

1 Original Research Article
2 **Photosynthetic Efficiency and Antioxidant**
3 **Activity of Cotton under Drought Stress during**
4 **Early Floral Bud Development**

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15 **Author's contribution**

16 *This work was carried out in collaboration between all authors. Authors CP and DMO*
17 *designed the study and wrote the protocol. Author CP and EAP collected the data and*
18 *performed the laboratorial analyzes. Author CP analyzed the data and wrote the first draft of*
19 *the manuscript. Author GLR managed the field in Lubbock throughout the experiment period.*
20 *Authors DMO and GLR reviewed the draft of the manuscript. All authors read and approved*
21 *the final manuscript.*

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24
25 **ABSTRACT**
26

Aims: Identify changes in photosynthetic efficiency, pigment concentration, and enzyme activity in cotton caused by water-deficit stress during early floral bud development; compare physiological stress responses among cotton cultivars.

Study design: A strip plot design with two water regimes at Lubbock, and a strip split plot design with two water regimes and three cultivars at Marianna, with five replications.

Place and Duration of Study: Fields at Quaker Avenue Research Farm of Texas Tech University in Lubbock, TX in 2012 and at the Lon Mann Cotton Research Station of University of Arkansas in Marianna, AR in 2013.

Methodology: Water was withheld for 14 days from the water-deficit stress treatment at the appearance of floral buds on the cotton plants. Stomatal conductance and chlorophyll *a* fluorescence were measured *in situ* seven and fourteen days after the onset of stress; tissue samples were also collected and analyzed for enzyme activity and pigment concentration.

Results: Lower stomatal conductance was observed in plants under water-deficit stress in all instances. Actual quantum yield of photosystem II (Φ_{PSII}) varied among the cultivars, with DP 0912 having the highest Φ_{PSII} , followed by ST 5288 and PHY 499. The Φ_{PSII} and electron transport rate also decreased over time. Pigment concentrations, including Chlorophyll *a* and *b*, were reduced by water-deficit stress over time among all cultivars and sampling dates, with the lowest pigment concentrations occurring in DP 0912. Enzyme activity was significantly increased by water-deficit stress, with stressed plants having a 4-fold increase in superoxide dismutase activity, a 10-fold increase in catalase activity, and a 57% increase in ascorbate peroxidase concentration compared with the control.

Conclusion: Stomatal conductance and pigment concentration were sensitive to water-deficit stress at squaring development; however, chlorophyll *a* fluorescence was not responsive to the stress. Increased antioxidant activity appeared to be associated with scavenging of free radicals in cotton. ST 5288 and PHY 499 seemed to have improved tolerance to water-deficit conditions compared to DP 0912. However, further research is needed to identify traits related to drought tolerance of these cultivars.

Keywords: Gossypium hirsutum; photosystem II; electron transport rate; enzymes; photosynthetic pigments; water-deficit stress.

1. INTRODUCTION

Upland cotton (*Gossypium hirsutum* L.) yield is compromised by water-deficit stress in multiple ways. As water becomes limiting, stomatal conductance decreases, resulting in a decrease in photosynthesis. The decreased photosynthetic rate in turn decreases the production and metabolism of carbohydrates, leading to a reduction in plant growth and fruit abscission [1]. The effects of water-deficit stress in crops vary with the severity and duration of the stress, plant growth stage, and genotype, as well as the interaction between these factors [2]. Cotton yields in the U.S. have shown great variability across the recent years [3], and this year-to-year variability might be attributed to differences in plant genetics and physiological responses to environmental stresses throughout the season [4].

Upland cotton uses an array of mechanisms to alleviate and survive water-deficit stress, such as increased activity of antioxidants, heat shock proteins, accumulation of osmolytes, and osmotic adjustment; however, due to domestication and cultivation as an annual crop, modern cotton cultivars differ in their ability and level of tolerance to a water-deficit period [5,6].

Stomatal closure and increased mesophyll resistance occur shortly after the onset of water-deficit stress, decreasing CO₂ absorption used in the photosynthesis process [7]. Under severe water-deficit stress, photosynthesis is also impaired by nonstomatal factors, including a reduction in carboxylation efficiency, which leads to an excess of absorbed light energy in photosystem II (PSII). This could result in damage to the photosynthetic apparatus through increased production of chlorophyll triplet if excess energy cannot be properly dissipated [8].

Due to the sensitivity of photosynthesis to water scarcity conditions, the photosynthetic efficiency of plants under water-deficit stress has been used successfully as an indicator for tolerance [9]. Chlorophyll fluorescence is a fast, precise, and non-destructive measurement, with a positive relationship between the actual quantum yield of PSII and the quantum efficiency of CO₂ fixation [10]. In cotton plants, chlorophyll fluorescence has been documented to decrease in plants grown under water-deficit stress conditions [11,12], decreasing photosynthetic rate and sugar production.

Additionally, the quantity and activity of photosynthetic pigments are affected by low water availability. The pigments commonly found in plants are chlorophyll *a*, chlorophyll *b* and carotenoids. Chlorophyll *a* is the main pigment absorbing energy for further conversion into adenosine triphosphate (ATP) and nicotinamide adenine dinucleotide phosphate (NADPH) during the light reactions of photosynthesis, and also responsible for the green color in plants [13]. Chlorophyll *b* helps to increase the absorption band of light to be used in photosynthesis. Under high light intensity, plants can absorb more light energy than actually used in photosynthesis process. The overexcitation of chlorophyll may result in increased formation of reactive oxygen species (ROS) such as chlorophyll triplet and singlet oxygen.

