

## Growth and photosynthesis of Upland and Pima cotton: response to drought and heat stress

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### ABSTRACT

The effects of drought and heat stress on physiology of two common cotton species, *Gossypium hirsutum* L. (Upland) and *G. barbadense* L. (Pima) were investigated in this study. Four consecutive 28-day pot experiments were carried out in a fully controlled growth chamber under four temperatures (25, 30, 35 and 40°C), while two water treatments (well-watered and drought) were imposed for 10 days to both cotton species. Growth according to dry matter accumulation of *G. barbadense* was more limited by sole drought conditions (50%), whereas heat stress was more remarkable on growth of *G. hirsutum* (64%). Chlorophyll (Chl) *a* and *b* content were more temperature-sensitive in *G. hirsutum* than *G. barbadense* under well-watered conditions. The most noticeable decrease in drought-induced rates of photosynthesis rate ( $P_n$ ), transpiration rate (*E*) and stomatal conductance ( $g_s$ ) were recorded under 35°C, although genotypic variation was found under 25°C. Higher water use efficiency was associated with higher temperature. Relative decrease in Chl *a* content,  $g_s$  and maximal quantum efficiency of PSII exhibited by both species were distinctive physiological traits for heat and drought tolerance.

**Keywords:** fiber crops; rainfall; climate change; water stress; photosynthetic parameters

*Gossypium hirsutum* L. (Upland) and *G. barbadense* L. (Pima) are the most important fiber crops grown worldwide in more than 50 countries. However, rising temperatures, variable rainfall patterns, and increasing drought affect areas because of climate change and threaten cotton production on a global scale. Drought and high temperatures are the main limiting factors on plant growth and photosynthesis in cotton production areas. These two abiotic stresses often occur simultaneously; however, the combined effects of both factors have not been thoroughly dissected at the physiological level (Xu and Zhou 2006).

Drought and high temperatures have independently opposing effects on cotton growth via altering its main plant processes including photosynthesis (Cornish et al. 1991). Decrease in stomatal conductance (Ackerson 1980) and photosynthetic rate (Genty et al. 1987), mainly due to stomatal closure, is a primary physiological response of cotton to drought stress. On the

other hand, stomatal conductance increases with temperature up to a certain level (Radin et al. 1994) with no apparent increase in photosynthetic rate (Lu and Zeiger 1994). Temperatures over 35°C highly restrict photosynthesis, growth and productivity of cotton (Bibi et al. 2008).

In addition, the photosynthetic efficiency of PSII decreases due to the loss of light energy as fluorescence from PSII reaction centres under both drought (Deeba et al. 2012) and heat stress (Bibi et al. 2008). Decrease in dark-adapted maximum yield of PSII ( $F_v/F_m$ ) indicates photo-inhibition response to dry and hot conditions (Gamon and Pearcy 1989).

*G. hirsutum* and *G. barbadense* are morphologically similar species having different dry accumulation strategies. *G. hirsutum* is heliotropic unlike *G. barbadense* and its leaves maximize light absorption during daytime (Wise et al. 2000). On the other hand, *G. barbadense* has large and fairly flat leaves (Sassenrath-Cole 1995) with higher stomatal density (Lu et al. 1997, Wise et al. 2000).

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The objective of this study is to characterise photosynthesis-involved specific response of cotton to drought and high temperatures. In addition, an examination was carried out whether two major cotton species, *G. hirsutum* and *G. barbadense*, had different strategies under both stress conditions.

**MATERIAL AND METHODS**

**Plant material and growth and experimental conditions.** Four consecutive pot experiments were carried out in a growth chamber. Upland cotton (*G. hirsutum* L., cv. Carmen) and Pima

cotton (*G. barbadense* L., cv. Giza) were grown in controlled-environment chambers. Seeds of the cotton genotypes were placed on moistened filter paper in Petri dishes for a day. Then four germinated seeds were sown in quadratic pots of 11 cm height and 11 cm diameter and three seedlings were eliminated after emerging. The pots contained a mixture of sterilized commercial garden soil and perlite (3:1). Plants were watered once a week with Knop's nutrient solution and in the remaining days by distilled water. Light conditions 14 h/10 h (day/night) and light intensity at the plant level was at least 500  $\mu\text{mol}/\text{m}^2/\text{s}$  photosynthetically active radiation. The temperature and relative humidity

