Genotypic Differences in Thermotolerance are Dependent Upon Pre-Stress Capacity for Antioxidant Protection of the Photosynthetic Apparatus in Cotton

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RESEARCH PROBLEM

Cotton is exceptionally sensitive to high temperature during reproductive development, with a negative correlation existing between high temperatures during flowering and yield (Oosterhuis, 2002). Furthermore, reproductive thermosensitivity in cotton is closely associated with the photosynthetic thermosensitivity of the subtending leaf (Snider et al., 2009). For example, high temperature is known to cause significant declines in fertilization efficiency (Snider et al., 2009) and carbohydrate content of the cotton pistil (Snider et al., 2009) along with declines in net carbon fixation of major source leaves (Bibi et al., 2008; Snider et al., 2009).

BACKGROUND INFORMATION

Plants exposed to heat stress respond with increased antioxidant enzyme activity to prevent the accumulation of damaging reactive oxygen species (ROS) (Gong et al., 1998). Although the importance of antioxidant enzymes in acquired thermotolerance following an acclimative response to high temperature has been shown previously for wheat (Almeselmani et al., 2006), information on the relationship between pre-stress antioxidant enzyme activity and innate photosynthetic thermotolerance is lacking. We recently obtained seeds for a cotton cultivar reported to have high fruit retention under maximum daily temperatures as high as 45 °C (VH260). The objective of this study was to quantify the relationship between PSII threshold temperature and pre-stress levels of antioxidant enzyme activity. We hypothesized that pre-stress antioxidant enzyme activity would be highest in a more thermotolerant cultivar and that the high temperature threshold for PSII efficiency will be dependent upon pre-stress antioxidant enzyme activity.

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RESEARCH DESCRIPTION

Two consecutive experiments were conducted to evaluate the effects of heat stress on reproductive development and source leaf activity in *Gossypium hirsutum* L. Experiments were conducted in January 2009 using the cotton cultivars cv. ST4554 B2RF (thermosensitive) and VH260 (thermotolerant) planted in two-liter pots and placed in two walk-in growth chambers (Model 36; Controlled Environments Limited, Winnipeg, Canada) at the Altheimer Laboratory, Arkansas Agricultural Research and Extension Center, Fayetteville Ark. under 30/20 °C day/night temperature regimes. Plants were grown under a 12 h photoperiod at a 500 μmol m⁻² s⁻¹ photosynthetically active radiation (PAR) and were watered daily with half-strength Hoagland’s solution.

To quantify in situ genotypic differences in actual quantum yield (ΦPSII) temperature responses (measured using a pulse amplitude modulated fluorometer), first-position sympodial leaves subtending open flowers on the day of anthesis at the tenth main-stem node above the cotyledon nodes from both cultivars were selected. Leaves were continually illuminated at 500 μmol photons m⁻² s⁻¹ of growth chamber irradiance. Leaf temperature was increased in 5 °C increments up to 50 °C, and ΦPSII was determined after 5 min of incubation at each temperature. Both the temperature at which ΦPSII is maximal (Topt) and the temperature at which ΦPSII declines 15% from Topt (T15ΦPSII), were determined from a best fit curve for both *G. hirsutum* cv. ST4554 (Fig. 1A) and VH260 (Fig. 1B) of ΦPSII versus leaf temperature data. The threshold T15ΦPSII was used as an indication of heat stress and is comparable to the method of Froux et al. (2004), which is an acceptable method for quantifying high temperature thresholds. Temperature control was accomplished using a thermoelectric cooler/heater and leaf temperature was monitored using a type K fine-wire thermocouple and a digital thermometer.

Unheated sections of the leaves utilized for high temperature threshold determination were collected for pre-stress antioxidant enzyme quantification. The activity of superoxide dismutase (SOD) was quantified spectrophotometrically by comparing the SOD dependent inhibition of NBT reduction of known SOD standards with the inhibition of NBT reduction of the sample in a xanthine-xanthine oxidase coupled system at 560 nm. Glutathione reductase (GR) activity was quantified by monitoring the NADPH-dependent reduction of oxidized glutathione at 340 nm using a plate reader.

