical temperature for the pollen viability transition and could be used to screen cotton cultivars that have pollen grains with high-temperature resistance. Before the high-temperature stage, cultivars with rates of decrease in the percentage of pollen germination of less than 41 % at 35 °C relative to the rates at 30 °C might be considered as high-temperature tolerance cultivars, and cultivars with rates of decrease in the percentage of pollen germination greater than 41 % might be considered as susceptible cultivars. The high-temperature stress for pollen grain germination in vitro was greater than 30 °C, and the high-temperature stress for square development might be greater than 33 °C. Boll retention was significant; it was positively correlated with the pollen germination percentage and negatively correlated with temperature during the high-temperature stage. This study provided a method for rapidly screening cultivars (lines) with high-temperature tolerance pollen in upland cotton breeding.

Keywords *Gossypium hirsutum* · Pollen germination · Pollen viability · High-temperature tolerance · Boll retention

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Introduction

A persistent warming trend, driven largely by the anthropogenic production of greenhouse gases, is projected to cause the global surface temperature to increase between 1.4 and 5.8 °C by the end of the 21st century (Misra et al. 2012). The temperature will increase by 2.1 and 4.2 °C by 2050 and 2090, respectively, based on data from 2010 (Lee et al. 2012). Unexpected periodic episodes of heat stress are predicted to occur more frequently in the future (Ganguly et al. 2009). Sexual reproduction in flowering plants, especially the process of

pollen formation and fertilization, is sensitive to high temperatures (Zinn et al. 2010). *Gossypium hirsutum* (Reddy et al. 1992a), *Zea mays* (Herrero and Johnson 1980), *Capsicum* (Erickson and Markhart 2002) and *Arachis hypogaea* (Prasad et al. 1999) are sensitive to high temperatures during the pollination stage of anthesis. Therefore, episodes of high temperature are unfavorable to sexual reproduction before or during the blossom period, resulting in the abscission of a large number of flowers or buds as well as a decrease in yield (Reddy et al. 1992a; Wheeler et al. 2000).

Sporophytic cells contain diploid cells that undergo meiosis and produce haploid male spores or microspores, and the microspores then divide mitotically and differentiate into multicellular male gametophytes or pollen grains (Goldberg et al. 1993). The microscopic analysis of anthers in plants grown continuously at a high temperature (32/26 °C) indicates a disruption of development in the pollen of *Lycopersicon esculentum* (Sato et al. 2002). At least three stages of reproductive growth of *Hordeum vulgare* are sensitive to high temperature (30/25 °C), namely, the early differentiation stage, the pre-meiotic stage and the meiosis of the pollen mother cells, and high temperatures during these stages result in abnormal pollen or sterility (Sakata et al. 2000). Reduced fruit-set is a consequence of fewer pollen grains and pollen viability in *A. hypogaea* (Prasad et al. 1999). Short episodes of high temperature (35 or 40 °C) will affect the fruit set and yield of *G. hirsutum* during the early reproductive period (Reddy et al. 1992b). In the cotton-growing region of the Yangtze River Valley in China, several periodic episodes of high temperature, defined as temperatures greater than 35 °C that continue at least 10 days, usually occur from June to August each year (Su et al. 2006), which is the stage of reproductive development in upland cotton. When cotton is flowering and boll setting is at its peak, high temperature results in boll abscission and yield reduction (Reddy et al. 1992b; Ma et al. 2010; Mei et al. 2014). The daily maximum temperature 15–16 days before anthesis and the percentage of sterile anthers are positively correlated in *G. hirsutum* (Meyer 1966). Pollen grain germination on pistils in flowering plants is related to the growth of pollen tubes, double fertilization and the development of seeds or fruits. High-temperature stress generally decreases pollen grain germination. Pollen germination (PG) under high-temperature conditions is highly correlated with yield, and the relationship between PG and temperature can be a reference for screening high temperature-tolerant *A. hypogaea* cultivars (Kakani and Prasad 2002; Craufurd et al. 2003). The results from in vitro pollen experiments have shown that variation exists in terms of PG and pollen tube growth response to different temperatures in *A. hypogaea* cultivars (Kakani and Prasad 2002) and *G. hirsutum* genotypes (Kakani et al. 2005) depending on the screening of high temperature-tolerance cultivars. The cultivars of *Capsicum annuum* have been screened for high-temperature tolerance via in vitro pollen germination percentage (PGP) (Reddy and Kakani 2007).

Although PGP and pollen tube length (PTL) under different germination temperatures are widely used as criteria for screening high-temperature tolerance cultivars of crops, it is difficult to distinguish the various effects of high-temperature stress during stamen development and PG. The effective method is to measure pollen vitality and the temperature of the pollen development process every day and use the PGP as a standard for the selection of heat-tolerant cultivars. However, the effect of high temperature on pollen vitality during the flower development process is rarely reported. The effects of high-temperature stress on cotton pollen grain germination during square development remain unknown. The main purpose of this study was to establish a rapid screening method and criteria for the high temperature-tolerance of pollen grains of upland cotton cultivars. We screened high-temperature tolerance upland cotton cultivars by measuring the PGP and temperature during the square development process in a natural environment before and during the high-temperature stage, and we also measured the boll setting rate. The results provided a basis for the identification of high-temperature tolerance upland cotton cultivars.

