The Americas Journal of Plant Science and Biotechnology ©2010 Global Science Books

Comparison of Responses of a Ruderal *Gossypium hirsutum* L. with Commercial Cotton Genotypes to High Temperature Stress

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ABSTRACT

Global warming has focused attention on the need for improved understanding of crop response to heat stress and the need for enhanced tolerance. Cotton originates in hot climates and native cottons may have the ability to tolerate high temperature stress, however, research is lacking. In this study it was hypothesized that a ruderal cotton from coastal Oaxaca, Mexico will be more tolerant to high temperature stress than USA commercial cotton genotypes. The objective was to compare the responses to high temperature of quantum yield of PSII, leaf extension growth, and antioxidant enzymes of a ruderal *G. hirsutum* L. race Palmeri (PI681044) and four commercial cotton genotypes popular in the USA (Tamcot Sphinx, Fibermax 960BR, Stoneville 474, and Deltapine 444BR) to high temperature. The ruderal *G. hirsutum* race Palmeri was significantly more tolerant to high temperature stress than the commercial cultivars, and this observation was supported by all three methods of assessment. Palmeri had less decrease in quantum yield of PSII, increased catalase and glutathione reductase activity, and less decrease in leaf extension growth compared to the commercial cultivars. Among the commercial cultivars, Tamcot Sphinx showed some tolerance to high temperature however this was not supported by all techniques. This study showed that the rudural cotton has better ability than the commercial cultivars to withstand high temperature stress. This knowledge is of particular importance for germplasm improvement in Upland cotton for high temperature tolerance.

Keywords: antioxidant enzymes, commercial genotypes, high temperature tolerance, leaf extension growth, quantum yield of photosystem II, ruderal *G. hirsutum* L

INTRODUCTION

A United Nations scientific panel on global warming (Anonymous 2007) declared that the evidence of a warming trend is undebatable. Global warming is projected to have significant impact on agriculture. Crop physiological responses to temperature largely determine plant adaptation to different climatic zones and seasons. Temperature is a primary controller of the rate of cotton plant growth, developmental events, and fruit maturation (Baker 1965). The primary centers of diversity for cotton (*Gossypium hirsutum* L.) are in Australia, in northeast Africa and Arabia, and in west-central and southern Mexico (Brubaker 1999) areas of hot and often dry weather. Although cotton originates from areas with hot, dry climates, it suffers under conditions of extreme high day temperature (Oosterhuis 2002). The maximum growth rate for rapid dry matter accumulation during the fruiting period in commercial cultivars of cotton occurs at day/night temperatures of 30/20°C (Reddy *et al.* 1991).

Breeders have improved yields in Pima cotton (*Gossypium barbadence* L.) by increasing high temperature tolerance (Kittock *et al.* 1988), however little has been done to improve high temperature tolerance in Upland cotton (*G. hirsutum* L.). The answer to this may derive from ruderal genetic material collected from the areas where cotton grows under conditionas of extreme heat such as southern Mexico.

The quantum yield of PSII is closely related with the efficiency of CO_2 assimilation (Genty *et al.* 1989) and was used as a tool for investigating photochemical mechanisms underlying photosynthesis (Papageorgiou 1975). In cotton quantum yield of PSII decreases with high temperature and has been used as an indication of high temperature stress (Bibi *et al.* 2008). Therefore, it is important to identify genetic material that can maintain high levels of quantum yield of PSII during high temperature stress.

Heat stress causes damage to photosynthetic and mitochondrial electron transport probably due largely to the formation of reactive oxygen species such as O_2^-, H_2O_2, and NO (Gould 2003). Plants scavenge and dispose of these reactive molecules formed during stress by the use of the antioxidant defense system. An increase in the concentrations of antioxidant enzymes constitutes an important mechanism for avoiding oxidative stress. According to Upadhyaya *et al.* (1991) enhanced synthesis of an antioxidant by plant tissues may increase cell tolerance to heat. Therefore, an increase in the level of antioxidant enzymes at high temperatures may indicate a potential for high temperature tolerance.

Leaf growth can also be detrimentally affected by high temperature. Walter and Shurr (2005) reported that short-term variations of environmental conditions caused rapid, but transient changes in leaf expansion in all investigated monocotyledonous species. Difficulty has been experienced in studying leaf growth dynamics in broad-leaved species (dicotyledonous) due to the absence of suitable methods (Walter and Shurr 2005). Leaf growth has been measured in eucalyptus (*Eucalyptus marginata* L.) in response to water deficit (Stoleman *et al.* 1994), while leaf expansion has been measured in cotton as an indicator of water stress (Oosterhuis *et al.* 1990). However, limited information exists about leaf extension in cotton as affected by elevated temperatures and the use of this technique for cultivar screening for high temperature tolerance.

