The Effects of Genotype and Irrigation Regime on PSII Heat Tolerance in Cotton

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Authors' contributions

This work was carried out in collaboration between all authors. Authors CP, DL and DMO designed the study. Author CP wrote the protocol. Authors CP and DL took all the measurements on the plants. Authors CP and JLS analyzed the data statistically and author CP wrote the first draft of the manuscript. Authors JLS, DL and DMO revised the manuscript. All authors read and approved the final manuscript.

Original Research Article

ABSTRACT

Aims: Differences in tolerance to drought and heat stresses exist among cotton species and between modern and obsolete genotypes. However, it is not clear if increases in thermostability under water deficit are associated with genotypic differences in drought tolerance. Therefore, the objective was to identify differences in heat tolerance and physiological acclimation of contrasting cotton genotypes under water-deficit stress and recovery conditions.
Study Design: The experimental design was a randomized complete block design with five replications. Treatments consisted of three cotton genotypes, DeltaPine (DP) 0912 B2RF, Pima 32, and Siokra L23 and two water regimes, a well-watered control and a water-stressed treatment.
Place and Duration of Study: The experiment was conducted in 2012, and repeated in 2013, at the Altheimer Laboratory, University of Arkansas in Fayetteville, Arkansas.
Methodology: Heat tolerance was assessed through maximum quantum yield of Photosystem II at

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temperatures 25°C to 45°C and measurements of stomatal conductance were also performed.

Results: Differences (P = .05) in heat tolerance and physiological acclimation exist among the genotypes under water-deficit stress and recovery conditions. Pima 32 showed higher heat tolerance and improved stomatal conductance at recovery. Siokra L23 was relatively heat sensitive and showed moderate recover in stomatal conductance after plants were re-watered. DP 0912 was the least heat tolerant.

Conclusion: Thermostablity under water-deficit stress was associated with drought tolerance of genotypes, with Pima 32 having the highest heat tolerance acclimation in response to water-deficit stress, followed by Siokra L23 and DP 0912.

1. INTRODUCTION

Water-deficit stress is the most restricting factor to plant development and yield on a global scale due to limiting water supplies and climate variability. The severity and duration of drought as well as plant growth stage and genotype determine the effects of water stress in plants [1]. Plants are usually exposed to drought-recovery cycles throughout the season and the effects of these episodic droughts on crop productivity depend on the plant's ability to prevent cellular damage during the stress and to recover after relief of the stress [2,3].

Drought and heat stresses commonly co-occur in the field, which makes control of plant water use more difficult [4]. Stomatal closure is one of the primary plant response to water limiting conditions. It limits evaporative cooling of leaves, increases leaf temperature and reduces heat avoidance [5]. Cotton plants have developed mechanisms to ensure their survival under water limiting conditions, such as stomatal closure and osmotic adjustment [6]. Although differences in drought tolerance have been shown to exist between modern and obsolete genotypes [7,8], the heat tolerance acclimation of PSII as a characteristic of drought tolerant cotton genotypes has not been explored.

Photosynthesis in leaves is reduced under drought stress due to stomatal closure, which results in lower CO_2 internal concentrations, and decreased CO_2 fixation [9]. Photosystem II (PSII) is the initial complex in the photosynthetic electron transport chain, responsible for oxidation of water and generation of molecular oxygen [10]. Its efficiency and stability can be measured by means of maximum quantum yield of PSII (F_v/F_m) through chlorophyll fluorescence. Chlorophyll fluorescence assesses many different parameters, which give information on changes in efficiency of photochemistry and increases in heat dissipation. Actual quantum yield of PSII (ϕ_{PSII}) provides information on the quantum efficiency of electron transport through PSII under actinic light and can be used to calculate electron transport rates *in vivo* [11]. Photochemical quenching (qP) is the proportion of PSII reaction centres that are open under a given set of conditions. Maximum quantum yield of PSII is measured in leaves that are in the dark-adapted state (F_v/F_m), to ensure that all reaction centres are open and any change in F_v/F_m can be attributed to non-photochemical quenching (NPQ) processes such as those resulting from damage to PSII [11]. Thus, F_v/F_m provides a very accurate measure of intrinsic quantum efficiency of PSII, and has been widely used to quantify PSII-specific impacts of high temperature [11-13]. Increasing temperature blocks PSII reaction centers and dissociates antennae pigment-protein complexes from the central core of PSII light harvesting apparatus, therefore, impairing the photosynthesis process [14]. Previous research has established that F_v/F_m presents tolerance to drought and high temperature conditions [13,15,16]. Snider et al. [13] recently reported that PSII heat tolerance was greatest for rain fed *G. hirsutum* plants at times during the growing season coinciding with maximal drought stress exposure. This suggests that increased PSII heat tolerance may be part of the acclimation response to water deficit, although controlled environment studies demonstrating improved PSII heat tolerance in drought stressed, relative to control plants, are limited in *G. hirsutum*. *Gossypium hirsutum* cv. Siokra L23 is regarded as one of the most drought tolerant Upland cotton cultivars available, as evidenced by gas exchange and yield responses to drought [17]. However, the possibility that heat tolerance acclimation could partially account for improved performance in Siokra L23 under drought is unexplored. Finally, *G. barbadense* (Pima cotton) is grown under extremely high temperature conditions in the