Damage caused by singlet oxygen and its reactive products reduce the efficiency of photosynthesis through photoinhibition. Carotenoids are capable to receive the triplet excitation energy of chlorophyll and thus help to prevent the formation of ROS [14]. Studies have shown degradation in photosynthetic pigments concentration in several crops grown under drought conditions, such as cotton, potato (*Solanum tuberosum* L.), wheat (*Triticum aestivum* L.), chickpea (*Cicer arietinum* L.), and sunflower (*Helianthus annuus* L.) [15-19].

Overproduction of reactive oxygen species (ROS) also damage plant cells irreversibly by degradation of lipids, proteins and nucleic acids [20]. Antioxidant enzymes, such as superoxide dismutase (SOD), catalase (CAT), and ascorbate peroxidase (APX) increase their activity to control the balance between production and scavenging ROS. Reactive oxygen species are eliminated by SOD through a reaction that produces hydrogen peroxide (H_2O_2). Then, the H_2O_2 is converted into oxygen and water by CAT or into water by APX. Research has been reported on antioxidant activity of plants under water-deficit stress, such as maize (*Zea mays* L.) [21], canola (*Brassica napus* L.) [22], quinoa (*Chenopodium quinoa* L.) [23], and potato [16]. Studies on antioxidant responses of cotton plants under drought conditions are still controversial and not well elucidated. Ratnayaka et al. [24] indicated increase in APX activity and no alteration in the activity of SOD or CAT of cotton plants under drought stress. However, studies reported by Deeba et al. [25] suggested higher CAT activity in plants under severe stress and no changes in CAT in plants under mild stress, while SOD was higher only in plants under mild stress and similar activity of SOD in plants under severe stress and control.

Throughout cotton development, the reproductive phase of flowering is generally accepted as the most sensitive stage [1]. In addition, there is evidence that the early stage of square (floral bud) development when meiosis is taking place is also a sensitive stage [26]. However, there is very little information on the physiological responses of cotton plants that experience water-deficit stress during the early squaring stage. Knowledge on the effect of water-deficit conditions on cotton at squaring development and the potential cultivars that are tolerant to the stress at this stage would assist producers in selection of most adapted cultivars to their location. Therefore, we hypothesize that activity of enzymes increases and photosynthetic efficiency is impaired as cotton plants experience water-deficit stress during the early squaring stage and that variation in drought tolerance exists among modern cultivars being utilized in the U.S. production. The objectives of this study were to evaluate changes in the photosynthetic efficiency, concentrations of photosynthetic pigments, and activity of enzymes in cotton plants caused by water-deficit stress during early floral bud development, and identify differences in physiological responses among the cultivars.

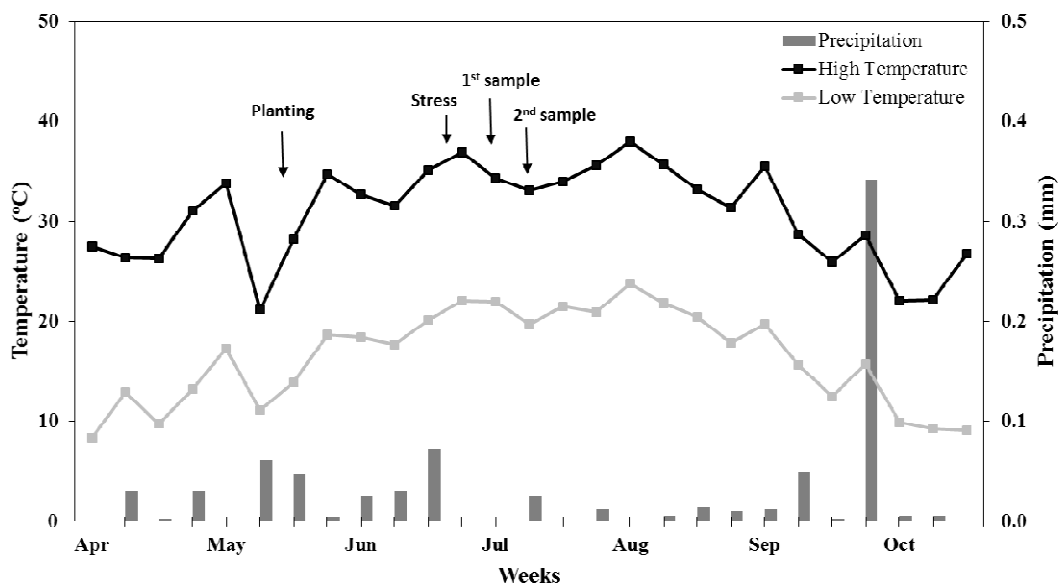
2. MATERIALS AND METHODS

2.1 Study Sites, Plant Material, and Sampling Protocol

Field experiments were conducted at the Quaker Avenue Research Farm of Texas Tech University in Lubbock, TX (N 33°59'93", W 101°90'72") and at the Lon Mann Cotton Research Station of University of Arkansas in Marianna, AR (N 34°43'50", W 90°45'34") in 2012 and 2013, respectively. Seeds of one commercial cotton cultivar, ST 5288B2F (Stoneville, Bayer CropScience, Lubbock TX) were sown on May 23, 2012 (Lubbock, TX) and three, DP 0912 B2RF (Delta and Pine Land, Monsanto Company, St. Louis MO), PHY 499 WRF (PhytoGen, Dow AgroSciences, Indianapolis IN), and ST 5288B2F (Stoneville, Bayer CropScience, Lubbock TX) on May 8, 2013 (Marianna, AR) at a 0.96 m inter-row spacing and at a rate of 11 seeds m^{-1} row. The soil series in Lubbock is an Amarillo-Acuff