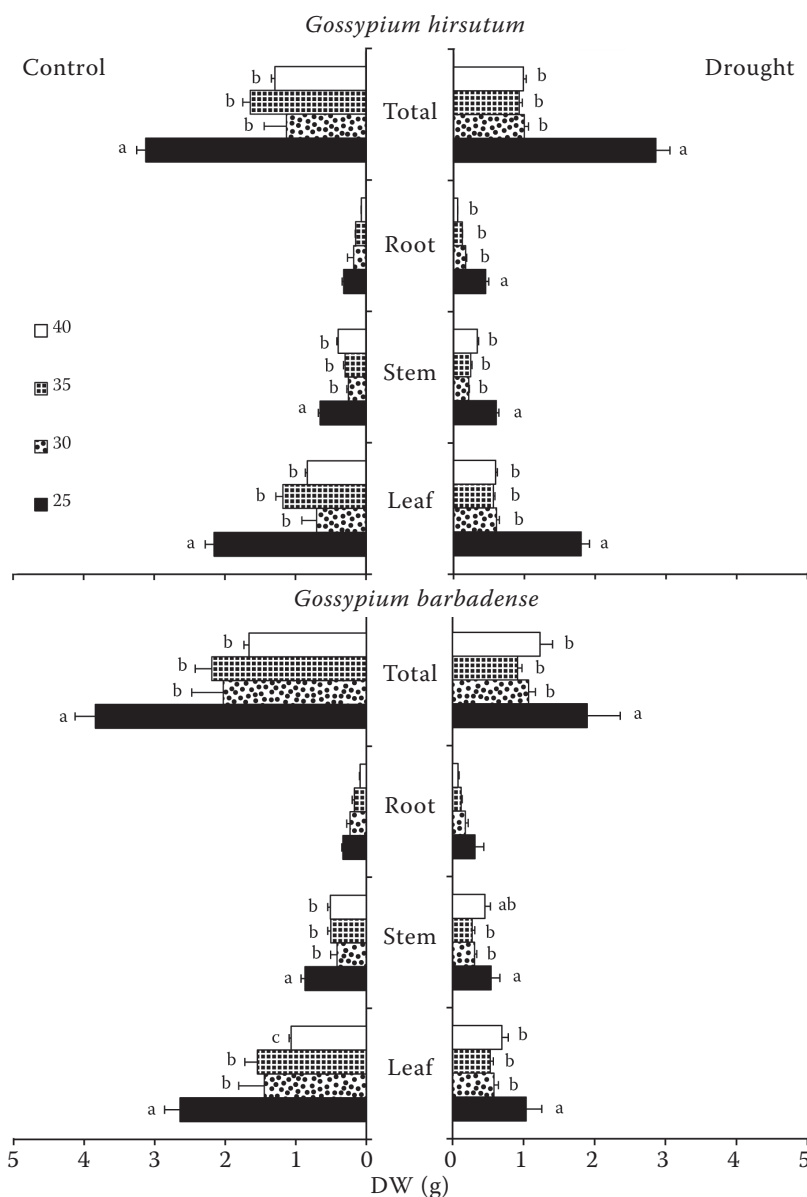


Figure 1. Effects of drought and temperature (25, 30, 35 and 40°C) on dry weight (DW) of *Gossypium hirsutum* L. and *G. barbadense* L. Statistically significant difference is given by letters according to the LSD ( $P < 0.05$ )

were kept at 25°C and 45% respectively until the onset of the treatments. Plants were grown under well-watered conditions until 5<sup>th</sup> true leaves of the plants appeared, after which stress treatment was initiated. Drought stress was applied during 10 days. Air temperature in the growth chamber was set at 25, 30, 35 and 40°C during four successive experiments for heat stress treatments.