In this study it was hypothesized the ruderal cotton will be more tolerant to high temperature stress than the commercial cotton genotypes. In addition we hypothesized that the superiority of the ruderal genotype to high temperature conditions would be reflected by the maintenance of both physiological functions and growth. Our objective was to

Received: 26 April, 2009. Accepted: 10 December, 2010.

Original Research Paper

Table 1 Pedigree information for the *G. hirsutum* L. genotypes used in this study.

Genotypes	Area of origin	Parent lines
Fibermax 960BR	U.S.A.	Stoneville 7 (1952)
Stoneville 474	Mississippi Delta	(St 453) x (DES 119)
Tamcot Sphinx	Texas U.S.A.	MAR-CDP3HPIH-1-1-86
Deltapine444BR	Stuttgart, AR	-
G. hirsutum L. race Palmeri	Oaxaca, Mexico	Land race
PI631044	Ulloa *et a*l. 2007	

compare a rudural type of *G. hirsutum* L. with four commercial cotton genotypes in their responses to high temperature using quantum yield of PSII, leaf extension growth, and amounts of antioxidant enzymes.

MATERIALS AND METHODS

Seeds of the five genotypes were planted in two large growth chambers (Model PW36, Conviron, Winnipeg, Canada) at the Altheimer Laboratory of the University of Arkansas, Fayetteville, AR. FM960BR, ST474, Sphinx, DP444BR and *G. hirsutum* (PI 681044) originating from Oaxaca, Mexico (**Table 1**), were planted in 2L pots filled with Sunshine potting media (MIX #1, Sun Gro Horticulture Distribution Inc., Bellevue, WA) and watered with half-strength Hoagland's nutrient solution (Hoagland and Arnon 1950). Three seeds were planted in each pot and thinned to one seed per pot after emergence. The growth chambers were maintained at 29/20°C (day/night temperature), and 75% relative humidity. The photoperiod was set at 10 hours because, even though the commercial cotton genotypes are not photoperiod sensitive, the ruderal genotype required a short photoperiod in order to become reproductive. The photosynthetic active radiation (PAR) followed a typical diurnal pattern with the highest rate of insolation (850 μmol/m^2/s) being between 10:00 a.m and 2:00 p.m. The plants were maintained at control conditions (29/20°C) until the appearance of floral buds (pinhead square stage), after which in one of the two growth chambers the maximum temperature was increased from 29°C to 44°C in 3°C increments during a 20-day period. The humidity, photoperiod, night temperature and PAR were the same in both growth chambers. The plants were allowed to acclimate at each new temperature for 4 days before measurements were taken. Each time measurements were taken from 60 (5 genotypes × 6 replications × 2 treatments) different plants. To maintain adequate soil moisture and fertility at the higher temperature treatments the plants were watered once daily with half-strength Hoagland's nutrient solution while additional water was applied as needed.

Measurement of quantum yield of PSII of light-adapted leaves

Quantum yield of the PSII was measured using the light-adapted test of the modulated chlorophyll fluorometer OS1-FL (Opti-Sciences, Tyngsboro, MA). With this system, chlorophyll fluorescence is excited by a 660 nm solid-state light source with filters blocking radiation longer than 690 nm. The average intensity of this modulated light was adjusted from 0 to 1 μE. Detection was in the 700 to 750 nm range using a PIN silicon photodiode with appropriate filtering to remove extraneous light. Saturation of the photosystem was accomplished with a filtered 35W halogen lamp (350~690 nm). For the light-adapted test (yield-test) an open-body clip held the fiber optic light guide steady at an angle of 50° to the leaf surface while allowing the ambient light to fall onto the leaf surface. Measurements were taken of plants in both growth chambers between 11:00 a.m. and 1:00 p.m. upon the fourth main-stem leaf from the terminal with one reading per leaf from a plant at each temperature. The percentage change in quantum yield of PSII due to the temperature treatment was compared with that of the control plant at 29°C.