sandy clay loam (Fine-loamy, mixed, superactive, thermic Aridic Paleustalfs), and the soil series in Marianna is a Memphis silt loam (fine-silty, mixed, active, thermic Typic Hapludalfs). Fertilization was performed according to soil tests prior to planting and recommended rates for cotton at both sites. Herbicide and pesticide applications were also applied according to Texas A&M AgriLife extension recommendations at Lubbock and University of Arkansas Cooperative Extension Service recommendations at Marianna. Mepiquat chloride was applied as needed to control vegetative growth and all plots within a location received identical applications. Irrigation was performed as necessary using a subsurface drip system at Lubbock according to an on-site GRW 100 weather station (Campbell Sci.) and furrow system at Marianna according to University of Arkansas Cooperative Extension Service recommendations until the appearance of floral buds (squaring stage). When plants reached the pinhead square stage, on June 25 at Lubbock and June 11 at Marianna, water was withheld from the water-deficit stress treatment for fourteen days at both sites. To further characterize site conditions, the weekly average maximum and minimum temperatures, and weekly average precipitation of each location throughout the season along with the sample dates are presented in Figures 1 and 2.

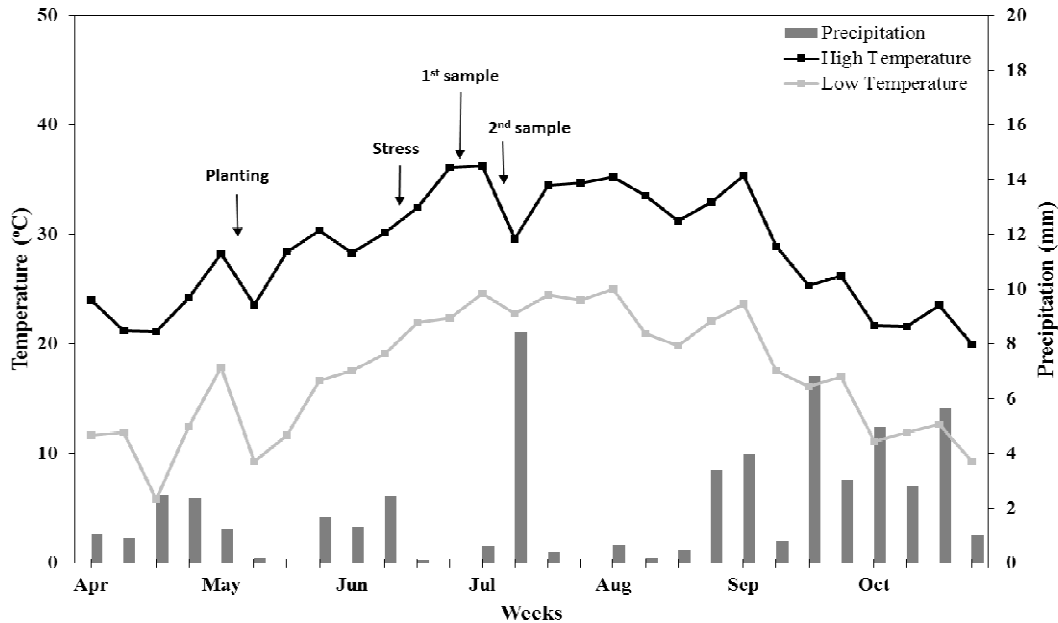
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133

134 **Fig. 1. The average weekly precipitation (mm), and average weekly high and low**
 135 **temperatures (°C) for Lubbock, TX from April to October, 2012. Arrows indicate**
 136 **planting date (on May 23), onset of the water-deficit stress (on June 25), and first and**
 137 **second sample dates (on July 2 and July 9, respectively)**

138



139

140 **Fig. 2. The average weekly precipitation (mm), and average weekly high and low**
 141 **temperatures (°C) for Marianna, AR from April to October, 2013. Arrows indicate**
 142 **planting date (on May 8), onset of the water-deficit stress (on June 11), and first and**
 143 **second sample dates (on June 18 and June 25, respectively)**

144

145 For the Lubbock site, field measurements of stomatal conductance were performed seven
 146 and fourteen days after the onset of the stress and samples for laboratory determinations of
 147 activities of antioxidant enzymes (SOD, CAT, and APX) were collected fourteen days after
 148 the onset of the stress. For Marianna, field measurements of stomatal conductance and
 149 chlorophyll *a* fluorescence, and samples for laboratory determinations of concentrations of
 150 pigments (chlorophyll *a*, chlorophyll *b*, and carotenoids) were taken seven and fourteen days
 151 after the onset of the stress. Both field and laboratory measurements were performed on the
 152 fully-expanded main-stem leaves from the fourth node below the apical meristem from the
 153 two middle rows of each plot at Lubbock and Marianna.