**Physiological measurements.** Leaf gas exchange parameters – the net photosynthetic rate ( $P_n$ ), rate of transpiration ( $E$ ) and stomatal conductance ( $g_s$ ) were measured 5 times on the youngest fully developed leaf during 10 days after the onset of the treatments (DAT), using the portable gas exchange system LCpro+ (ADC BioScientific Ltd., Hoddesdon, UK). The water use efficiency (WUE) was calculated, as  $P_n/g_s$ . The chlorophyll fluorescence parameters – minimum ( $F_0$ ) and maximum ( $F_m$ ) were measured 5 times on the youngest fully developed leaf during 10 DAT by the portable ADC:OSI FL 1 analyser (ADC BioScientific Ltd., Hoddesdon, UK) with 1 s excitation pulse (660 nm) and saturation intensity 3000  $\mu\text{mol}/\text{m}^2/\text{s}$  after 20 min dark-adaptation of the leaves. The maximal

quantum efficiency of PSII was calculated as  $F_v/F_m$  ( $F_v = F_m - F_0$ ) and the efficiency of the water-splitting complex on the donor side of PSII (as inferred from  $F_v/F_0$ ) (Kalaji et al. 2011). Plants were harvested 10 DAT. Leaves, stem and roots were separated and dried at 65°C for 72 h in the drying chamber. Then dry weights (DW) of the plant parts were determined.

Leaf samples were extracted in 80% acetone and the absorbance of the centrifuged extract was recorded at 480, 645 and 663 nm using a spectrophotometer. Chlorophyll contents were calculated according to Arnon (1949).

**Statistical analysis.** A mixed-model procedure, with a repeated statement for each parameter, was used to analyse the data set. Data from each plant were tested separately. ANOVA (the Wilks' lambda,  $P < 0.05$ ) was used to determine significant differences and means were compared by *LSD*, relative decrease (RD) in stomatal conductance to control conditions. All statistical tests presented in this study were performed using a Statistica 9.1 (StatSoft Inc., Tulsa, USA) software package.

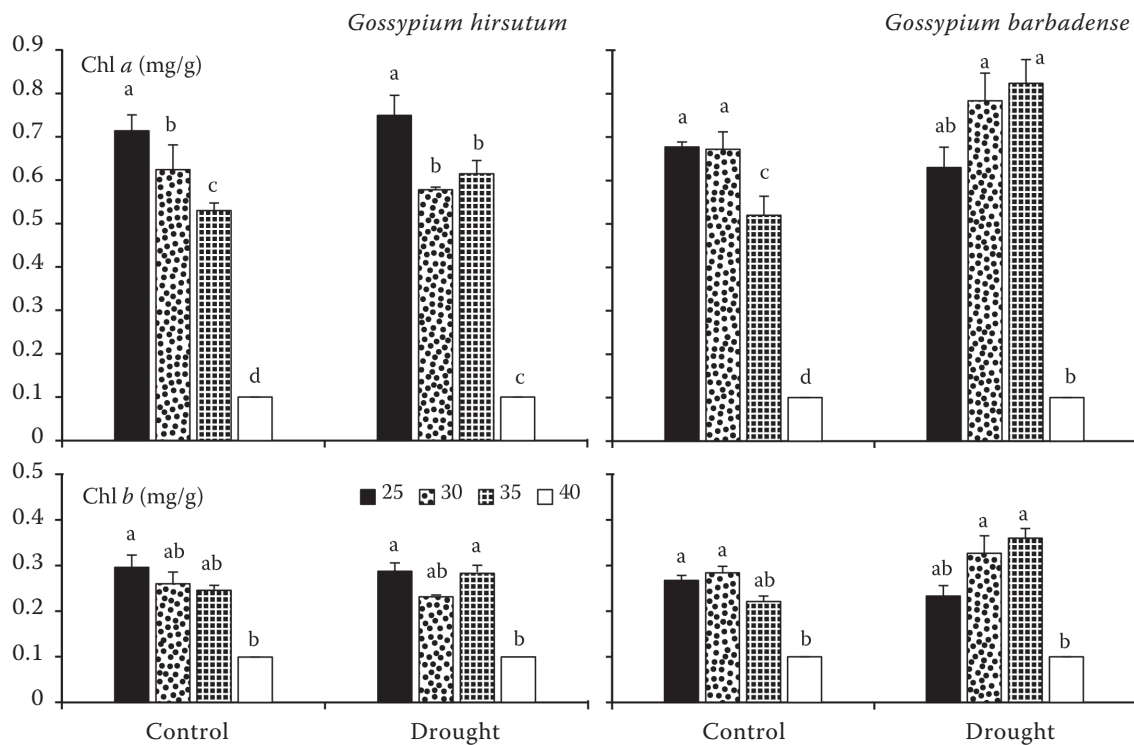


Figure 2. Effects of drought and temperatures (25, 30, 35 and 40°C) on leaf chlorophyll (Chl) *a* and *b* contents of *Gossypium hirsutum* L. and *G. barbadense* L. Statistically significant difference is given by letters according to the *LSD* ( $P < 0.05$ )