Measuring the activity of catalase, glutathione reductase, and peroxidase

At the end of the experiment leaf tissue was collected from plants in both growth chambers and was immediately stored in an ultra low temperature (-80°C) freezer for subsequent extraction of catalase, glutathione reductase, and peroxidase. Antioxidant enzyme activities were measured on extracts obtained by the procedure of Anderson *et al.* (1992) with a BioSpec-1601 enzyme analyzer (Shimadzu Inc., Columbia, MD). Initially, the frozen tissue was ground in liquid nitrogen in a mortar with a pestle. The pulverized sample was placed in a 35-mL centrifuge tube containing 0.5 g insoluble polyvinylpyrrolidone, one drop of antifoam A (Sigma-Aldrich, Milwaukee, WI) and 4 mL of ice-cold extraction buffer, and homogenized with a Polytron homogenizer. The tubes were then centrifuged at 15.000 × *g* and 4°C for 20 min, and the supernatant fraction was passed through a PD-10 column (Amersham Biosciences, Uppsala, Sweden) for purification before measurement in a spectrophotometer at the wavelengths described below.

The protocol of Beers and Sizer (1952) was followed to measure catalase activity. The disappearance of H_2O_2 was measured as the decrease in absorbance at 240 nm for 1 min at 25°C. The assay described by Schaedle and Bassham (1977) was to measure glutathione reductase activity. The glutathione dependent oxidation of NADPH+H at 340 nm for 1 min at 25°C was recorded. Finally, peroxidase activity was measured by monitoring the hydrogen peroxide-dependent oxidation of 2,3,6 trichloroindophenol at 675 nm for 1 min at 25°C, as described by Nickel and Cunningham (1969). For each antioxidant enzyme assay three measurements per sample were recorded.

Measurement of leaf extension

Uniform, fully unfurled young main-stem leaves with a length of about 3.5 mm from each cultivar were tagged on the first day of each temperature increment using Dennisson marking tags (Dennisson Manufacturing Co, Framingham, MA) carefully placed around the base of the petiole in order to measure leaf length. The length of the tagged leaves, from the leaf tip to the point of attachment of the petiole, was measured with a ruler the first day that the leaves were tagged and the fourth day that the plants were exposed to each temperature regime. The initial measurement of leaf length was subtracted from that at the fourth day to calculate the increase in leaf length of both treated and control plants. Finally, the percentage change of leaf length (hereafter, leaf extension) relative to control plants maintained at 29°C was calculated.

Statistical analysis

The experimental design was completely randomized with six replications and a two-factor factorial arrangement of the treatments. The main factor was temperature and the sub-factor was "cultivars". The significance of treatment differences were detected by analysis of variance (ANOVA) and were considered significant at probability values of $\alpha \leq 0.05$. The statistical analyses were performed with the JMP 6 software (SAS Institute Inc., Cary, NC).

RESULTS

Effect of high temperature on quantum yield of PSII

The data for the percentage change in quantum yield of PSII of the temperature treated plants compared to the control plants at 29°C showed that there was no significant "cultivar x temperature" interaction (P=0.4775). However, the "cultivar" effect on the percentage change of the quantum yield of PSII was highly significant (P<0.0001) and showed that with increasing temperature the ruderal genotype [*G. hirsutum* L. (Palmeri)] had a significantly smaller decrease in the quantum yield of PSII at 44ºC compared to the other genotypes (**Fig. 1A**). Additionally, DP444 experienced the largest decrease in quantum yield of PSII compared to the other genotypes, while Sphinx, FM960, and ST474 showed a decrease in quantum yield of PSII greater than the ruderal genotype but less than DP 444. The "temperature" effect was also highly significant (P<0.0001) indicating that quantum yield of PSII significantly decreases in the temperature treated plants compared to the control at