southeastern United States, and when compared side-by-side in the field with *G. hirsutum*, *G. barbadense* leaves have much lower stomatal conductance and higher foliage temperatures [18]. However, to our knowledge, studies evaluating differences in PSII heat tolerance between Pima and Upland cotton genotypes in response to water deficit are also non-existent.

Considerable variation in tolerance to drought and heat stresses exists between cotton species, *Gossypium hirsutum* and *G. barbadense*, and between modern and obsolete genotypes [19-21]. The development of new drought-tolerant cultivars has been hampered by lack of knowledge on physiological mechanisms utilized by plants to cope under water limiting conditions [4]. Progress has been made in developing high-yielding cotton cultivars; however, this selection has narrowed the genetic variability for drought tolerance [22]. Some obsolete genotypes have traits for drought tolerance and they could be used in cotton biotechnology programs to assist in development of modern cultivars widely adapted. We hypothesize that different physiological responses will exist among contrasting cotton genotypes exposed to water-deficit stress and that more drought tolerant genotypes will exhibit the greatest PSII thermostability. Therefore, the objective of this study was to identify differences in heat tolerance and physiological acclimation of contrasting cotton genotypes under water-deficit stress and recovery conditions.

2. MATERIALS AND METHODS

2.1 Location, Plant Materials and Sampling Protocol

An experiment was conducted in 2012, and repeated in 2013, at the Altheimer Laboratory, University of Arkansas in Fayetteville, Arkansas. The experimental design was a randomized complete block design with five replications. Treatments consisted of three cotton genotypes, DeltaPine (DP) 0912 B2RF, Pima 32, and Siokra L23 (Table 1) and two water regimes, a well-watered control and a water-stressed treatment. Each pot with a single plant represented one experimental unit. Pima 32 is a *Gossypium barbadense* that has no introgressed genes from Upland cotton [23].

Cotton genotypes were planted in 2-L pots filled with Sunshine potting media (Sun Gro Horticulture Distribution Inc., Bellevue, WA) in a large growth chamber (Model PW36, Conviron, Winnipeg, Canada). Temperatures of 32/24°C (day/night), 14 h photoperiod, 60% relative humidity, and a typical diurnal pattern of the photosynthetically active radiation (PAR) with the highest PAR (850 µmol $m^{-2}s^{-1}$) between 10:00 am and 2:00 pm were maintained in the growth chamber. The plants were watered once daily with half-strength Hoagland's nutrient solution [24] to maintain adequate moisture until the appearance of floral buds (pinhead square stage) approximately 35 days after planting. At this stage, water-deficit stress was imposed by withholding water from the water-stressed plants until stomatal conductance (g_s) reached approximately 20 mmol $m^{-2}s^{-1}$ indicating that the stomata were virtually closed. Well-watered control plants received an optimum quantity of water throughout the duration of the experiment and a figure is presented to illustrate the typical pattern of g_s in well-watered and drought stressed plants (Fig. 1). Once the water-stressed plants reached the required stress, samples for maximum quantum yield of photosystem II (F_v/F_m) measurements were collected using the fourth uppermost fully expanded main-stem leaf of all 30 plants. After the measurements and samples were taken, the water-stressed plants were re-watered. Twenty-four hours after re-watering, stomatal conductance and maximum quantum yield of photosystem II (F_v/F_m) were measured on the third uppermost fully expanded main-stem leaf for the recovery evaluation.