Materials and methods

Plant materials and experimental design

The experiments were performed at Nanjing Xiaozhuang University in Nanjing, Jiangsu, China (31°95′ N and 118°83′ E). The experimental materials consisted of four upland cotton cultivars (lines): 9650Duanguozhi, Yankang1107, Sumian12 and Sumian16, which were screened from 200 varieties (data not published) in 2009. Seeds were sown in pots (0.09 m in height and 0.05 m in diameter) on April 10, 2010. The

seedlings were transplanted into the experimental field on May 11, 2010. A randomized complete block design was used, with three replicates for each cultivar. The plants were spaced 0.3 m apart in the rows, and the row spacing was 0.7 m. Each plot was composed of 4 rows, and each row consisted of 15 plants. The application of fertilizer, pesticides, irrigation and other culturing methods followed the conventional procedures.

Pollen grains collected from July 23 to August 24, 2010 were cultured at 30, 33, 35, 37 and 39 °C, with three replicates for each culture temperature. The daily maximum temperature in Nanjing according to Weather China was recorded from June 17 to August 24, 2010. The duration of periods with temperatures greater than 33 and 35 °C every day was also recorded.

Pollen grain collection

Pollen grains from the four cultivars were collected from the first flowering stage to the peak flowering stage. Fresh cotton flowers were picked randomly from the same position on the fruiting branches of plants of each cultivar at the time of anther indehiscence between 6:00 and 7:00 in the morning, and they were immediately placed in plastic bags and carried to the laboratory. The flowers were placed for 1–2 h at room temperature until anther dehiscence. The temperature of the laboratory was maintained at 28 °C, and the relative humidity was maintained at 65–70 % with a hygrothermograph (HS35D-1, Hangzhou, China). The pollen grains were cultured upon anther dehiscence. Observations of PGPs were not conducted from August 17 to August 21: because of high temperatures, the anthers of all cultivars did not release pollen grains from August 17–21. Based on the average daily maximum temperature at August 17–21, we included August 17–21 in the period after the high-temperature period.

Pollen in vitro culture

The pollen culture medium used in this study consisted of the following components (weight/volume, w/v): 0.07 % $MnSO_4$, 0.04 % $Ca(NO_3)_2$, 0.02 % H_3BO_3, 0.01 % serine, 0.01 % glutamate, 0.01 % lysine, 0.01 % proline and 40 % sucrose dissolved in 100 mL of deionized water. The medium was stored at 4 °C, and it was placed at room temperature before being used. Culture medium (50 μL) was placed on slides and temperature equilibrated before the pollen was sprinkled on the medium. Pollen grains were sprinkled on the medium by gently tapping the flowers above the surface of the medium on each slide. Approximately 300–500 pollen grains were sprinkled on the surface of the medium on each slide. Three slides of each cotton cultivar at each temperature treatment were used as replications. The slides were placed into a Petri dish (with a diameter of 20 cm) with three layers of filter paper at the bottom and 8 mL of deionized water. The Petri dishes were then covered with plastic wrap to maintain 70–80 % relative humidity. The relative humidity in the Petri dishes was measured with a hygrometer (testo 605-H1, Germany). After the Petri dishes were covered with plastic wrap, they were gradually placed in the incubators. The Petri dishes with medium containing pollen grains were incubated in the dark at 30, 33, 35, 37 and 39 °C for 3–4 h in the incubators (SPX-1000B-2, Shanghai, China). The incubators were maintained at the setting temperature, and the temperature of the medium was the same as the incubator temperature.

Pollen germination measurement

Coverslips were placed gently on the medium (50 μL), and PG was determined by microscopic observation (Jiangnan XS-213, Nanjing, China). A pollen grain was considered to have germinated when the PTL was equal to or greater than the grain diameter (Kakani and Prasad 2002). The germination percentage was determined by dividing the number of germinated pollen grains per field of view by the total number of pollen grains per field of view. Ten fields of view, approximately 200 pollen grains, were measured.

The following formula was used to determine PGP:

$$\mathrm{PGP} = \frac{\text{The number of germinated pollen grains per field of view}}{\text{The total number of pollen per field of view}} \times 100\,\%$$

Decreases in the rate of pollen germination

To analyze variation in the PGP at various culture temperatures during different stages, we used decreases in the rate of pollination germination (pollen germination percentage decreased rates, PGDR) for analysis. The following formula was used to determine the PGDR:

$$\mathrm{PGDR} = \frac{\text{PGP of culture temperature X °C} - \text{PGP of culture temperature 30 °C}}{\text{PGP of culture temperature 30 °C}} \times 100\,\%$$

Investigation of boll set rate

The boll set rate of each cultivar was measured with tagged flowers at the same nodal position on nodes not used for the determination of PG. Flowers of selected plants were tagged to record the number of open flowers on July 28 and 29 and August 9, 10, 11 and 12. The numbers of retained bolls were counted for the same flowers that had been tagged at harvest. The boll set rate was calculated by dividing the number of bolls retained from their previously tagged flowers by the total number of tagged flowers and was expressed as a percentage.