154

155 2.2 Measurements

156 2.2.1 Stomatal Conductance

157 Stomatal conductance ($\text{mmol H}_2\text{O m}^{-2}\text{s}^{-1}$) was measured between 1100 and 1400 h
 158 in five leaves per plot and two readings per leaf due to the small surface area of the cuvette
 159 (6.35 mm^2) on each sample date using a steady-state leaf porometer (SC-
 160 1 Leaf Porometer, Decagon, Pullman, WA) on the abaxial surface of fully expanded leaves.

161

162 2.2.2 Chlorophyll *a* Fluorescence

163 Actual quantum yield of electron transport through photosystem II (Φ_{PSII}) was
164 measured *in situ* in five leaves per plot under natural field irradiance between 1200 and 1400
165 h using a portable fluorometer Model OS1-FL (Opti-Sciences, Hudson, NH). Steady-state
166 fluorescence prior to a saturation pulse was measured to obtain F_t , followed by induction of
167 maximum fluorescence on the adaxial surface of the leaves with a saturating white light
168 pulse for 0.95 s for estimation of F'_m when all reactions centers are closed due to infinite light
169 intensity. Φ_{PSII} was obtained according to the equation $\Phi_{PSII} = (F'_m - F_t) / F'_m$ [27]. Electron
170 transport rate (ETR) through photosystem II was obtained by calculation according to the
171 equation $ETR = \Phi_{PSII} \times PAR \times 0.5 \times 0.84$, where PAR is the absorbed light (in $\mu\text{mol photon}$
172 $\text{m}^{-2}\text{s}^{-1}$) at the leaf surface, 0.5 is a factor on the partitioning of energy between PSII and PSI
173 and 0.84 is a common leaf absorbance coefficient for C_3 plants.

174

175 **2.2.3 Pigments Concentration**

176 Two leaf discs (10 mm diameter) were collected from five leaves of each plot, placed
177 in vials filled with 1.5 mL dimethylformamide and incubated at ambient temperature (25 °C)
178 for 48 h for pigment extraction. After the incubation period, the samples were measured in a
179 UV-visible spectrophotometer (UV-1700 PharmaSpec, Shimadzu, Kyoto, Japan) at
180 wavelengths of 480, 646.8, and 663.8 nm for carotenoids, chlorophyll *a* and chlorophyll *b*
181 concentrations, respectively, according to calculations described by Inskeep and Bloom [28].

182

183 **2.2.4 Activities of Enzymes**

184 Leaves were collected from each plot and immediately frozen for further laboratory
185 determination of activities of enzymes. Superoxide dismutase (SOD) activity was obtained
186 by identification of the enzyme's ability to inhibit photochemical reduction of
187 nitrobluetetrazolium with one unit of SOD, indicating the amount of enzyme required to
188 inhibit 50% of the rate of nitrobluetetrazolium reduction measured at 560 nm according to
189 Giannopolitis and Ries [29]. Catalase (CAT) activity was determined by the variation in H_2O_2
190 absorption in an interval of 60 seconds using a spectrophotometer at 240 nm, according to
191 Peixoto et al. [30]. A molar extinction coefficient of $39.4 \text{ mM}^{-1} \text{ cm}^{-1}$ was used for CAT activity
192 determination. Ascorbate peroxidase (APX) activity was determined by measuring the
193 negative variation of H_2O_2 absorption in a spectrophotometer at 290 nm, according to
194 Koshiba [31]. A molar extinction coefficient of $2.8 \text{ mM}^{-1} \text{ cm}^{-1}$ was used for the calculations.
195 The protein concentration ($\mu\text{g } \mu\text{L}^{-1}$) was considered in the calculations for the specific activity
196 of the three enzymes.

197

198 **2.3 Statistical Analysis**

199 The experiments were arranged in a strip plot design with water treatments running across
200 all blocks in strips at Lubbock and a strip split plot design with water treatments as the main
201 unit running across all blocks in strips in a randomized complete block design and the
202 cultivars were randomly assigned in the sub unit for each whole plot in each block at
203 Marianna, with five replications for the two sites. Data were subjected to analysis of variance
204 using JMP Pro 11 (SAS Institute, Cary, NC). The treatments water regime, cultivar, and
205 sample date were treated as fixed effects. The blocks and the block x treatments interaction
206 were treated as random effects. Tukey's HSD test ($P = .05$) was used to separate treatment

207 combination mean performance. Differences between main factors for a variable were
208 indicated in tables and, when interaction between the factors was observed, a graph was
209 plotted. Sigma Plot 12.5 (Systat Software Inc., San Jose, CA) was used to plot the graphs.

210

211 **3. RESULTS AND DISCUSSION**

212 **3.1 Stomatal Conductance**

213 Significant effect of cultivar or interaction between the factors (cultivar, water regime, and
214 sample date) was not observed for leaf stomatal conductance of cotton plants from Marianna
215 site (Table 1). However, stomatal conductance was significantly affected by water regime (P
216 = .01) and sample date (P = .01) separately. When stomatal conductance results were
217 combined from all cultivars and sample dates, the rates were significantly lower for the
218 water-deficit stressed plants than the well-watered control (Table 1). For the effect of sample
219 date, leaves collected on July 25 had significantly higher stomatal conductance rates than
220 the leaves collected on July 18 (Table 1).