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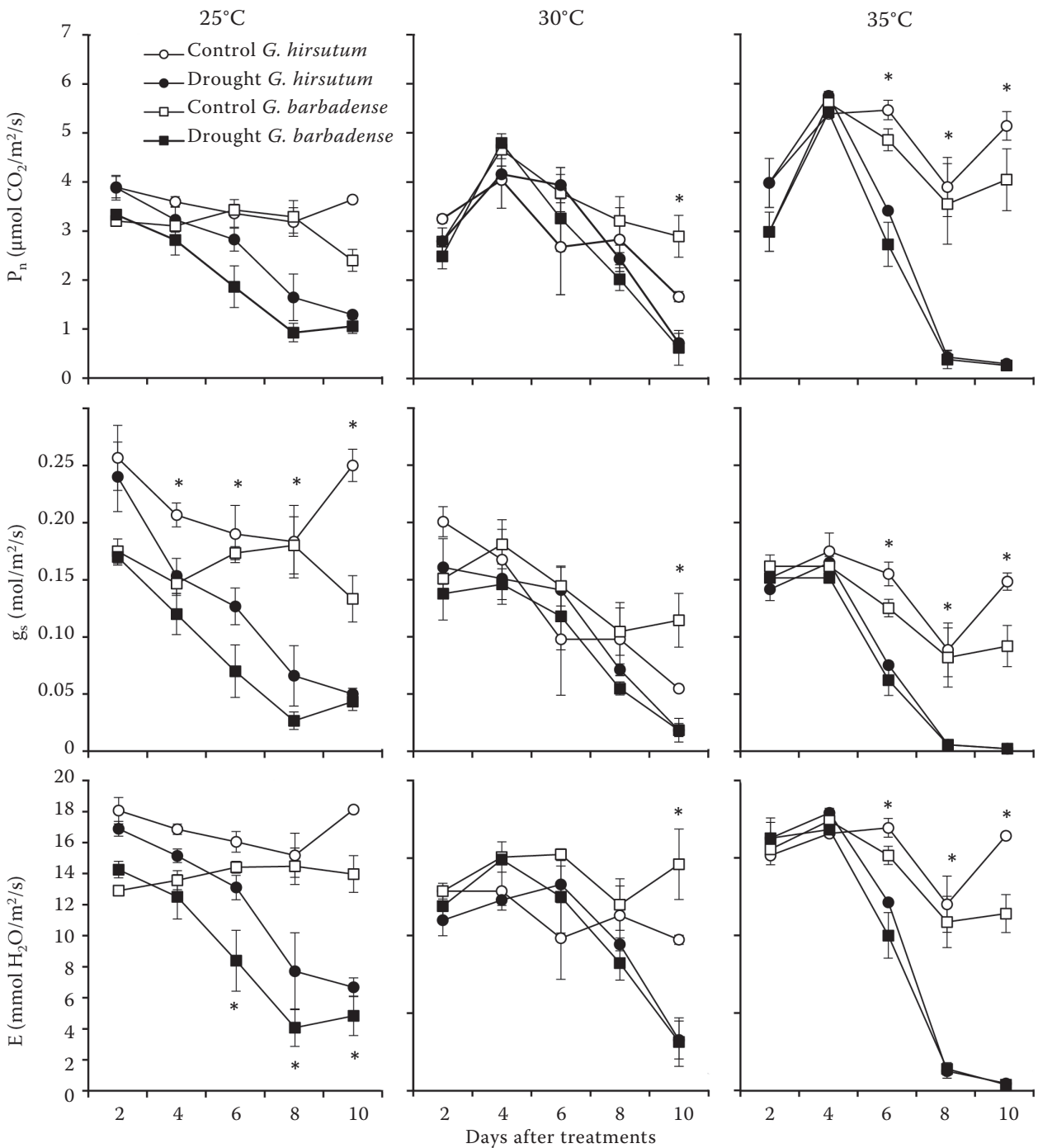