Table 1. Background information for the obsolete and modern cotton genotypes used in the experiment

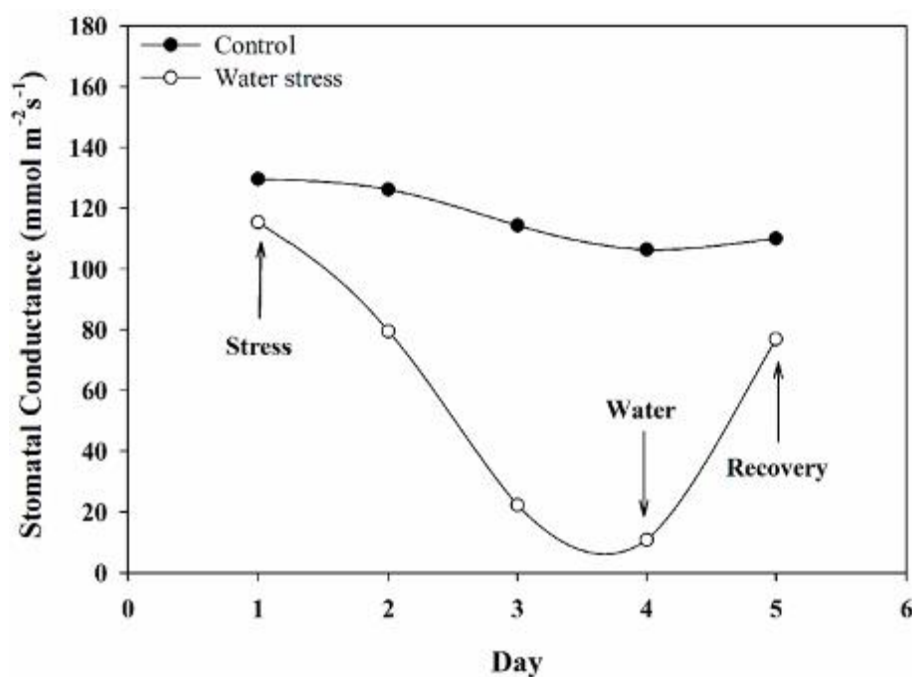| Genotype	Species	Origin	Leaf type	Characteristic	Year of development
DP 0912 B2RF	*G. hirsutum*	Mid-South United States	Normal	Relative drought sensitive	2009
Pima 32	*G. barbadense*	West United States	Normal	Drought tolerant	1949
Siokra L23	*G. hirsutum*	South-East Australia	Okra	Drought tolerant	1991

Fig. 1. Stomatal conductance from the onset of the stress period until the first day of recovery of an individual leaf of *Gossypium barbadense* cv. Pima 32. As indicated with arrows, water-deficit stress started when plants reached the pinhead square stage. Water was withheld from the water-deficit stress treatment until stomatal conductance reached 20 mmol $m^{-2}s^{-1}$. Then the stressed plants were re-watered and recovery measurements were taken one day after the water-stressed plants received the same amount of water as the control plants

2.2 Measurements

2.2.1 Stomatal conductance

Stomatal conductance was measured daily starting at the first day of the stress until one day of recovery using a steady-state leaf porometer (SC-1 Leaf Porometer, Decagon, Pullman, Washington, USA) on the abaxial surface of fourth uppermost fully expanded main-stem leaves until the last day of the stress and third uppermost fully expanded main-stem leaves one day of recovery. Measurements were taken between 12:00 pm to 2:00 pm, during plateau time of stomatal conductance for these genotypes, with two readings per leaf.

2.2.2 Maximum quantum yield of photosystem II (F_v/F_m)

Leaves were collected from the fourth main-stem node below the plant terminal, wrapped in a moist paper towel and stored in ziploc plastic bags in the dark for 24 h in order to dark adapt. Then, 5 cm^2 leaf segments were cut from each