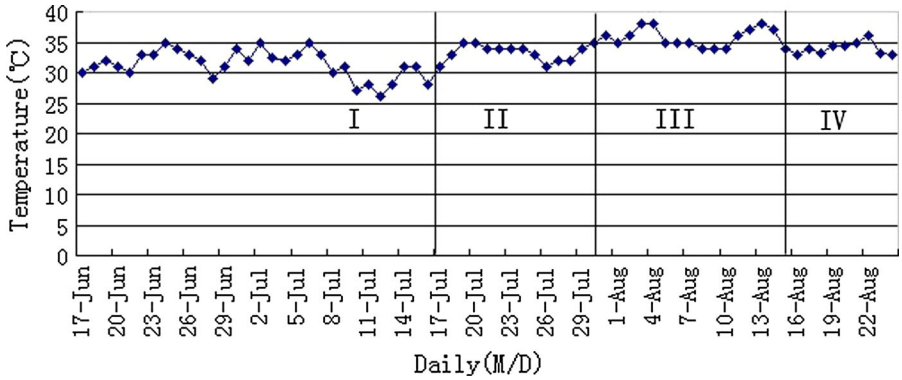
Data analysis

The PGP data were analyzed using Excel XP (Microsoft Corporation, 2003), and OriginPro7.5SR1 (Originlab Corporation, 2003) was used for graphing. The effect of temperature on in vitro PGP was analyzed using SPSS18.0 (IBM, PASW Statistics V18.0, 2010). The PGP data were subjected to an analysis of variance (ANOVA) using SPSS 18.0. Differences between the means for PGP and for the boll set rate were analyzed with an LSD test.

Results

Variation in daily maximum temperature

The curve of daily maximum temperature variation from June 17 to August 24, 2010 at Nanjing, Jiangsu Province, was divided into four different stages, indicated by I, II, III and IV (Fig. 1). Stage I, the cotton square development stage, extended from June 17 to July 17. During this stage, the average daily maximum temperature was 31.3 °C. The time during which daily maximum temperature was above 33 °C and the time during which daily maximum temperature was above 35 °C were less than 3 h daily (Fig. 2). During Stage II, from July 18–30, the average daily maximum temperature was 33.5 °C. This average daily maximum temperature was lower than 35 °C. The time during which daily maximum temperature was above 33 °C was less than 5 h daily. During Stage III, from July 31 to August 15, the average daily maximum temperature was 35.8 °C. A daily maximum temperature above 35 °C occurred on 12 of these 16 days. This period was the high-temperature period in the Yangtze River Valley. The daily maximum temperature was greater than 33 °C for less than 12 h, and the daily maximum temperature was greater than 35 °C for less than 10 h. This long duration of high-temperature stress influenced seriously the development of squares. During Stage IV, from August 16–24, the average daily maximum temperature was 34 °C. Two days had a maximum temperature greater than 35 °C. The variation of the daily maximum temperature (Fig. 1) was consistent with the curve of the duration of the daily maximum temperature above 33 and 35 °C (Fig. 2). The daily maximum temperature was used to define a classification consisting of the following three periods from June 17 to August 24: before the high temperatures (BHT) (June 17–July 30), the high temperatures (HT) (July 31–August 16) and after the high temperatures (AHT) (August 17–24). In upland cotton, the optimum temperature for square development is 30 °C, and a temperature of 35 °C will inhibit square formation and development (Reddy et al. 1992a; Meyer 1966). Cotton reproduction is most vulnerable to average daily maximum temperatures above 32 °C (Reddy et al. 1999).



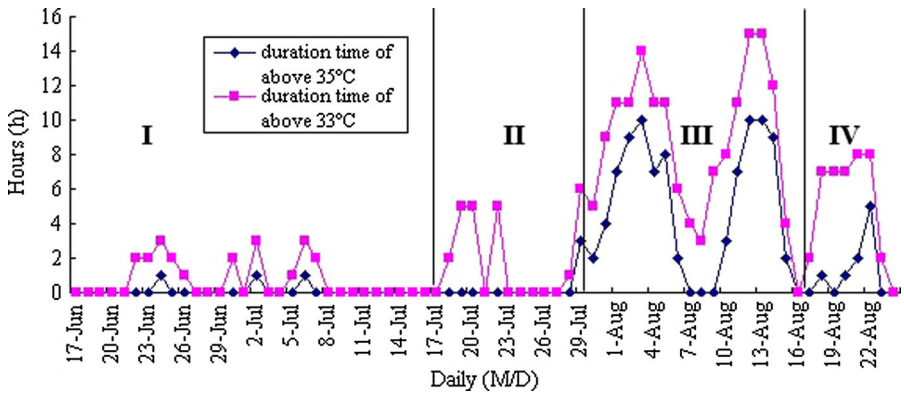


Fig. 1 Daily high temperatures of the cotton growth stages

Fig. 2 Durations of periods of temperatures above 33 °C and above 35 °C daily

According to these results, the temperature during Stage I and II was generally close to the optimum temperature for square development, but a few days with temperatures greater than 33 °C may affect the development of cotton squares. The temperature during Stage III and IV was higher than the optimum temperature for square development, thus influencing square growth during the two periods. Daily maximum temperatures above 33 °C or above 35 °C may affect square development. We suggest that temperatures above 30 °C are high temperatures for square development.