Figure 3. Effects of drought and different temperatures (25, 30, and 35°C) on photosynthetic rate ( $P_n$ ), stomatal conductance ( $g_s$ ) and transpiration ( $E$ ) of *Gossypium hirsutum* L. and *G. barbadense* L. Statistically significant difference is given by asterisk according to the  $LSD$  ( $P < 0.05$ )

## RESULTS AND DISCUSSION

Total dry-weight of both genotypes (*G. hirsutum* and *G. barbadense*) significantly decreased in drought imposed plants in all heat treatments (Figure 1).

However, reduction in DW was more marked in *G. barbadense* (50%) than *G. hirsutum* (8%) under sole drought conditions. This reduction was associated with a significant decrease in leaf DW rather than roots and stems of *G. barbadense* (Figure 1).

Dry weights of both genotypes were lower under higher temperatures (30, 35 and 40°C) than at 25°C under well-watered control conditions. However, increasing temperatures above 25°C caused greater reduction in DW of *G. hirsutum* (64%) than *G. barbadense* (47%).

Many studies reported separately on changes in growth and physiology of cotton genotypes under drought stress (Pace et al. 1999, Ullah et al. 2008) and heat stress (Reddy et al. 1992). However, these stress factors often accompany each other and occasionally appear alone. Therefore, it is generally difficult to distinguish between the effects of sole drought and heat stress on plants *in situ*.

Growth of *G. barbadense* in the present study was more limited by drought without heat stress, whereas sole heat stress was more remarkable on the growth of *G. hirsutum*. *G. hirsutum* is heliotropic, unlike *G. barbadense* and its leaves maximise light absorption during daytime (Wise et al. 2000). The solar tracking behavior of *G. hirsutum* leaves may lead to higher plant sensitivity to heat stress. Leaves of *G. barbadense*, however, are stationary and provide shading to mitigate photo-bleaching (Sassenrath-Cole 1995). However, *G. barbadense* has relatively larger leaf areas (data not presented) and more stomata than *G. hirsutum* (Wise et al. 2000). High stomatal density, which has a crucial role in leaf conductivity and larger transpiring organs, may cause lesser resistance to drought stress in *G. barbadense*.

Chl *a* and *b* content as a function of drought stress remained similar in both species (Figure 2). Reduction in Chl *a* content of *G. hirsutum* leaves began significantly by 30°C and Chl *a* content reached minimum at 40°C. However, Chl *a* content remained nearly constant at 25°C and 30°C in *G. barbadense* and decreased by 35°C. Chl *b* content of both species significantly decreased only in severe heat stress conditions (40°C). Extreme temperatures led to malfunction and structural disruption in chloroplasts (Xu et al. 1995). Almeselmani et al. (2012) suggested that the level of Chl synthesis and breakdown under heat stress determine tolerance levels of genotypes. *G. barbadense*, a relatively heat tolerant species, exhibited better protection and maintained higher pigment content than *G. hirsutum* (at least under mild heat stress conditions, Figure 2).

Photosynthesis measurements were not taken under 40°C treatments because leaves dried just 1 DAT. Drought stress significantly reduced  $P_n$ ,  $g_s$

and *E* of both species 2 DAT under 25°C conditions (Figure 3). However, all these parameters were lower in leaves of *G. barbadense* during drought stress than *G. hirsutum*. Drought induced decrease in  $P_n$ ,  $g_s$  and *E* were significant 4 DAT in 30°C whereas more remarkable 2 DAT in 35°C.

The gas exchange parameters of both *G. barbadense* and *G. hirsutum* were higher at the end of 30°C and 35°C, under well-watered conditions. Lu and Zeiger (1994) reported that the higher productivity of *G. barbadense* was associated with  $g_s$ , whereas Levi et al. (2009) found no relation between yield and  $g_s$  in *G. hirsutum*. Our results supported these earlier findings under well-watered and 30°C conditions.