leaf sample and placed on a moist filter paper in contact with a thermoelectric heating/cooling block [25]. The temperature of the thermal block was adjusted to 25°C and leaf segments were allowed to incubate for five min. After the incubation period, maximum quantum yield of photosystem II (F_v/F_m) was measured using the OS5p Chlorophyll Fluorometer (Opti-Sciences, Hudson, New Hampshire, USA). Briefly, F_0 was measured under a low-intensity modulation light source immediately prior to exposure of the leaf sample to a saturating light pulse for 0.8 s to determine F_m. F_v/F_m was calculated as follows: $F_v/F_m=[(F_m-F_0)/F_m]$. This procedure was repeated for the temperatures 30°C, 35°C, and 40°C in 2012 and 30°C, 35°C, 40°C and 45°C in 2013. Thermotolerance was assessed by quantifying percent decline in F_v/F_m for individual leaves at 40°C (2012 and 2013) and 45°C (2013) in relation to 25°C (Fig. 2). Higher % decline is indicative of greater sensitivity to high temperature. To assess the impact of drought stress alone on PSII function in each cotton genotype, the F_v/F_m value obtained at 25°C (prior to increasing leaf temperature) was recorded for each leaf.
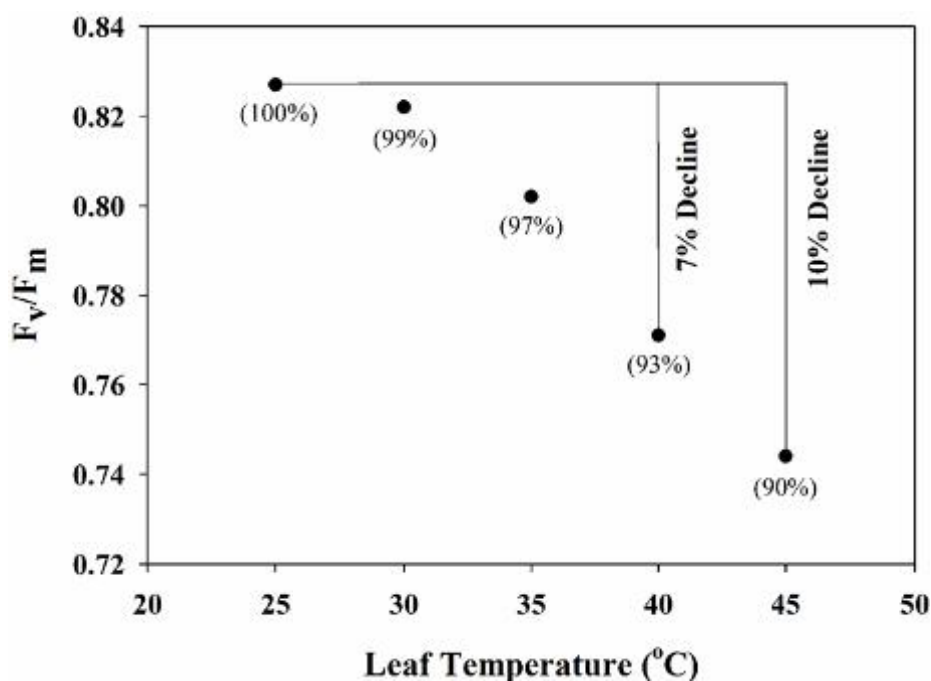
Fig. 2. The response of F_v/F_m to leaf temperature for an individual leaf of growth chamber-grown *Gossypium hirsutum* cv. DP 0912 B2RF. The figure illustrates how % decline was calculated at 40°C and 45°C in relation to 25°C

2.3 Statistical Analysis

As similar trends and no significant differences were observed for stomatal conductance and F_v/F_m at 25°C in the two years of experiment, the results were pooled and the means were taken. A two-way analysis of variance (ANOVA) was performed for each sample date using JMP Pro 11 (SAS Institute, Cary, NC). The treatments genotype and water regime were considered as fixed effects. The blocks and the block x treatment interaction were considered as random effects. Treatment means were separated using Tukey's HSD test at the 0.05 probability level. Graphs were plotted using Sigma Plot 12.5 (Systat Software Inc., San Jose, CA).

3. RESULTS AND DISCUSSION

Stomatal function is known to be one of the first mechanisms affected by the onset of water-deficit stress [26]. In our experiment, stomatal conductance was measured from the first day of the stress until one day after the water-stressed plants were re-watered, as shown in Fig. 1. Water was withheld in the water-stressed plants until g_s reached approximately 20 mmol m^{-2}s^{-1}. Then, the plants were re-watered and the recovery was recorded one day after the water-

stressed plants received the same amount of water as the control plants. The stress period of all genotypes was four days in average.

Stomatal conductance was affected by genotype x water regime interaction (Table 2) at the last day of the stress and one day of recovery. Stomatal conductance of all genotypes was significantly decreased by the water-deficit stress (Fig. 3a) when evaluated on the last day of the stress. Several reports on cotton plants cultivated in pots under drought stress demonstrated that stomatal closure increases considerably under moderate or severe water-deficit stress [27-30]. Under well-watered conditions, Siokra L23 showed the highest g_s followed by DP 0912 and Pima 32. However, there was no significant difference in g_s of the water-stressed plants among the genotypes, and the overall average was approximately 17 mmol m^{-2}s^{-1}. Stomatal closure occurs in order for the plants to prevent water losses through stomata. The differences in the stomatal conductance rate among the genotypes under well-watered conditions (control) are due to the different genetic backgrounds contained in the obsolete and modern genotypes. Siokra L23 had the highest stomatal conductance among the genotypes studied under well-watered control conditions.