Effects of high temperature during the square development process on PGP in vitro

Stamen development in cotton occurs approximately 22–28 days before flowering. Pollen development in this stage tends to be influenced by high temperature. To detect the effects of high temperature on PGP during the stamen development process, we measured the PGP at the culture temperature of 30 °C for 28 consecutive days. The results showed that the PGP of four cultivars could be divided into three distinct stages (Fig. 3) that were the same as those for the high-temperature curve (Fig. 1). At the BHT stage (July 23–30), the average PGP values for 9650Duanguozhi, Sumian12 and Yankang1107 did not differ significantly. However, the average PGP of Sumian16 was the lowest, and it differed significantly from those of the other cultivars. At the HT stage (July 31 to August 16), the average PGP of the four cultivars was significantly lower than that at the BHT stage, and the differences in PGP among cultivars were higher than those at the BHT stage. The average PGPs of 9650Duanguozhi and Sumian12 were still the highest, followed by the PGP of Yankang1107; and the average PGP of Sumian16 was the lowest. During the AHT stage (August 22–24), the average PGPs of all cultivars were between the values for the BHT and HT stage, but the average PGP of Sumian16 was still

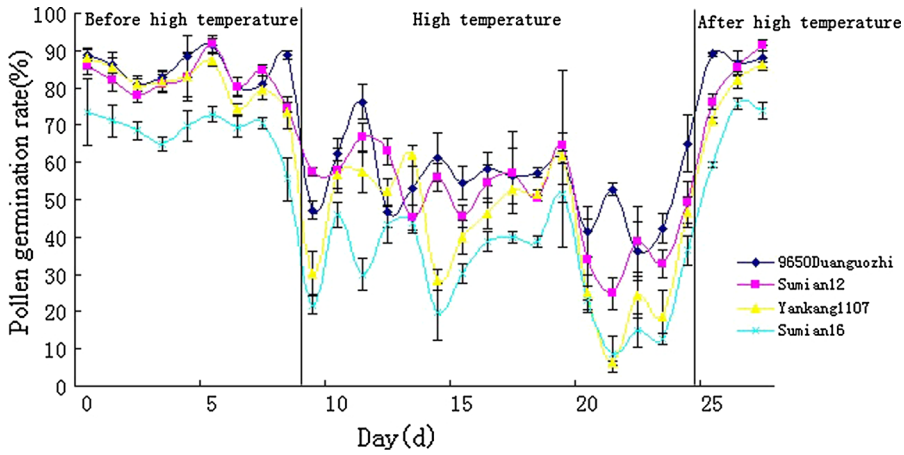


Fig. 3 PGP of four cotton cultivars in vitro at a 30 °C culture temperature

the lowest. The results showed that the PGPs of different cultivars differed significantly at a culture temperature of 30 °C. Because the 30 °C culture temperature is generally the optimum temperature for the germination of cotton pollen grains, the variation in PGP at the 30 °C culture temperature was primarily the result of the high temperatures during stamen development. Pollen viability declined with increasing temperature during the stamen development stage. During the same period, the PGP was generally at the same level but showed several changes. During the BHT stage, the PGPs of the four cultivars decreased on July 25, 26, 29 and 30 due to the effects of transient high temperature on the pollen formation process. The square development process was affected by a transient high temperature of 33 and 35 °C at the BHT stage (Fig. 1, Stage I and II) resulting in decreased PGPs from July 31 to August 13. Because of sustained high temperatures during the HT stage (Fig. 1, Stage III), the PGPs on August 14–16 were significantly lower than those before August 14 (Fig. 3). Heat stress decreased after August 16, but PGP was gradually restored (Fig. 3; after high-temperature stage) to normal levels. The PGP differences for the BHT, HT and AHT stages resulted from the effects of high-temperature stress in the field on square development. The results showed that high temperature during square development may affect the germination vigor of pollen grains of upland cotton cultivars at flowering, although the pollen grains are cultured at temperatures under 30 °C, an optimum temperature for the germination of pollen grains.

Effects of culture temperature on in vitro pollen germination percentage

The PGP of the four cotton cultivars decreased significantly under 33, 35, 37 and 39 °C culture temperatures (Fig. 4a–d), showing that PGP was significantly influenced by the culture temperature. Higher pollen grain culture temperatures resulted in larger PGP decreases. At the same culture temperature, the PGPs of the four cultivars differed significantly (Tables 1, 2). The PGP of Sumian16 was the lowest at all culture temperatures, and the PGPs of Sumian12 and 9650Duanguozhi were the highest at all culture temperatures. The four cultivars showed the same trend of variation in the three periods (BHT, HT and AHT stages). Under the same culture temperature, the variation of PGP during the BHT and HT stages was due primarily to the influence of environmental temperature on pollen development. The PGP decreased more markedly as the daily maximum temperature increased and as the duration of the daily high temperature increased (Figs. 1, 2). As the culture temperature increased (33, 35, 37 and 39 °C), PGP decreased significantly, and the PGP at the high culture temperature was significantly lower than that under a culture temperature of 30 °C. The germination rates of pollen grains were influenced by high-temperature stress, including the processes occurring during pollen development and cultivation in vitro. The differences in PGP among cultivars were more obvious because of the varying tolerance to high temperature, and the overlapping effects of high-