221 **Table 1. Effect of cultivar, water regime, sample date, and the interaction between the factors on stomatal conductance (g_s), actual**
 222 **quantum of PSII (Φ_{PSII}), electron transport rate (ETR), and pigments concentration (chlorophylls *a* and *b*, and carotenoids) of**
 223 **cotton leaves of plants grown in Marianna, 2013**

Source of variation	g_s mmol m ⁻² s ⁻¹	Φ_{PSII}	ETR μmol electrons m ⁻² s ⁻¹	Chl <i>a</i>	Chl <i>b</i> μg cm ⁻²	Carotenoids
Cultivar						
DP 0912	667	0.401 a	237	8.43 b	2.11 b	2.63 b
PHY 499	697	0.360 b	216	8.96 a	2.35 a	2.67 ab
ST 5288	645	0.381 ab	230	8.97 a	2.38 a	2.74 a
Water Regime						
Control	848 a [†]	0.380	223	9.36 a	2.37 a	2.85 a
Water Stress	491 b	0.381	232	8.22 b	2.18 b	2.51 b
Sample Date						
June 18	579 b	0.423 a	246 a	9.13 a	2.32 a	2.67
June 25	760 a	0.338 b	209 b	8.45 b	2.23 b	2.69
ANOVA						
Cultivar (C)	.75	.04 *	.13	<.0001 *	<.0001 *	.01 *
Water Regime (WR)	.01 * §	.91	.45	.001 *	.01 *	.001 *
Days after Stress (SD)	.01 *	<.0001 *	0.001 *	<.0001 *	.001 *	.50
Interaction C x WR	.06	.45	.06	.39	.68	.67
Interaction C x SD	.61	.40	.74	.34	.53	.03 *
Interaction WR x SD	.53	.04 *	.10	.04 *	.36	.84
Interaction C x WR x SD	.93	.79	.51	.15	.15	.26

224 [†] Means not sharing a common letter in column within each main factor are not significantly different (Tukey's HDS test, $P = .05$)

225 [§] Asterisks indicate significant effect of the main factor or interaction between the factors according to Tukey's HDS test at $P = .05$

For the Lubbock site, there was a significant interaction between water regime and sample date ($P = .001$) on stomatal conductance (Fig. 3). Leaf stomatal conductance of plants measured on July 2 (one week after the onset of the stress) did not differ between the water-stressed and well-watered control treatments. Additionally, they were lower than the plants measured on July 9 (two weeks after the onset of the stress), regardless of the water regime. On the second sample date (July 9), water-deficit stress reduced leaf stomatal conductance compared with the well-watered control (Fig. 3).

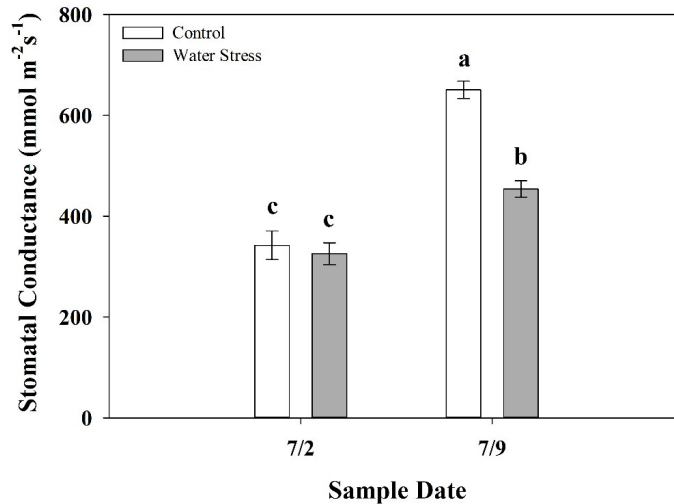


Fig. 3. Effect of the interaction of sample date (July 2, one week after the onset of the water-deficit stress, and July 9, two weeks after the onset of the water-deficit stress) and water regime (well-watered control and water-deficit stress during the early floral bud development) on stomatal conductance (mmol m⁻²s⁻¹) in cotton leaves cv. ST 5288B2F at Lubbock, TX

All values are means $\pm$ standard error (n = 5)

Columns not sharing a common letter are significantly different according to Tukey's HDS test at $P = .05$

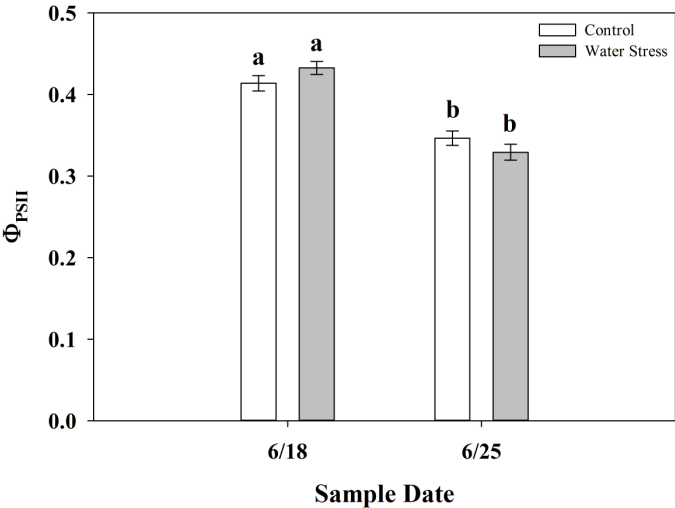
As water is one of the most important factors controlling plant growth and development [32], physiological processes in the plant, such as photosynthetic capacity and stomatal activity, are impaired under low water availability in the soil [33]. In accordance with this study, research has reported lower stomatal conductance rates in plants cultivated under low water availability in the soil leading to reduction in plant growth [34,35].