This response can be related to its Australian origin and leaf morphology (okra leaf), which has smaller boundary layer and higher evaporative cooling capacity [31]. Stressed plants of all genotypes increased stomatal conductance after re-watering compared with the last day of the stress (Fig. 3). However, one day of recovery, the increase in stomatal conductance was not sufficient to reach similar rates of the well-watered plants, except for Pima 32 which had the stomatal conductance of the stressed plants statistically similar to the well-watered plants (Fig. 3b) indicating that this genotype was able to adjust the stomatal mechanism and recover from a short period of water scarcity. Siokra L23 showed the highest g_s compared with the other genotypes under either well-watered or water-stressed conditions one day of recovery.

Maximum quantum yield of photosystem II (F_v/F_m) at 25°C was affected by genotype at the last day of the stress and one day of recovery and by water regime at the last day of the stress (Table 2). DP 0912 showed the highest F_v/F_m under both water regimes compared with the other genotypes at the last day of the stress (Fig. 4a). DP 0912 and Pima 32 had the F_v/F_m significantly decreased by the water-deficit stress, while the F_v/F_m of Siokra L23 was not significantly affected by the water-deficit stress. The reduction in F_v/F_m in stressed plants was likely due to damage to PSII and a resultant increase in NPQ [11]. One day of recovery, DP 0912 was the only genotype that had significantly lower F_v/F_m in the stressed plants compared with the well-watered plants (Fig. 4b).

Table 2. Analysis of variance (ANOVA) of stomatal conductance and F_v/F_m at 25°C at the last day of the stress and one day of recovery

Source of variation	d.f.	*F*-value			
		Stomatal conductance		F_v/F_m at 25°C	
		Last day	Recovery	Last day	Recovery
Genotype (G)	2	8.63**	10.75**	10.75**	19.22**
Water regime (WR)	1	71.27**	6.56*	6.56*	0.73[ns]
Interaction G x WR	2	8.55**	0.19[ns]	0.19[ns]	0.72[ns]

[ns] = nonsignificant; *Significant at $P < 0.05$; **Significant at $P < 0.001$

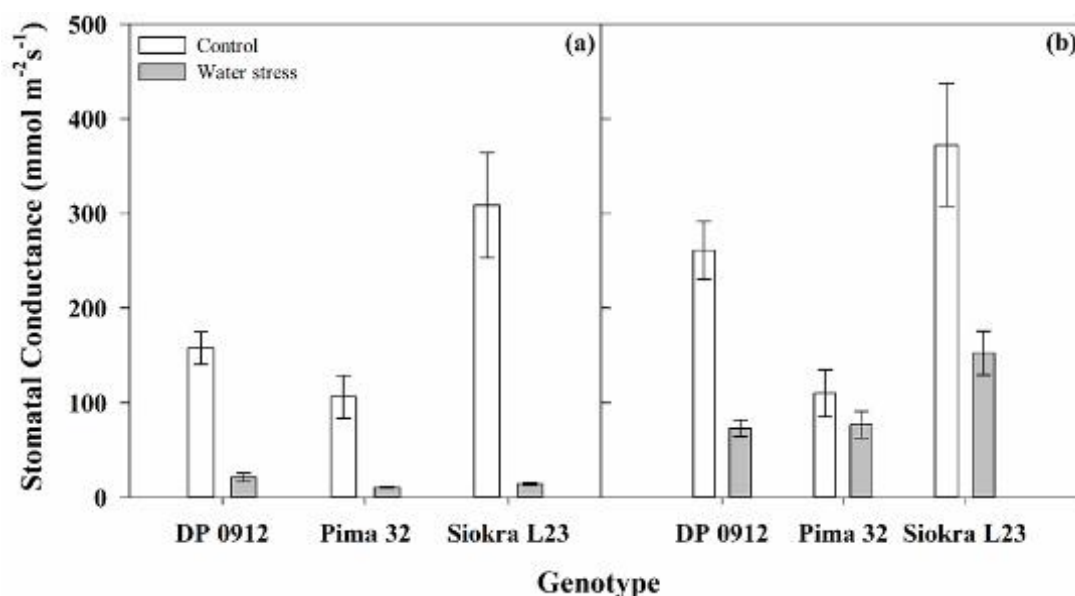**Fig. 3. Stomatal conductance (mmol m^{-2}s^{-1}) on the last day of the stress (a) and first day of recovery (b) of three cotton genotypes (DP 0912 B2RF, Pima 32 and Siokra L23)**
All values are means ± standard error (n=5)