*G. hirsutum*, on the contrary, had higher  $P_n$  and  $g_s$  under both drought and control conditions than *G. barbadense* if the temperature was lower (25°C). Thus it is possible that higher  $g_s$  associated with the productivity of *G. barbadense* is temperature-dependent and revealed under warmer conditions. Cornish et al. (1991) reported that heat tolerance of *G. barbadense* was regulated via higher  $g_s$ . *G. barbadense*, in our results, has also higher  $P_n$  and  $g_s$  at the end of 30°C treatments in well-watered plants than *G. hirsutum*, but contrary results were found in 35°C treatments. Heat tolerant *G. bar-*

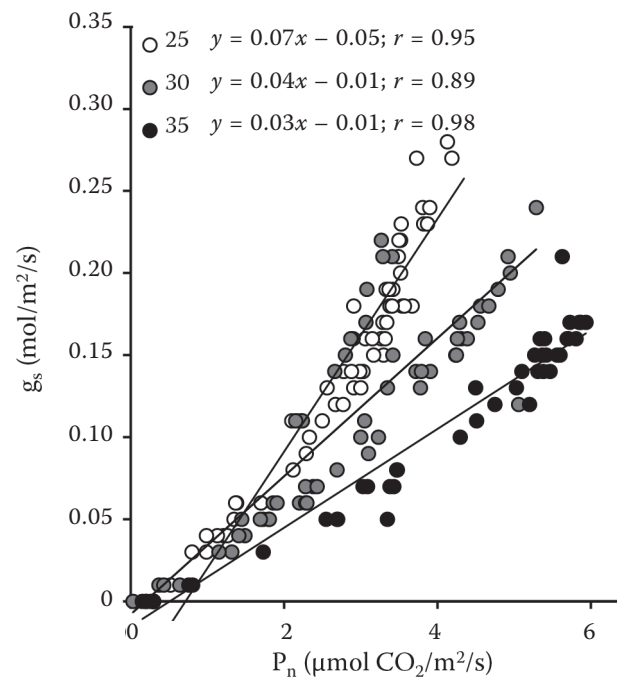


Figure 4. Correlations between photosynthetic rate ( $P_n$ ) and stomatal conductance ( $g_s$ ) of cotton under three different temperatures (25, 30, and 35°C)



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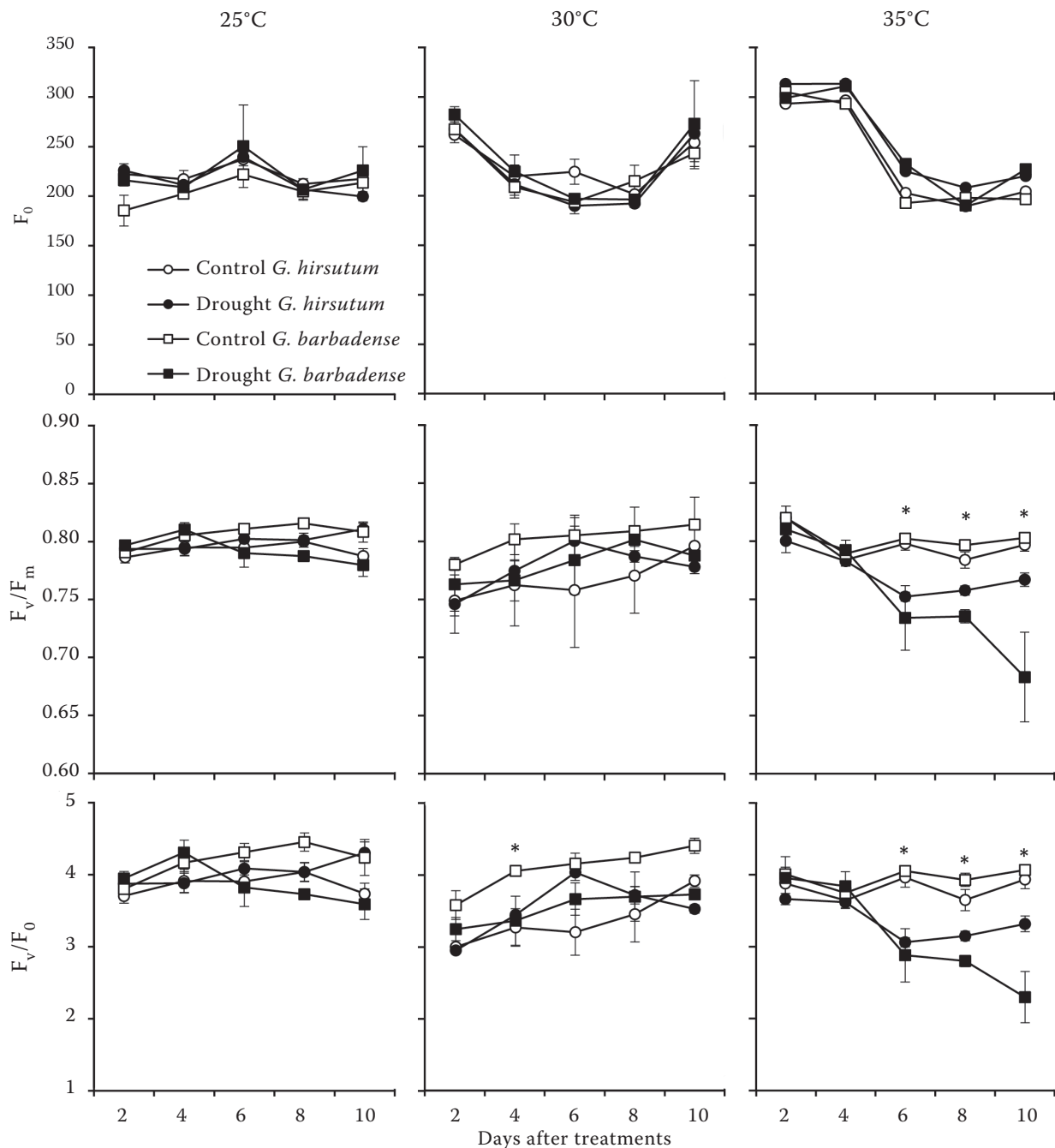