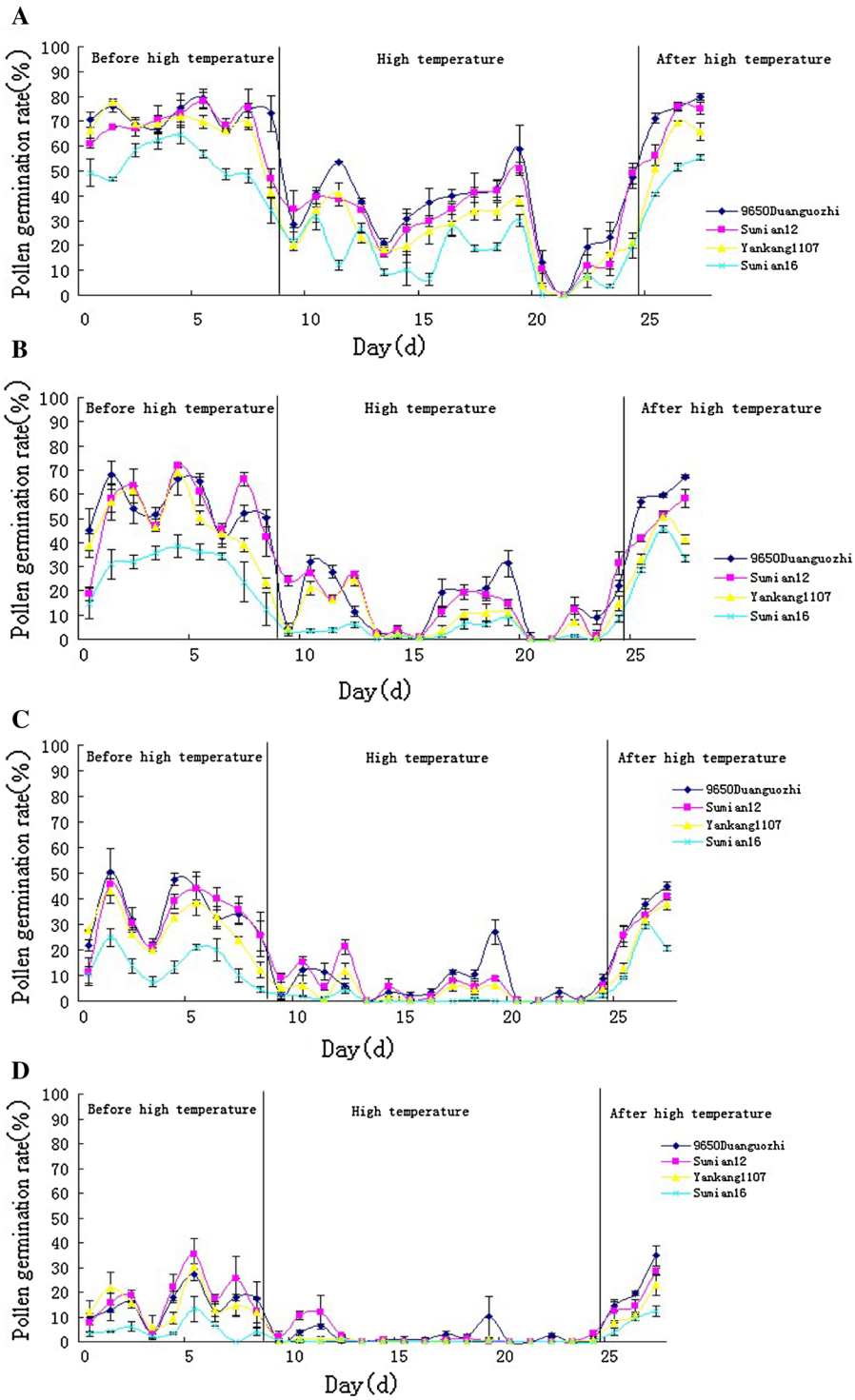


Fig. 4 PGP of four cotton varieties in vitro at different culture temperatures **A**, **B**, **C** and **D** are PGPs at 33, 35, 37 and 39 °C, respectively

temperature stress during pollen development and culture were more obvious in the susceptible cultivars (Table 1). The PGP values at different temperatures and in different cultivars showed significant differences, but the interaction of temperature and cultivar showed no significant differences (Table 2). The

Table 1 The average germination percentage of cotton pollen grains at different temperatures