High temperatures cause an increase in the fluidity of the thylakoid membranes in cells, forming non-bilayer lipid structures. The lack of stability of lipid-protein interaction leads to a perturbation of PSII activity. Therefore, heat tolerance can be assessed in cotton genotypes by means of F_v/F_m, which is an indicator of heat sensitivity of PSII complex in vivo in plants [32]. When heat tolerance was assessed at 40°C, genotype and water regime treatments influenced thermotolerance differently depending upon year and sample date (Table 3). Heat tolerance was either unaffected by water-stressed treatment in Siokra L23 (Figs. 5a-c) or

decreased (decline indicates more heat sensitive) following a short recovery period (Fig. 5d) only in 2013. However, there were a few consistent trends across sample dates and years. DP 0912 was always more (numerically or statistically) heat tolerant at 40°C in water-stressed plants than well-watered whether plants were sampled during water-deficit exposure or following a short recovery period. Percent decline in F_v/F_m for Pima 32 was unaffected by water regime treatment, regardless of sample date, and Pima 32 was, in all instances, the most heat tolerant or statistically equivalent to the most heat tolerant cultivar at any sample time (Fig. 5).
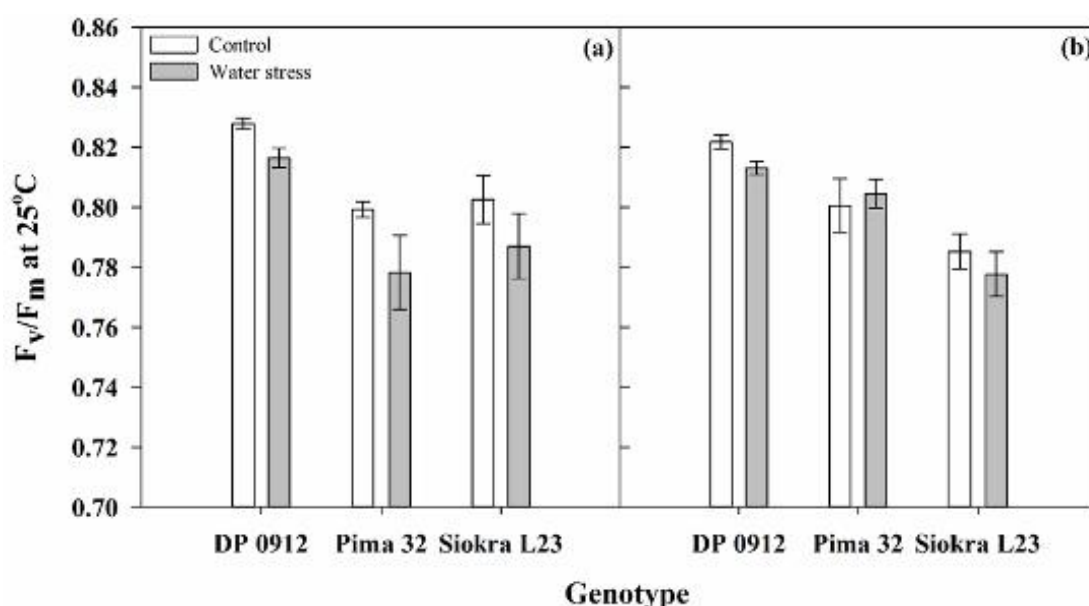

Fig. 4. The response of F_v/F_m at 25°C to water-deficit stress on the last day of the stress (a) and first day of recovery (b) of three cotton genotypes (DP 0912 B2RF, Pima 32 and Siokra L23)
All values are means ± standard error (n=5)

Table 3. Analysis of variance (ANOVA) of percent decline in F_v/F_m at 40°C and 45°C at the last day of the stress and one day of recovery

Source of variation	d.f.	*F*-value					
		[a] % F_v/F_m at 40°C				[b] % F_v/F_m at 45°C	
		Last day	Recovery	Last day	Recovery	Last day	Recovery
		2012				2013	
Genotype (G)	2	12.65**	10.02**	50.06**	49.04**	125.13**	76.41**
Water regime (WR)	1	9.38**	2.42[ns]	3.88[ns]	14.31**	0.10[ns]	9.65**
Interaction G x WR		0.97[ns]	0.60[ns]	1.88[ns]	36.59**	9.69**	25.16**