Leaf age also has an effect on stomatal conductance rates. Young leaves usually have higher concentrations of abscisic acid (ABA) in guard cells inducing stomatal closure and therefore increasing resistance to transpiration [36]. With the maturity of the leaves, a decline in ABA concentration decreases the stomatal resistance, which helps explain the higher stomatal conductance of leaves measured two weeks after the onset of the stress compared with one week after the stress had started.

3.2 Photosynthetic Efficiency

Stomatal closure leads to a reduction in CO₂ assimilation, reducing photosynthesis. Photosynthetic efficiency of plants can be evaluated by measuring chlorophyll fluorescence. Light energy absorbed by the pigments in the chloroplast can be directed to be used in the photosynthesis, with excess energy being dissipated as heat, or it can be re-emitted as light through chlorophyll fluorescence [27]. Due to a competition between these processes, the chlorophyll fluorescence measurement is used as indication of changes in the efficiency of photochemistry and heat dissipation [27]. In our study, quantum yield of PSII (Φ_{PSII}) was significantly affected by cultivar ($P = .04$), sample date ($P < .001$) and the interaction water regime x sample date ($P = .04$) (Table 1). The cultivar DP 0912 showed the highest Φ_{PSII} followed by ST 5288 and lastly PHY 499 with the lowest Φ_{PSII} . Quantum yield of PSII was decreased over time, with lower values on June 25 compared with June 18, regardless of the cultivars and water regimes (Table 1 and Fig. 4).

269



270

Fig. 4. Effect of the interaction of sample date (June 18, one week after the onset of the water-deficit stress, and June 25, two weeks after the onset of the water-deficit stress) and water regime (well-watered control and water-deficit stress during the early floral bud development) on actual quantum yield of photosystem II (Φ_{PSII}) in cotton leaves at Marianna, AR

276

*All values are means $\pm$ standard error ($n = 5$)
Columns not sharing a common letter are significantly different according to Tukey's HDS test at $P = .05$*

279

Electron transport rate (ETR) was significantly affected only by sample date ($P = .001$), with lower rates on June 25 in relation to rates observed on June 18, regardless of the cultivar or water regime (Table 1). Measurements of the proportion of the light absorbed by chlorophyll associated with PSII and the ETR in the light reaction are indication of overall photosynthesis. Thus, results of this research suggested that water-deficit stress during the squaring stage did not impair photosynthetic efficiency of the cotton cultivars studied, even with lower stomatal conductance rates found in all cultivars under water-deficit stress regardless of the sample date. Li et al. [37] found that cotton plants have photosynthetic efficiency impaired by drought stress during the flowering stage, with reduction in quantum yield of PSII and ETR, as well as concentrations of chlorophylls. However, studies on

290 physiological responses of cotton plants under drought conditions during the early floral bud
291 development have not been reported. Additionally, studies on soybean, wheat and sorghum
292 also showed reduction in the photosynthetic efficiency of plants grown under drought stress
293 [38-40].

294

295 **3.3 Concentrations of Pigments**

296 In addition to the quantum yield of PSII and electron transport rate, photosynthetic pigments,
297 such as chlorophylls *a* and *b*, and carotenoids are essential for maintenance of the
298 photosynthesis process at high rates. Chlorophylls *a* and *b* were significantly affected by
299 cultivar, water regime and sample date (Table 1). Concentrations of chlorophylls *a* and *b*
300 varied among the cultivars, with ST 5288 and PHY 499 showing higher concentrations of
301 these pigments than DP 0912. Low water availability for plants is known to cause
302 degradation of pigments in the cells. The concentrations of chlorophylls *a* and *b* were lower
303 in plants grown under water-deficit stress compared with the well-watered control.
304 Concentrations of these pigments were also affected by sample date, with significantly
305 reduction in concentration on June 25 in relation to June 18 (Table 1).

306 Chlorophyll *a* concentration was significantly affected by the interaction water regime x
307 sample date ($P = .04$) (Table 1). Regardless of cultivar, chlorophyll *a* concentration was
308 decreased by water-deficit stress either on June 18 and June 25 (Fig. 5). There was also a
309 reduction in chlorophyll *a* concentration in cells over time, with lower concentration being
310 observed on June 25 compared with June 18 for either water regime. The reduction in the
311 photosynthetic pigments might impair the photosynthetic process due to lower light
312 harvesting efficiency by the leaves, therefore resulting in reduced plant growth and
313 productivity. Accordingly to our results, Li et al. [37] indicated that concentrations of
314 chlorophylls *a* and *b* in cotton plants were reduced by drought stress over time throughout
315 the season, which included the squaring and flowering stages. In this research, even with
316 lower concentration of the pigments in plants under water-deficit stress, the quantum yield of
317 PSII and electron transport rate were maintained to similar rates found in well-watered plants
318 (Table 1 and Fig. 4), which might indicate that the cotton cultivars studied were able to
319 maintain photosynthesis process at lower concentration of photosynthetic pigments present
320 in the cells.