Figure 5. Effects of drought and different temperatures (25, 30, and 35°C) on chlorophyll fluorescence parameters ( $F_0$  (minimum fluorescence),  $F_v/F_m$  (maximal quantum efficiency of PSII) and  $F_v/F_0$  (maximum primary efficiency of PSII)) of *Gossypium hirsutum* L. and *G. barbadense* L. Statistically significant difference is given by asterisk according to the LSD ( $P < 0.05$ )

*barbadense* may control stomatal function under mild stress conditions, but not under the severe ones, due to degradation of its photosynthetic apparatus.

Correlation between  $g_s$  and  $P_n$  under different temperatures (25, 30 and 35°C) is shown in Figure 4. The slope of the linear regression line was steeper under lower temperatures. Higher temperature

was associated with higher WUE. Kakani et al. (2015) was in agreement with our results while Crawford et al. (1999) reported decreasing leaf WUE with increasing temperatures.

PSII complex is the most sensitive component of the electron transport chain (Havaux and Tardy 1996).  $F_v/F_m$  as an indicator of photoinhibition

decreases due to abiotic stress factors such as heat and drought. However,  $F_0$ ,  $F_v/F_m$  and  $F_v/F_0$  were not significantly different under 25°C and 30°C conditions (Figure 5).

In addition, drought induced a reduction in  $F_v/F_m$  and  $F_v/F_0$  2 DAT under 35°C conditions. The reduction was relatively higher in *G. barbadense* (15%) than *G. hirsutum* (3.6%). This suggests that *G. hirsutum* can maintain higher levels of photosynthetic efficiency than *G. barbadense* under drought stress at warmer conditions (35°C).

Relative decrease (RD) in  $g_s$  ( $r = 0.83$ ) and RD in PSII efficiency ( $r = 0.85$ ) were associated with RD in DW in *G. hirsutum* but not in *G. barbadense* under heat stress conditions (Figure 6). On the contrary, under drought conditions, significant positive correlations were found between both parameters and RD in DW in *G. barbadense* but not in *G. hirsutum*.

The different response of the species, relatively heat tolerant *G. barbadense* and drought tolerant *G. hirsutum*, revealed the role of fine-regulation of

stomata and maintaining PSII efficiency on plant performance under both stress conditions. Radin et al. (1994) also reported that new heat tolerant cultivars of *G. barbadense* mainly developed through changes in  $g_s$ . Our results indicated that the enhanced tolerance to drought and heat stress conditions of both cotton species was correlated with the traits  $g_s$  and efficiency of PSII.