[a] Percent decline in F_v/F_m at 40°C, [b] Percent decline in F_v/F_m at 45°C, [ns] = nonsignificant; * Significant at P < .05; ** Significant at P < .001
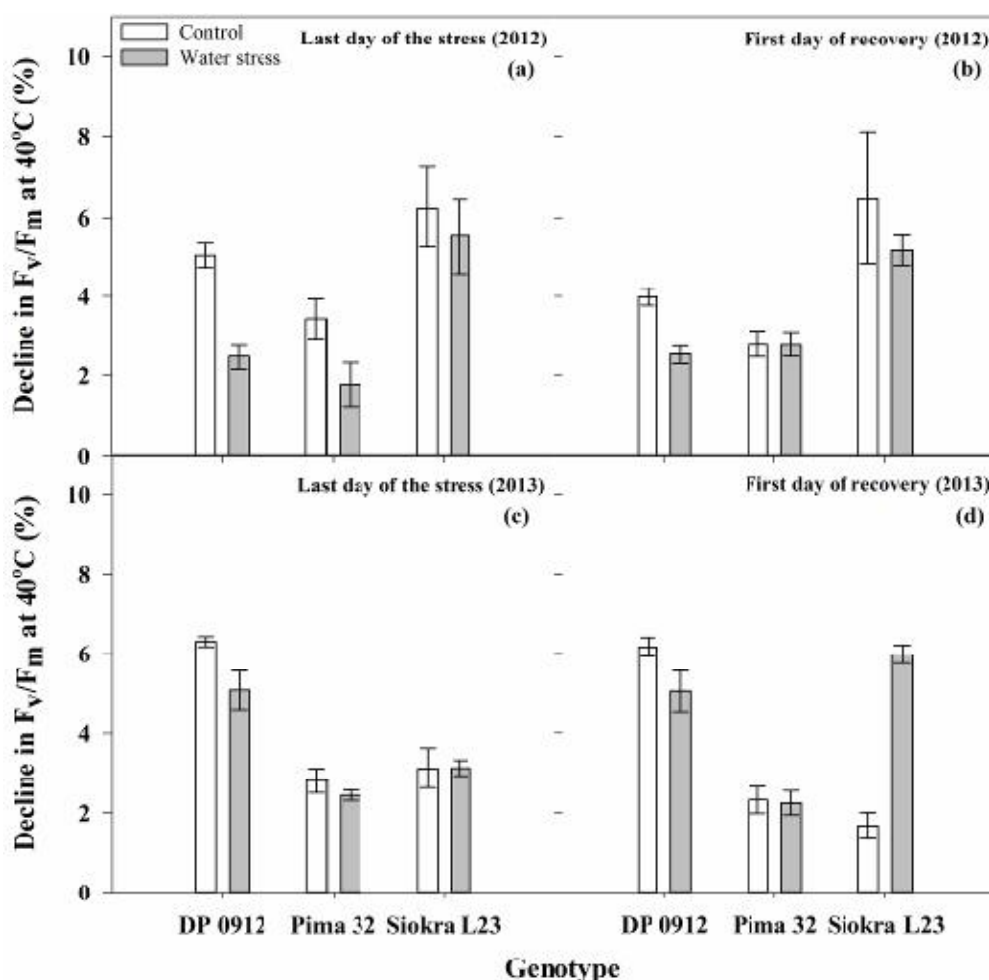
Fig. 5. The effect of genotypes and water regime on the percent decline in maximum quantum yield of photosystem II (F_v/F_m) at 40°C on the last day of the stress and first day of recovery in 2012 and 2013. Decline in F_v/F_m represents the percent decline in F_v/F_m at 40°C in relation 25°C
All values are means ± standard error (n = 5)

When leaf temperatures were increased to 45°C in 2013, a significant interaction was observed between genotype and water regime treatment for % decline in F_v/F_m (Fig. 6). Under well-watered conditions, DP 0912 exhibited the greatest heat sensitivity, as evidenced by having the highest % decline in F_v/F_m at 45°C (Fig. 6a), whereas exposure to water deficit slightly enhanced thermotolerance for DP 0912 in both sample dates. The reverse trend was observed for Siokra L23, where heat tolerance was higher under well-watered conditions than water-stressed conditions. Siokra L23 exposed to either water regime was more heat tolerant than DP 0912. Pima 32 exhibited the greatest heat tolerance of any genotype evaluated (except for Siokra L23 under well-watered conditions which was equally heat tolerant), and thermotolerance

was unaffected by irrigation treatment for this genotype.