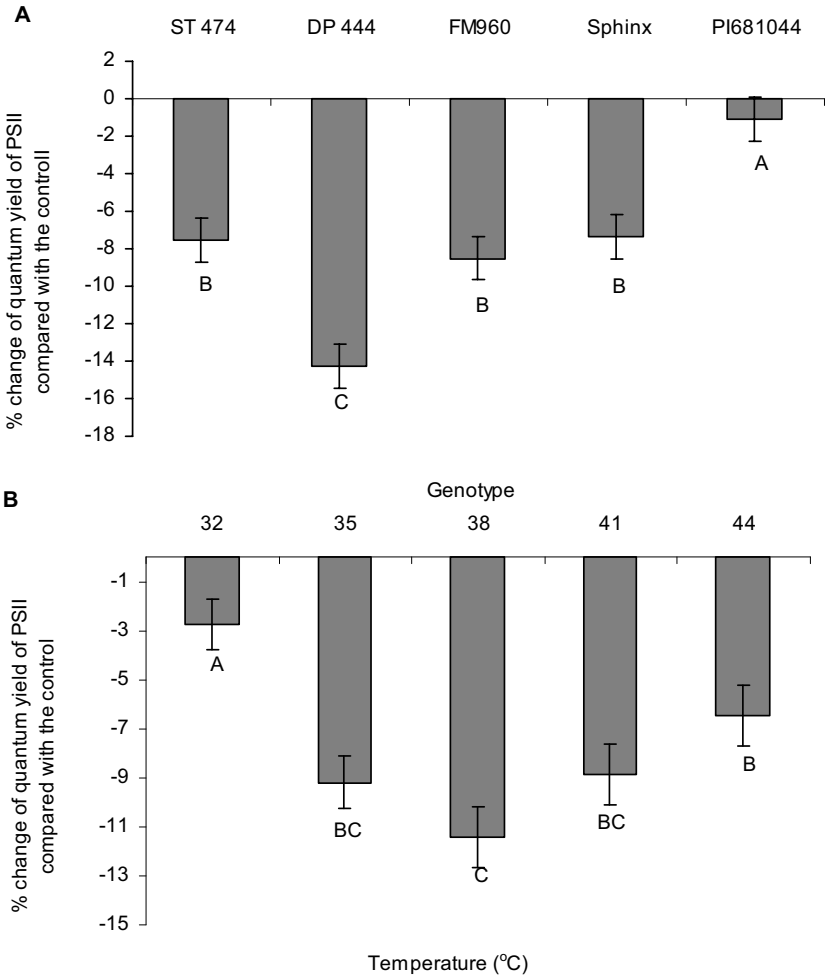

Fig. 1 Effect of genotype (**A**) and increasing air temperature (**B**) on the percentage change of quantum yield of PSII compared to the control at 29ºC. Columns with the same letter are not significantly different (P≤0.05).

29°C (**Fig. 1B**). At the higher temperatures quantum yield of PSII was more variable, and the apparent increase in the change in quantum yield of PSII above 38°C is puzzling, possibly due to protein (i.e. Rubisco) deactivation. However, it was still significantly lower than the decrease observed at 32°C.

Effect of high temperature on antioxidant enzymes

The "cultivar x temperature" interaction was significant (P=0.0414) for catalase activity, therefore we compared the effect of temperature on each genotype separately (**Fig. 2**). In general, catalase activity increased with increasing temperature, but this effect was statistically significant only for the ruderal genotype and two of the commercial genotypes, Sphinx and FM960.

On the other hand, analysis of the glutathione reductase activity showed that the interaction of "cultivar x temperature" was not significant (P=0.4206), so the "cultivar" and the "temperature" effects were analyzed separately (**Fig. 3A, 3B**). The cultivar effect was highly significant (P<0.0001) and showed that the ruderal genotype and the commercial genotype Sphinx had significantly higher glutathione reductase activity compared to ST474, DP444 and FM960 (**Fig. 3A**). The effect of temperature was also significant (P=0.0076). The plants at 44°C had significantly increased

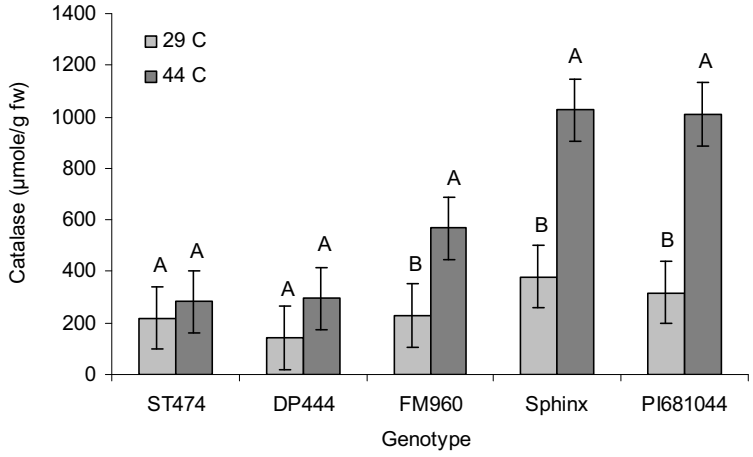

Fig. 2 Comparison of the catalase activity of the five genotypes at two temperatures, 29 and 44°C. Pairs of columns with the same letter are not significantly different (P≤0.05).