In conclusion, the growth of *G. barbadense* was more limited by sole drought conditions, whereas heat stress was more remarkable on *G. hirsutum* growth. The most noticeable decrease in drought induced gas exchange parameters,  $P_n$ ,  $g_s$  and  $E$ , were recorded under 35°C although genotypic variation was found under 25°C.

Contrary to some earlier reports, higher leaf WUE was associated with higher temperature in the present study. In addition, the heliographic properties of *G. hirsutum* leaves, RD in Chl *a* content, RD in  $g_s$  and RD in efficiency of PSII were distinctive physiological traits for heat and drought tolerance exhibited by both species.

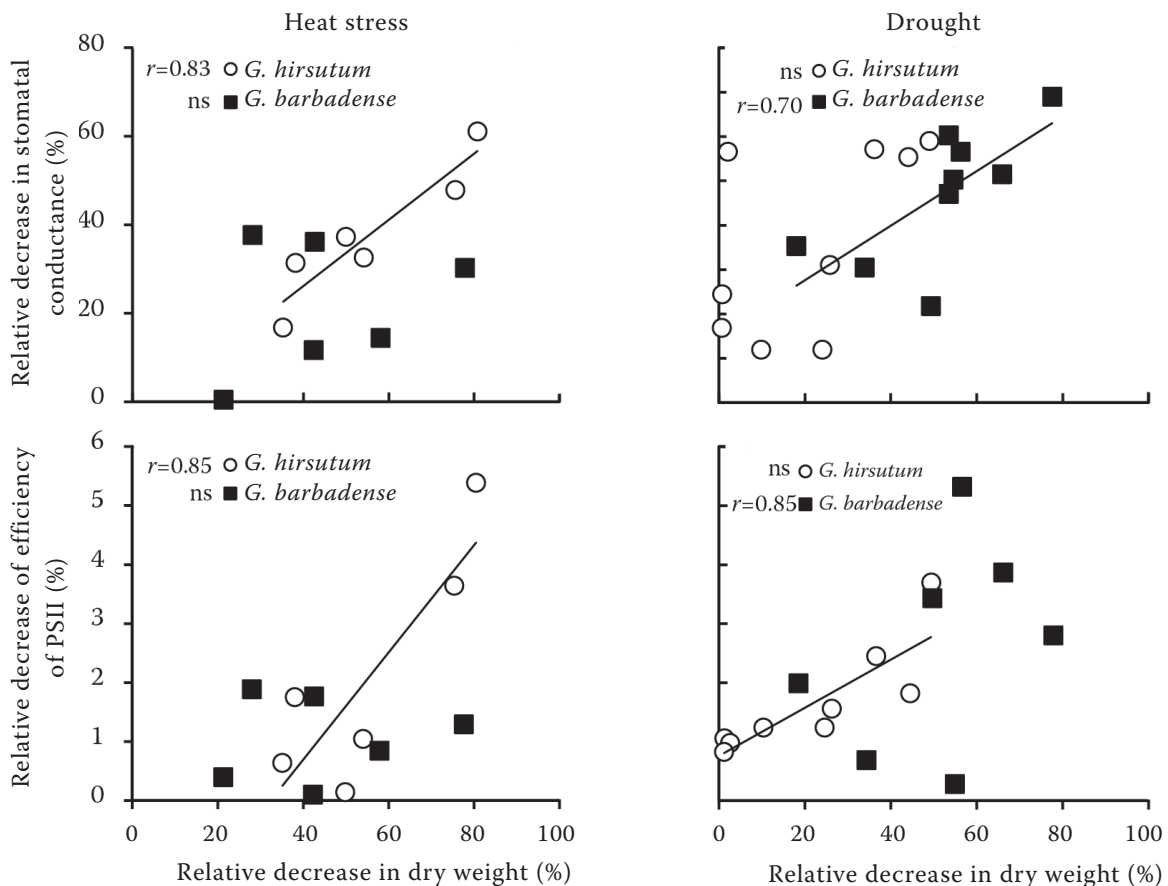


Figure 6. Correlations between relative decrease of efficiency of PSII, relative decrease in stomatal conductance and relative decrease in dry weight (DW) of *Gossypium hirsutum* and *G. barbadense* under drought and heat stress

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