Similar trends were observed following recovery (Fig. 6b), where DP 0912 was the least heat tolerant cultivar, and heat tolerance was higher in previously drought-stressed leaves of DP 0912 than those maintained under well-watered conditions. In contrast, heat tolerance was lower in Siokra L23 leaves that had been previously exposed to drought stress before a brief recovery period than Siokra L23 leaves that had been maintained under well-watered conditions throughout the experiment. Siokra L23 under well-watered conditions and Pima 32, under either irrigation treatment, were the most heat tolerant following the recovery period.
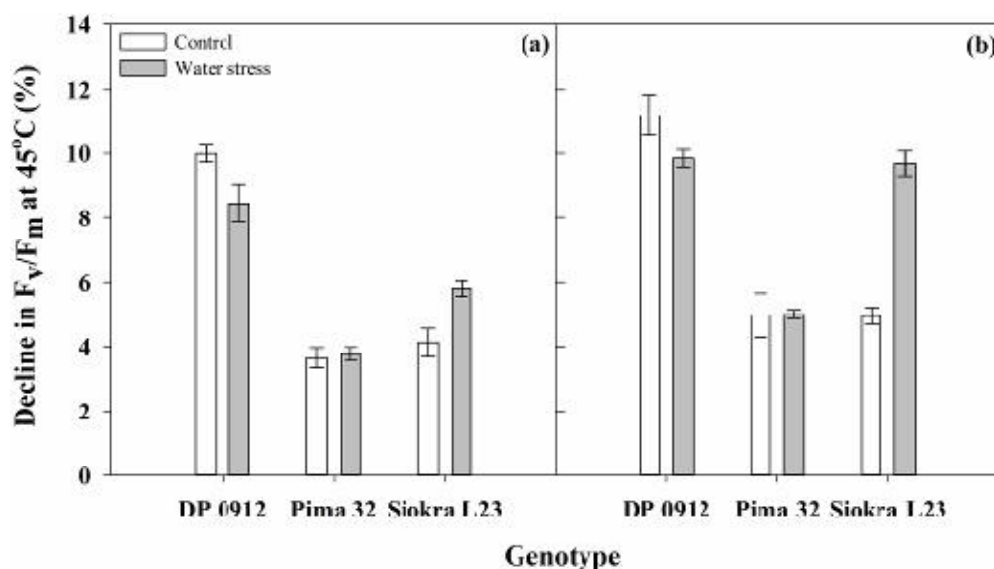
Fig. 6. The effect of genotypes and water regime on the percent decline in maximum quantum yield of photosystem II (F_v/F_m) at 45°C at the last day of the stress (a) and first day of recovery (b) in 2013. Decline in F_v/F_m represents the percent decline in F_v/F_m at 45°C in relation to 25°C
All values are means ± standard error (n = 5)

Data of % decline in F_v/F_m support the hypothesis that obsolete and modern genotypes differ in heat tolerance under water-stress conditions and after a short period of recovery. Pima 32 was consistently and statistically the most heat tolerant cultivar as the lower % decline of F_v/F_m indicates greater tolerance to high temperatures, and heat tolerance was unaffected by water regime. These findings indicate that Pima 32 is innately heat tolerant, and may not need to acclimate to drought by increasing its heat tolerance. This is likely due to its origin from hot growing environment and already lower stomatal conductance and higher leaf temperatures than *G. hirsutum* as reported previously [18]. Heat tolerance of Siokra L23 was either not influenced by water regime or more heat sensitive under water-stress conditions than well-watered control. Additionally, water-stressed Siokra L23 leaves increased heat sensitivity after a short period of recovery, suggesting that increasing heat tolerance in response to drought is a mechanism that does not play a central role for coping with drought for this genotype since it was less heat tolerant when exposed to water-deficit stress conditions. DP 0912 was generally the least heat tolerant genotype under well-watered conditions, but consistently increased heat tolerance following exposure to drought, similar to a previous report with this same cultivar [13]. This indicates that increasing heat tolerance

(acclimation) is an important mechanism that this particular cultivar uses to cope with water-deficit stress.

4. CONCLUSION

Differences in heat tolerance and physiological acclimation exist among the genotypes studied under water-deficit stress and recovery conditions. Pima 32 was the most heat tolerant genotype and had higher recovery capacity in stomatal conductance after the plants were re-watered. Siokra L23 was relatively heat sensitive and had stomatal conductance reduced by water-deficit stress. DP 0912 was the least heat tolerant genotype under well-watered conditions; however, it increased heat tolerance in the water-stressed plants after the recovery. Stomatal conductance in DP 0912 was greatly decreased by water-deficit stress. Overall, it appeared that the obsolete genotype Pima 32 had superior heat tolerance acclimation, which was indicated as a characteristic of drought tolerant cotton genotypes, while Siokra L23 and the modern genotype DP 0912 were considered heat sensitive under both water regimes.

COMPETING INTERESTS

Authors have declared that no competing interests exist.

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