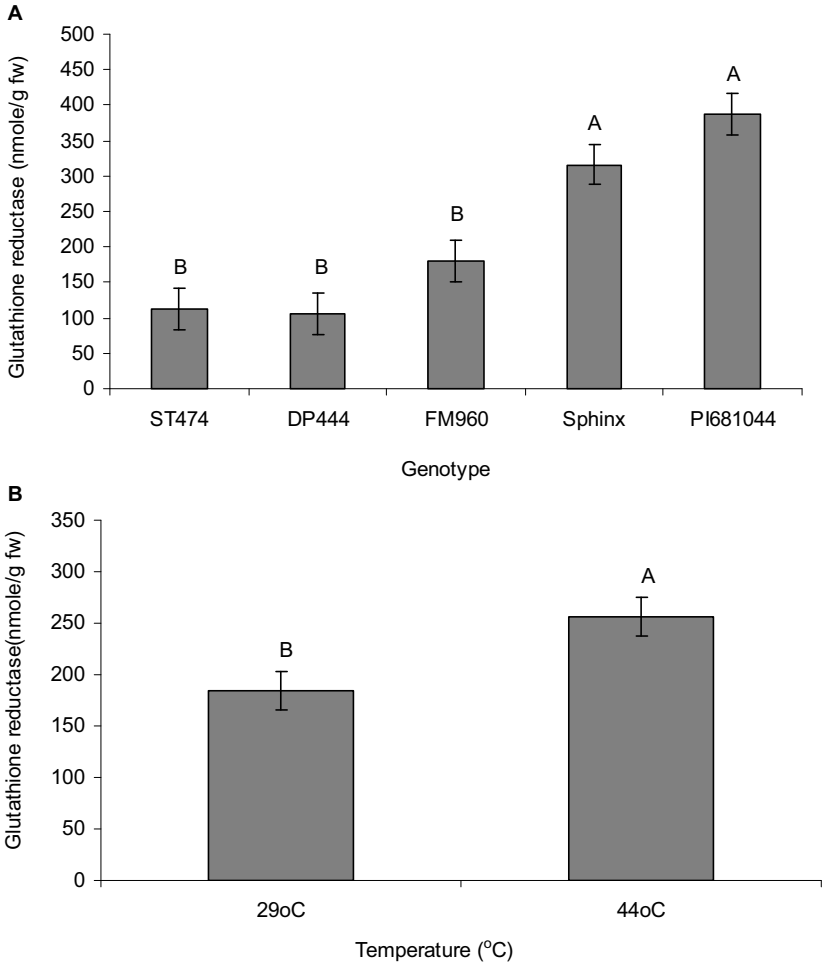

Fig. 3 Effect of genotype (**A**) and increasing air temperature (**B**) on glutathione reductase activity. Least square means with the same letter are not significantly different (P≤0.05).

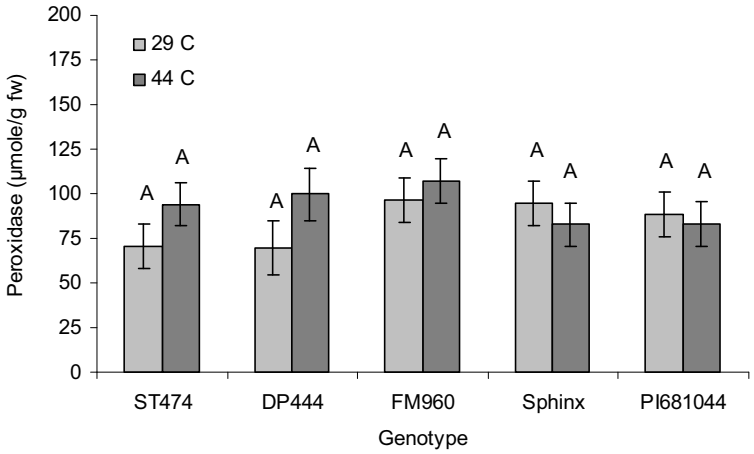

Fig. 4 Comparison of the peroxidase activity of the five genotypes at two temperatures 29°C and 44°C. Pairs of columns with the same letter are not significantly different (P≤0.05).

glutathione reductase activity compared to the control plants at 29°C (**Fig. 3B**).