	July 23–30				July 31–August 16				August 22–24			
	9650Duanguozhi	Sumian12	Yankang1107	Sumian16	9650Duanguozhi	Sumian12	Yankang1107	Sumian16	9650Duanguozhi	Sumian12	Yankang1107	Sumian16
PGP of 30 °C	85.02 ± 1.50a	83.30 ± 1.51a	82.65 ± 1.58a	70.09 ± 0.94a	56.54 ± 3.11a	51.32 ± 3.22a	43.10 ± 4.41a	32.68 ± 3.38a	87.93 ± 0.69a	84.35 ± 4.43a	79.88 ± 4.42a	69.65 ± 5.12a
PGP of 33 °C	72.28 ± 1.67b	70.19 ± 1.90b	69.83 ± 1.30b	54.31 ± 2.45b	35.82 ± 4.30b	30.51 ± 3.22b	24.00 ± 3.01b	16.41 ± 2.72b	75.56 ± 2.51b	69.19 ± 6.45b	61.92 ± 5.61b	49.19 ± 4.44b
PGP of 35 °C	55.55 ± 3.46c	54.04 ± 5.96c	50.66 ± 3.88c	30.74 ± 2.78c	15.74 ± 3.40c	14.90 ± 3.22c	8.96 ± 2.03c	3.68 ± 0.91c	61.12 ± 3.21c	50.26 ± 4.77c	41.64 ± 5.08c	35.81 ± 5.09c
PGP of 37 °C	35.59 ± 3.87d	33.44 ± 4.20d	30.64 ± 2.67d	15.07 ± 2.17d	7.58 ± 2.01d	6.68 ± 1.85d	3.48 ± 0.98d	0.98 ± 0.37c	36.32 ± 5.51d	33.23 ± 4.35d	27.56 ± 7.61d	19.61 ± 5.82d
PGP of 39 °C	14.59 ± 2.47e	18.30 ± 3.49e	15.24 ± 2.68e	4.78 ± 1.40e	2.92 ± 1.13d	2.68 ± 1.06d	1.10 ± 0.69d	0.36 ± 0.24c	22.82 ± 6.15e	18.53 ± 5.07e	13.63 ± 4.66e	8.57 ± 2.43e

PGP of 30, 33, 35, 37 and 39 °C pollen grains germination percentage under 30, 33, 35, 37 and 39 °C culture temperature, respectively

Mean ± SD (standard deviation) was used for the value of pollen grains germination percentage, significant level of pollen grains germination percentage at $P < 0.05$

results showed that PGP was affected by the overlap of the negative effects of high temperature (higher than 30 °C) during stamen development and culture in vitro at flowering. Four different cotton cultivars showed different resistance to high temperature during pollen grain development.

Variation in pollen germination percentage under different culture temperatures

The PGDRs of the four cultivars in different periods varied under the culture temperatures of 30, 33, 35, 37 and 39 °C (Table 1). The PGDR reflected the heat tolerance levels of pollen grains at different culture temperatures. Under the same culture temperature, the PGDR was negatively related to heat resistance ability. At the BHT stage, the differences in PGDRs among the cultivars under a culture temperature of 35 °C were the most obvious. The PGDRs for 9650Duanguozhi, Sumian12, Yankang1107 and Sumian16 were 34.66, 35.13, 38.71 and 56.14 %, respectively, with an average of 41.16 %. At the HT stage, the PGDRs of 9650Duanguozhi, Sumian12, Yankang1107 and Sumian16 at 35 °C were 72.16, 70.97, 79.21 and 88.74 %, respectively, with an average of 77.77 %. However, the differences in PGDRs among the cultivars increased at culture temperatures of 30–33 °C and decreased at culture temperatures of 35–37 °C. Because the PGP was low (Fig. 4d) at the 39 °C culture temperature, no PGP differences among cultivars were observed at this temperature. The results showed that a culture temperature of 35 °C might be a critical temperature for the pollen viability transition and could be used to screen cotton cultivars for pollen grains that have high temperature resistance.

The relationship between boll set rate and pollen germination percentage in vitro

The boll set rates of 9650Duanguozhi, Sumian12, Yankang1107 and Sumian16 were not identical. At the BHT stage, the boll set rate of Sumian12 was the highest, whereas the boll set rate of Sumian16 was the lowest. The boll set rate and PGP variation showed the same trend (Table 3), and they were positively correlated. The correlation coefficients were 0.866, 0.858, 0.906 and 0.837 ($P < 0.05$), respectively. During the HT stage, the boll set rate of

Table 2 Analysis of variance for the tested pollen grains germination percentage at BHT, HT and AHT

Variables	MS		SS		*F* value		Significant level of treatment effect		
	T	C	T	C	T	C	T	C	T × C
df	4	3	4	3	4	3	4	3	19
1PGP	23751.93	2753.69	95007.70	8261.06	361.50	41.91	***	***	NS
2PGP	22894.72	2780.15	91578.86	8340.44	196.81	23.90	***	***	NS
3PGP	8075.50	1123.35	32302.00	3370.06	111.71	15.54	***	***	NS

df degree of freedom, *1PGP* the pollen grains germination percentage in July 23–30, *2PGP* the pollen grains germination percentage in July 31–August 16, *3PGP* the pollen grains germination percentage in August 22–24, *T* temperature, *C* cultivar, *MS* mean square, *SS* sum of square, *NS* not significant

*** Significant at $P < 0.05$

9650Duanguozhi was the highest, and the boll set rates of Yankang1107 and Sumian16 were the lowest. Compared with the BHT stage, the boll set rate and PG both decreased. The PGP was positively correlated with the boll set rate for the same period. The results indicated that pollen viability was a key factor for the boll set rate. The PGP was negatively correlated with the daily maximum temperature, and the correlation coefficients were −0.873, −0.952, −0.942 and −0.896 ($P < 0.05$), respectively. The boll set rate was negatively correlated with the daily maximum temperature, and the correlation coefficients were −0.941, −0.767, −0.803 and −0.822, respectively. The results showed that the lower PGPs could be the reason for the decrease in the boll set rate.