RESULTS

The optimal temperature (Topt) and the high temperature threshold (T15ΦPSII) were both significantly affected by cultivar (P < 0.0001 and P = 0.012, respectively). For example, *G. hirsutum* cv. VH260 had a 7.5 °C and 5.5 °C lower mean Topt (27.7 °C Fig. 1A) and T15ΦPSII (38 °C; Fig. 1A), respectively, than ST4554 (Fig. 1B; 35.2 and 43.5 °C, respectively) when both were initially grown under control temperature conditions (30/20 °C). The average SOD activity was numerically
34.8% higher in VH260 than in ST4554, but there was no significant effect of cultivar on SOD activity (P = 0.154; Fig. 2A). However, GR activity of *G. hirsutum* grown under 30/20 °C day/night temperature regime was 225% higher in VH260 compared with ST4554 (P = 0.025; Fig. 2B). Figure 3 shows that the threshold temperature for efficiency of electron transport through photosystem II (T<sub>15PSII</sub>) is nonlinearly dependent upon pre-stress levels of both GR (Fig. 3A; r<sup>2</sup> = 0.532) and SOD (Fig. 3B; r<sup>2</sup> = 0.669) activity. The initial effect of both GR and SOD antioxidant enzyme activity on T<sub>15PSII</sub> is initially positive, followed by a gradual plateau above which additional antioxidant enzyme activity does not lead to a substantial increase in T<sub>15PSII</sub> (Fig. 3A-B).

**DISCUSSION AND PRACTICAL APPLICATION**

The results presented in Figs. 1-3 support our hypothesis that innate thermostolerance would be dependent upon pre-stress capacity for antioxidant defense in *G. hirsutum*. For example, Fig. 2B shows that VH260 has higher GR activity under control temperatures than ST4554 and likely contributes to the higher T<sub>15PSII</sub> observed for VH260 (Fig. 1), since antioxidant enzymes are an essential component of the heat stress response (Gong et al., 1998). We conclude that maintenance of sufficient levels of GR prior to heat stress is a genotypic mechanism for coping with rapid increases in leaf temperature under field conditions (Wise et al., 2004). These findings also suggest that pre-stress GR levels may be an important criterion for selecting heat tolerant cultivars without first exposing them to high temperature conditions as previously described (Almeselmani et al., 2006; Bibi et al., 2008).

**LITERATURE CITED**


Fig. 1. The optimal temperature for \( \Phi_{\text{PSII}} \) (\( T_{\text{opt}} \)) and the temperature resulting in a 15% decline in \( \Phi_{\text{PSII}} \) from \( T_{\text{opt}} \) (\( T_{15\%\Phi_{\text{PSII}}} \)) for thermosensitive (ST4554; A) and thermotolerant (VH260; B) \( G. \) \textit{hirsutum} leaves illuminated with 500 µmol photons m\(^{-2}\) s\(^{-1}\). Both cultivars were grown under optimal (30/20 °C) temperature conditions prior to chlorophyll fluorescence-determination of temperature responses. All values are means ± standard error (\( n = 6 \)). Values not sharing a common letter are significantly different (Student’s t-test; \( P < 0.05 \)).
Fig. 2. Effect of cultivar on superoxide dismutase (SOD; A) and glutathione reductase (GR; B) activity of *G. hirsutum* grown under 30/20 °C day/night temperature regime. All values are means ± standard error (n = 6). Values not sharing a common letter are significantly different (Student’s t-test; P < 0.05).
Fig. 3. The relationship between glutathione reductase (GR; A) and superoxide dismutase (SOD; B) activity and $T_{15\text{PSII}}$ in *G. hirsutum* (solid circles = ST4554 and open circles = VH260) leaves grown initially under 30/20 °C day/night temperature regime prior to rapid leaf temperature increases.