The "cultivar x temperature" interaction for peroxidase activity was not significant (P=0.542). Also, main effects of "cultivar" and "temperature" had no significant effect on peroxidase activity (P=0.396 and P=0.231, respectively) (**Fig. 4**).

Effect of high temperature on leaf extension

Leaf extension, as an indicator of cotton response to elevated temperature, had no significant "cultivar x temperature" interaction (P=0.394). However, the "cultivar" effect on leaf extension was significant (P<0.0394) and showed that the ruderal genotype had a significantly smaller decrease in leaf extension compared to ST474, DP444, and FM960 with increasing temperature (**Fig. 5A**). The decrease in leaf extension of Sphinx was numerically higher than the ruderal genoype but numerically lower than the rest of the commercial genotypes. In addition, increasing temperature had a highly significant adverse effect on leaf extension (P<0.0001) (**Fig. 5B**). Leaf extension was increased at 32 and 35°C compared to the control at 29°C. However, significant decreases in leaf extension were observed at temperatures above 38°C, with the effect being more detrimental at the higher temperatures (41 and 44°C).

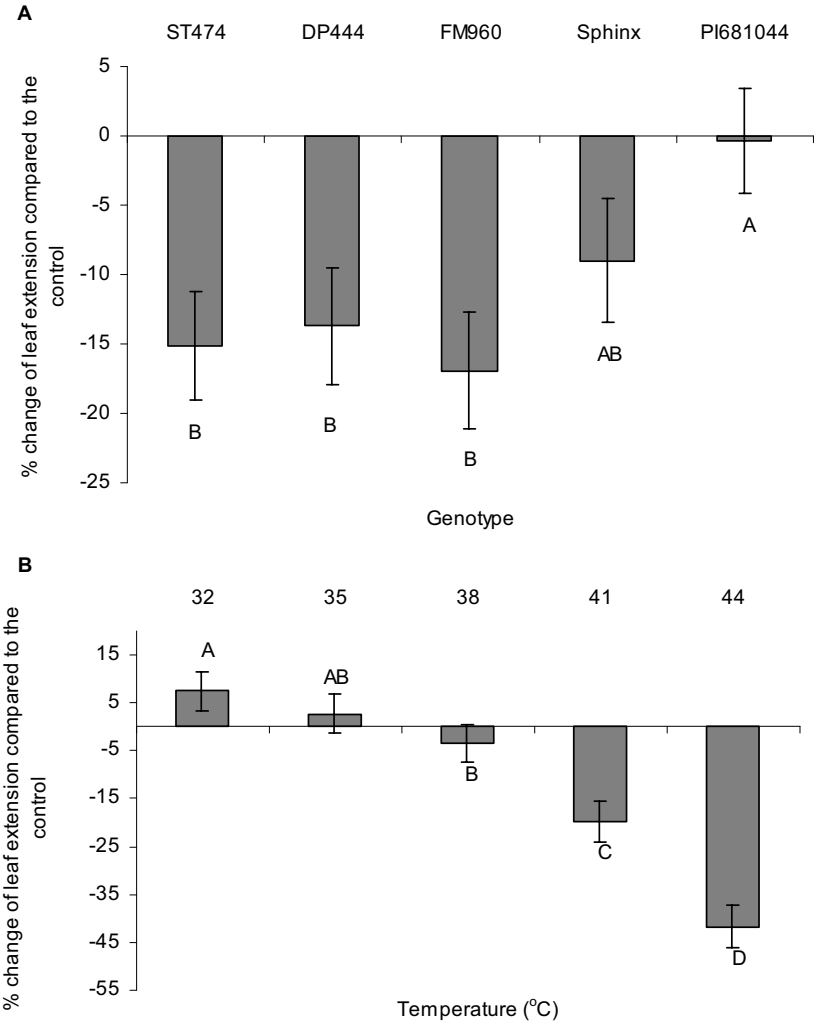

Fig. 5 Effect of genotype (**A**) and increasing air temperature (**B**) on leaf extension compared to the control at 29ºC. Least square means with the same letter are not significantly different (P≤0.05).