Discussion

The relationship between pollen germination percentage and culture temperature in vitro

The values of the PGP and PTL response to temperature in different cultivars are different, and the PGP at temperatures greater than the optimum temperature decreases with culture temperature increases in *A. hypogaea* and *C. annuum* (Kakani and Prasad 2002; Erickson and Markhart 2002). Our results agreed with these previous studies. PGP decreased as the germination temperature increased. Temperatures greater than 30 °C inhibit PG of upland cotton (Barrow 1983). PGP is low when the culture temperature is greater than 30 °C, and PGPs differ among cultivars (Salem et al. 2007; Singh et al. 2008). Cultivars with higher $PG\%_{max}$ and PTL_{max}, as well as an optimum temperature >32 °C for maximum PG in vitro on a simple defined medium, can be used for screening *G. hirsutum* cultivars for high-temperature tolerance (Kakani et al. 2005). Our study also found that the PGPs of the four cultivars differed significantly as the temperature increased and that 35 °C could be a critical temperature for pollen vigor. The pollen vigor was very low at a culture temperature above 35 °C. This finding could be used to screen cultivars for heat tolerance during pollen grain germination. High temperature stress for pollen grain germination occurs above 30 °C. Therefore, a temperature of 35 °C, below which pollen can germinate and grow well, can be used as a tool to identify high-temperature tolerance in cotton cultivars. Moreover, the PGDR at 35 °C compared with that at 30 °C could be used as the screening parameter.

The relationship between the temperature for square development and PGP in vitro

At least three stages of reproductive growth are sensitive to high temperature in *H. vulgare*, resulting in abnormal and sterile pollen (Sakata et al. 2000). Two stages of pollen development, namely, microspore mother cell meiosis and mature microspores at anthesis, have been reported to be highly sensitive to high temperature (33 °C) in *C. annuum* (Erickson and Markhart 2002). When the anther of a plant grows continuously at a high temperature (32/26 °C), pollen development is disrupted, e.g., in *L. esculentum*, where this disruption is decreased but still observable in

Table 3 Boll set rate and pollen germination percentage of four cotton cultivars

	9650Duanguozhi		Sumian12		Yankang1107		Sumian16	
	Boll set rate	PGP	Boll set rate	PGP	Boll set rate	PGP	Boll set rate	PGP
July 28	72.78 ± 1.47	91.43 ± 1.12	82.89 ± 1.98	91.96 ± 1.29	65.41 ± 0.30	87.47 ± 1.02	56.57 ± 0.72	72.88 ± 1.18
July 29	64.67 ± 2.60	80.28 ± 1.37	75.44 ± 1.37	80.18 ± 1.56	52.67 ± 1.45	74.40 ± 0.86	44.33 ± 2.33	69.57 ± 1.60
August 9	53.33 ± 2.40	56.33 ± 4.33	40.67 ± 1.76	57.23 ± 6.36	32.67 ± 1.76	52.47 ± 2.10	32.00 ± 1.15	40.01 ± 0.90
August 10	54.67 ± 2.91	57.26 ± 0.79	43.33 ± 1.76	50.25 ± 0.43	43.33 ± 2.40	51.45 ± 0.60	35.00 ± 0.58	38.68 ± 0.87
August 11	49.33 ± 1.76	57.26 ± 1.15	54.33 ± 2.33	64.73 ± 1.89	43.67 ± 2.33	61.46 ± 0.92	32.67 ± 1.45	51.64 ± 1.47
August 12	42.00 ± 2.00	57.26 ± 3.84	45.00 ± 2.52	64.73 ± 0.58	32.00 ± 1.15	25.17 ± 2.73	32.00 ± 1.15	23.26 ± 2.05

PGP pollen grains germination percentage

Mean ± SD (standard deviation) was used for the value of pollen grains germination percentage and boll set rate, correlation analysis of the PGP and boll set rate was at the 0.05 level in results

plants relieved from high temperatures for 10 days before anthesis (Sato et al. 2002). Micro- and mega-sporogenesis are easily injured by high temperatures (35 °C). The injuries to micro- and mega-sporogenesis result in low pollen fertility (Cross et al. 2003; Young and Wilen 2004). Low pollen fertility of *Brassica napus* reduces PG and pollen viability at high temperatures (35 °C) (Young and Wilen 2004). These studies show that high temperature influences pollen fertility and vigor during the development of the stamens of flowering plants.