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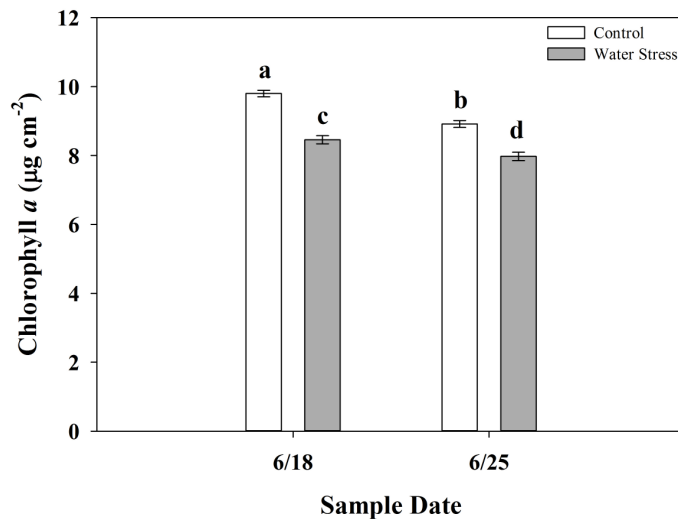


Fig. 5. Effect of the interaction of sample date (June 18, one week after the onset of the water-deficit stress, and June 25, two weeks after the onset of the water-deficit stress) and water regime (well-watered control and water-deficit stress during the early floral bud development) on chlorophyll a concentration ($\mu\text{g cm}^{-2}$) in cotton leaves at Marianna, AR

All values are means $\pm$ standard error ($n = 5$)

Columns not sharing a common letter are significantly different according to Tukey's HDS test at $P = .05$

Carotenoids are important accessory pigments present in cells to assist in harvesting light for the photosynthesis process [13]. Carotenoids concentration was significantly affected by cultivar ($P = .01$), water regime ($P = .001$) and interaction cultivar $\times$ sample date ($P = .03$) (Table 1). Regardless of water regime and sample date, ST 5288 showed the highest carotenoids concentration, followed by PHY 499 and DP 0912 with the lowest concentration. In addition, carotenoids concentration was lower in cells of plants grown under water-deficit stress compared to the well-watered control, regardless of cultivars (Table 1). The interaction between cultivar and sample date indicated that on June 18, ST 5288 showed the highest carotenoids concentration followed by DP 0912 and PHY 499, while on June 25, ST 5288 and PHY 499 had higher concentrations than DP 0912 (Fig. 6). Carotenoids work not only as an accessory pigment, but also as an effective non-enzymatic antioxidant in defense against ROS, which causes damage in cells. Carotenoids main role as antioxidant is in deletion of chlorophyll triplets produced during photosynthesis, restricting the production of ROS and therefore protecting the cells from oxidative damage [14]. Our results indicate that concentrations of carotenoids are not increased by the stress during the early floral bud development, suggesting that the cotton cultivars studied in this research do not make use of this mechanism to tolerate water-deficit stress periods.

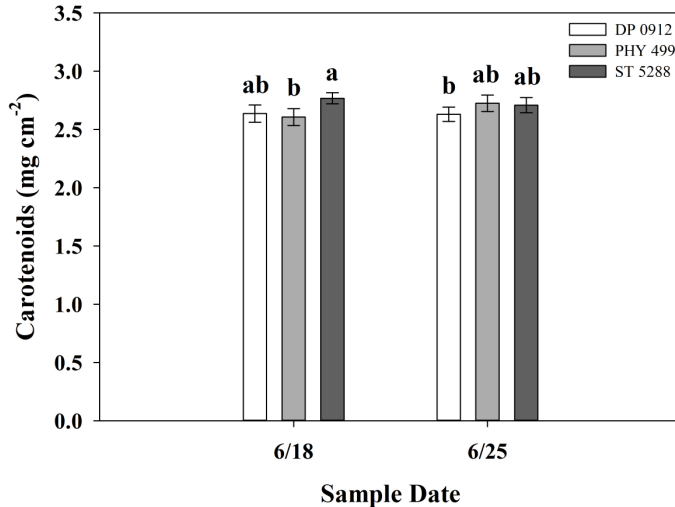


Fig. 6. Effect of the interaction of sample date (June 18, one week after the onset of the water-deficit stress, and June 25, two weeks after the onset of the water-deficit stress) and cultivar (DP 0912, PHY 499, and ST 5288) on carotenoids concentration ($\mu\text{g cm}^{-2}$) in cotton leaves at Marianna, AR

All values are means $\pm$ standard error ($n = 5$)

Columns not sharing a common letter are significantly different according to Tukey's HDS test at $P = .05$