DISCUSSION

This study showed that high temperature (≥32°C) caused a significant decrease in quantum yield of PSII for all genotypes. Particularly important was that the ruderal *G. hirsutum* race Palmeri experienced a significantly smaller decrease in quantum yield of PSII compared to the commercial cultivars suggesting that PI631044 is more tolerant of high temperature. The quantum efficiency of PSII measured with the dark-adapted test (Fv/Fm) has been used widely as an indicator of environmental stress and as a screening method for heat resistance in barley mutants (Georgieva *et al.* 2003). In addition, commercial cotton cultivars have been screened for tolerance to high temperature using the quantum yield of PSII measured with the light-adapted test (Bibi *et al.* 2008). However, this study is the first evidence for screening commercial cotton cultivars with a ruderal genotype of *G. hirsutum* for high temperature tolerance using measurements of the quantum yield of PSII.

In addition to quantum yield of PSII, increased temperature caused significant increases in the antioxidant activity of both glutathione reductase and catalase. The ruderal genotype and the commercial genotype, Tamcot Sphinx, had significantly higher activity of glutathione reductase compared to the other genotypes. Similarly, catalase activity increased significantly at the high temperature treated plants of *G.hirsutum* race Palmeri, Tamcot Sphinx, and FM960 compared to the genotypes at 29°C. Increased activities of antioxidant enzymes confirms stress tolerance (Upadhyaya *et al.* 1991); therefore mainly PI 631044 and Tamcot Sphinx showed tolerance to high temperature stress. This is the first report of a ruderal *G. hirsutum* showing significantly higher activity of glutathione reductase and catalase indicating tolerance to high temperature. Similarly, there are no previous reports of increased levels of antioxidant enzymes in the commercial cultivar, Tamcot Sphinx, to confirm its high temperature tolerance. However, it should be noted that according to its registration (El-Zik and Thaxton 1996) this genotype was selected for its improved tolerance to abiotic stresses. FM960 showed increased levels of catalase activity with higher temperatures but not glutathione reductase activity. Increased activities of glutathione reductase and catalase with high temperatures were also reported in wheat (*Triticum aestivum* L.) genotypes (Sairam *et al.* 1997) and in commercial cotton cultivars (Bibi *et al.* 2004). However, this report is the first to present data for high temperature tolerance in a ruderal genotype of *G. hirsutum* also well as the response of commercial cotton genotypes to high temperature using antioxidant enzymes activity.

Finally, the effect of increased high temperature was detrimental to leaf extension growth. Leaf extension of all the genotypes was decreased significantly with increased temperature. However, PI631044 was significantly less affected by high temperature stress compared to ST474, DP444, and FM960. This further supports the tolerance of this genotype to high temperature that was previously shown with quantum yield of PSII and the antioxidant enzymes catalase and glutathione reductase. In addition, the effect of high temperature on Tamcot Sphinx was intermediate, i.e, more from the ruderal genotype but numerically less than the other commercial genotypes. The increased levels of glutathione reductase and catalase observed in Tamcot Sphinx compared to the other commercial lines indicates that Sphinx has some tolerance to high temperature stress.

Previous work with cotton has focused on the effects of water-deficit stress on leaf extension for irrigation scheduling (Oosterhuis *et al.* 1990), while cotton leaf area expansion has been used for modeling purposes by Reddy *et al.* (1992). Our study is the first report of the use of leaf extension as a screen for differences in tolerance to high temperature between commercial genotypes and a ruderal genotype of *G. hirsutum*.

The superior high temperature tolerance of the ruderal genotype compared to the commercial cultivars was evident in this study. Among the commercial cultivars, Tamcot Sphinx showed some tolerance to high temperature however this was not supported by measurements of quantum yield of PSII. The results of all three measurements in this study supported our hypothesis that the ruderal genotype of *G. hirsutum* would be more tolerant to high temperature stress compared to the commercial cultivars of this species. The fact that the PI631044 exhibited greater tolerance to high temperature than commercial cultivars is of particular importance in germplasm improvement in Upland cotton.

CONCLUSIONS

The concern about global warming and the narrow genetic focus of the commercial genotypes has drawn attention to the search for temperature tolerance. Our results clearly supported the stated hypothesis that the ruderal cotton was more tolerant to high temperature stress than the commercial cotton genotypes. The superiority of the ruderal genotype to high temperature conditions was reflected by the maintenance of both physiological functions and growth during high temperature. Knowledge that tolerance to high temperature exists in native cottons indicates that ruderal genotypes of *G. hirsutum* L. need to be further examined as a promising source for high temperature tolerance in cotton.