We found that high temperature during the development of cotton squares and at the blossom stage (Fig. 1) affects PGP. High temperature at Stage II and III (Fig. 1) resulted in lower pollen grains viability and PGP (Fig. 2; Table 1), and high temperature even resulted in anther indehiscence. Our results suggest that PGP may be influenced by high temperature during all stages of square development. Meyer (1966) reported a positive correlation between cotton anther sterility and the maximum temperature at 15-16 days prior to anthesis, suggesting that microgametophyte development was exceptionally sensitive to high temperatures immediately after meiosis of the microspore mother cell. The reproduction of cotton is most vulnerable to average daily temperatures above 32.8 °C (Reddy et al. 1999). When cotton pollen is exposed to high temperature for a few hours, the viability of the pollen grains is reduced (Barrow 1983). The meiosis of microspore mother cells occurs 22–23 days before anthesis, and division of the microspore nucleus occurs earlier than 12 days before anthesis (Sarvella 1964; Meyer 1966). We consider that flowers open during the period August 1–16, whereas their microspore development stages may occur during the period July 18–August 4, according to the results of Sarvella (1964) and Meyer (1966). The duration of high temperatures during Stage II and III (Fig. 2) may influence the development of squares during this period, resulting in PGP reduction during the stage August 1–16. If the sensitive stage of square development suffers from high temperatures, the pollen grain viability and PGP may decrease. These results are consistent with the previous conclusions of Meyer (1966) and Reddy et al. (1999), who reported that temperature stress during square development affected pollen grain viability. We consider that temperatures above 33 °C may be a high-temperature stress for square development of upland cotton.

Relationship between pollen germination percentage and boll set rate

Sato et al. (2002) showed that *L. esculentum* plants treated with heat stress (32/26 °C) for 0–15 days before anthesis fail to set fruit due to decreased pollen viability. Young and Wilen (2004) used *B. napus* to perform crosses between male donor and female receptor plants exposed to 35 °C heat stress, and they measured reproductive output in terms of seed production. These results suggest that high temperature has a direct effect on pollen viability. In this study, the number of days of high temperature differed between the BHT and HT stages (Fig. 1) resulting in significant differences in PGP (Table 1) and boll set rates (Table 3). Our conclusions were consistent with those of Young and Wilen (2004) and Sato et al. (2002). The differences in seed setting rate might be due to the influence of high temperature during bud development, resulting in differences in pollen activity and seed setting rate among the cultivars. Our study results imply that PGP during the HT stage was positively correlated with the boll setting rate. Therefore, pollen viability is one of the important factors that influence boll setting. This conclusion is consistent with that of Sato et al. (2002).

Screening heat-tolerant cultivars would be an important measure for improving cotton production in high-temperature environment. Tolerant cultivars have higher yields than controls under high-temperature conditions (Sato et al. 2002). Overexpression of the cell wall arabinogalactan proteins (*AGP6* and *AGP11* genes) may improve pollen viability under high temperatures (Levitin et al. 2008). Overexpression of *TDF1*, a tapetum development and function gene, may reduce sterile pollen under high temperatures (Zhu et al. 2008). Transgenic technology may improve the stamen development process, increasing the heat resistance level and pollen vitality.

Screening methods for high-temperature tolerance

Upland cotton genotypes have been bred for heat tolerance by selecting progenies developed from pollen grains that survive exposure to 35 °C for 15 min (Rodriguez-Garay and Barrow 1988). This result suggests that pollen characters could be used to screen high-temperature tolerant cotton cultivars. Using PGP and PTL of peanut as parameters at different culture temperatures, *A. hypogaea* cultivars have been screened for high-temperature tolerance (Kakani and Prasad 2002; Craufurd et al. 2003). Kakani et al. (2005) and Liu and Yuan (2006) screened heat-tolerant cultivars of *G. hirsutum* based on a principal component analysis of PGP and PTL. However, they reported that the pollen grains used were not influenced by high temperature during bud development. We found that high temperature during square development influenced the germination of pollen grains in cotton. In the present study, we measured PGP continuously, and we found that the PGPs in the HT stage had greater differences than those in the BHT stage. The daily maximum temperature during square development caused a decrease in PGP. Our results were not completely consistent with those of Liu and Yuan (2006). In this study, 9650Duanguozhi, Sumian12 and Yankang1107 were classified as high-temperature tolerant cultivars, and Sumian16 was classified as a high-temperature susceptible cultivar. These cultivars were screened from 200 varieties (data not published). In contrast, Liu and Yuan (2006) considered Sumian16 as a heat-tolerant cultivar and Sumian12 as a moderately susceptible cultivar based on a principal component analysis. These conclusions were not consistent with our results. Our results suggested that Sumian16 was sensitive to high temperature in the HT and BHT stages because the PGP of Sumian16 was minimal and significantly lower than that of other cultivars. The optimal PG temperature of Sumian16 might be lower than 30 °C, as its square development was more susceptible to high temperatures. We also observed that the days of anther indehiscence for Sumian16 were more numerous than those for other cultivars. Moreover, Sumian16 frequently showed slightly smaller bolls at high temperatures.

Temperature is one of the most important environmental factors for plant sexual reproduction, especially for PG. PGP under high-temperature conditions can be used for screening as a heat tolerance parameter in *A. hypogaea* (Prasad et al. 1999). Moreover, we found that pollen heat tolerance of cultivars was the most significant difference at the 35 °C culture temperature during the BHT stage. Cultivars with PGDR decreases of approximately 41 % at 35 °C, compared with 30 °C, may be considered as high temperature-tolerant cotton cultivars, and cultivars with PGDR decreases greater than 41 % might be considered as high temperature-sensitive cotton cultivars. A PGDR of 41 % might be used as a reference for screening high-temperature tolerant cotton cultivars.

In the HT stage, a PGDR less than 77 % might be considered as a criterion for high-temperature-tolerant cotton cultivars, and a PGDR greater than 77 % might be considered as a criterion for high-temperature susceptible cotton cultivars.

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