3.4 Antioxidant Profile

Enzymes play a role in cell defense by detoxifying the overproduction of ROS, maintaining the balance between formation and removal of ROS in the cells [20]. Research on increased enzymatic activity to control rate of ROS in cells has been reported for several crops grown under drought stress, such as maize, canola, quinoa, and potato [21-23,16]. In cotton, there have been contradictory results in experiments of enzymatic activity of plants subjected to water-deficit conditions. Some studies identified an increase in APX under water-deficit stress and no alteration in SOD or CAT [24]. Other studies reported higher CAT activity in plants under severe stress and no changes in plants under mild stress, while SOD was higher only in plants under mild stress and similar levels of SOD in plants under severe stress and control [25]. In our study, significant increase in activity of the enzymes SOD, CAT, and APX was detected in plants grown under water-deficit stress (Fig. 7). Water-stressed plants showed a 4-fold increase in SOD and 10-fold increase in CAT activity, compared with the well-watered control plants (Fig. 7A and B). Ascorbate peroxidase activity was approximately 57% higher in the plants grown under water-deficit stress (Fig. 7C). Improved tolerance to cell damage caused by ROS is observed in plants with higher antioxidant activity. These results suggested that activation of antioxidant enzymes was involved in the mechanism controlling overproduction of ROS when the plants experienced water-deficit stress during the early floral bud development, maintaining a balance between production and scavenger of ROS in the cells of the cultivar ST 5288.

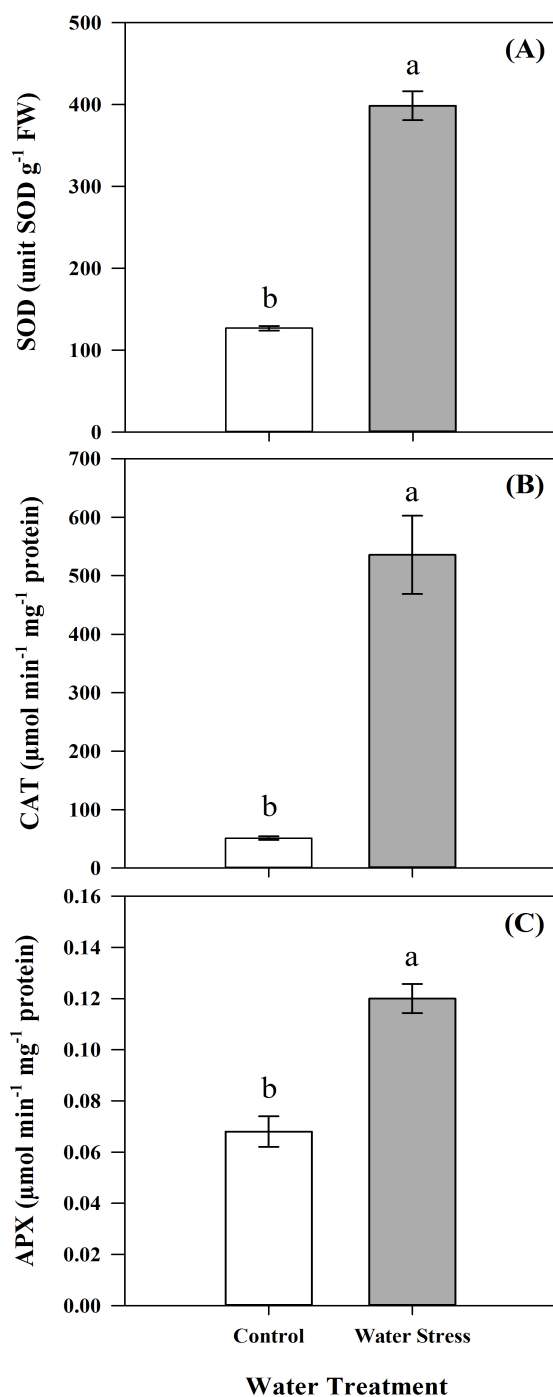


Fig. 7. Effect of water regime (well-watered control and water-deficit stress during the early floral bud development) on activities of the enzymes superoxide dismutase (SOD, unit SOD g⁻¹ FW) (A), catalase (CAT, μmol min⁻¹ mg⁻¹ protein) (B), and ascorbate peroxidase (APX, μmol min⁻¹ mg⁻¹ protein) (C) in cotton leaves cv. ST 5288B2F at Lubbock, TX

All values are means ± standard error (n = 5)

Columns not sharing a common letter are significantly different according to Tukey's HDS test at P = .05

389
390

4. CONCLUSION

391 Leaf stomatal conductance and pigments concentration of all cotton cultivars were sensitive
392 to water-deficit stress at the squaring development; however, photosynthetic efficiency was
393 not responsive to the stress, maintaining similar rates on stressed and non-stressed plants.
394 Actual quantum activity of PSII seemed to be insensitive to water-deficit stress, preventing
395 impairment of leaf photosynthesis. Increased antioxidant enzymes activity of water-deficit
396 stressed plants appeared to be associated with scavenger of free radicals as a mechanism
397 to withstand the stress period. ST 5288 and PHY 499 seemed to have improved tolerance to
398 water-deficit conditions than DP 0912. The knowledge of more tolerant cultivars to drought
399 stress assist producers to select the most appropriate cultivar for their location. However,
400 further research is needed on physiological traits related to drought at the reproductive
401 development of cotton to evidence tolerance of these cultivars.

402

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