REFERENCES

Anderson JA, Chevone BI, Hess JL (1992) Seasonal variation in the antioxidant system of Eastern white pine needless: Evidence for thermal dependence. *Plant Physiology* **98**, 501-508

Anonymous (2007) New York Times. Available online: http://topics.nytimes.com/top/news/science/topics/globalwarming/index.html#

Baker DN (1965) Effects of certain environmental factors on net assimilation in cotton. *Crop Science* **5**, 53-55

Beers R, Sizer I (1952) A spectrophotometric method for measuring the breakdown of hydrogen peroxide by catalase. *Journal of Biological Chemistry* **195**, 130-140

Bibi AC, Oosterhuis DM, Gonias EG (2008) Photosynthesis, quantum yield of photosystem II, and membrane leakage as affected by high temperatures in cotton genotypes. *Journal of Cotton Science* **12**, 150-159

Brubaker CL, Bourland FM, Wendel JE (1999) The origin and domestication of cotton. In: Smith CW, Cothren JT (Eds.) *Cotton: Origin, History, Technology, and Production*, Technology Production. John Wiley & Sons, Inc. Danvers, MA, pp 3-31

El-Zik KM, Thaxton PM (1996) Registration for "Tamcot Sphinx" cotton. *Crop Science* **36**, 1074

Genty B, Briantais J, Baker NR (1989) The relationship between the quantum yield of photosynthetic electron transport and quenching of chlorophyll fluorescence. *Biochimica et Biophysica Acta* **990**, 87-89

Georgieva K, Fedina IS, Maslenkova L, Peeva V (2003) Response of chlorina barley mutants to heat stress under low and high light. *Functional Plant Biology* **30**, 515-524

Gould KS (2003) Free radicals, oxidative stress, and antioxidants. In: Thomas B, Murphy D, Murray B (Eds) *Encyclopedia of Applied Plant Science*, Academic Press, London, pp 9-16

Hoagland DR, Arnon DI (1950) The water-culture for growing plants without soil. *California Agricultural Experimental Station Circulation* **347**, 32

Kittock DL, Turcotte EL, Hofmann WC (1988) Estimation of heat tolerance improvement in recent American pima cotton cultivars. *Journal of Agronomy and Crop Science* **161**, 305-309

Nickel RS, Cunningham BA (1969) Improved peroxidase assay method using leuco 2,3,6-trichlcroindophenol and application to comparative measurements of peroxidase catalysis. *Analytical Biochemistry* **27**, 292-299

Oosterhuis DM (2002) Day or night high temperature: A major cause of yield variability. *Cotton Grower* **46 (9)**, 8-9

Oosterhuis DM, Ball RA, Hampton RE (1990) Plant indicators of water stress for improved irrigation scheduling. *1990 Proceedings of the Cotton Research Meeting*, University of Arkansas, Special Report **144**, 33-44

Papageorgiou G (1975) Chlorophyll fluorescence: An intrinsic probe of photosynthesis. In: Govundjee X (Ed) *Bioenergetics of Photosynthesis*, Academic Press, New York, pp 319-371

Reddy VR, Baker DN, Hodges HF (1991) Temperature effect on cotton canopy growth, photosynthesis and respiration. *Agronomy Journal* **83**, 699-704

Reddy KR, Reddy VR, Hodges HF (1992) Temperature effects on early season cotton growth and development. *Agronomy Journal* **84**, 229-237

Sairam RK, Deshmukh PS, Saxena DC (1997) Tolerance to drought and temperature stress in relation to increased antioxidant enzyme activity in wheat. *Journal of Agronomy and Crop Science* **178**, 171-177

Schaedle M, Bassham JA (1977) Chloroplast glutathione reductase. *Plant Physiology* **59**, 1011-1012

Stoleman GL, Turner NC, Dell B (1994) Leaf growth, photosynthesis and tissue water relations of greenhouse-grown *Eucalyptus marginata* seedlings in response to water deficits. *Tree Physiology* **14**, 633-646

Ulloa M, Stewart JMcD, Garcia-C EA, Godoy-AS, Gaytan-M A, Acosta-N S (2006) Cotton genetic resources in the western states of Mexico: *In situ* conservation status and germplasm collection for *ex situ* preservation. *Genetic Research and Crop Evolution* **53**, 653-668

Upadhyaya A, Davis TD, Sankhla N (1991) Heat shock tolerance and antioxidant activity in moth bean seedlings treated with tetcyclacis. *Journal of Plant Growth Regulation* **10**, 215-222

Walter A, Schurr U (2005) Dynamics of leaf and root growth: Endogenous control versus environmental impact. *Annals of Botany* **95**, 891-900