

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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7

8 **ABSTRACT**

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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.

10

11 *Keywords: Gossypium hirsutum; photosystem II; electron transport rate; enzymes;*

12 *photosynthetic pigments; water-deficit stress.*

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15 **1. INTRODUCTION**

16 Upland cotton (*Gossypium hirsutum* L.) yield is compromised by water-deficit stress in

17 multiple ways. As water becomes limiting, stomatal conductance decreases, resulting in a

18 decrease in photosynthesis. The decreased photosynthetic rate in turn decreases the

19 production and metabolism of carbohydrates, leading to a reduction in plant growth and fruit

20 abscission [1]. The effects of water-deficit stress in crops vary with the severity and duration

21 of the stress, plant growth stage, and genotype, as well as the interaction between these
22 factors [2]. Cotton yields in the U.S. have shown great variability across the recent years [3],
23 and this year-to-year variability might be attributed to differences in plant genetics and
24 physiological responses to environmental stresses throughout the season [4].

25 Upland cotton uses an array of mechanisms to alleviate and survive water-deficit stress,
26 such as increased activity of antioxidants, heat shock proteins, accumulation of osmolytes,
27 and osmotic adjustment; however, due to domestication and cultivation as an annual crop,
28 modern cotton cultivars differ in their ability and level of tolerance to a water-deficit period
29 [5,6].

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

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

43 Additionally, the quantity and activity of photosynthetic pigments are affected by low water
44 availability. The pigments commonly found in plants are chlorophyll *a*, chlorophyll *b* and
45 carotenoids. Chlorophyll *a* is the main pigment absorbing energy for further conversion into
46 adenosine triphosphate (ATP) and nicotinamide adenine dinucleotide phosphate (NADPH)
47 during the light reactions of photosynthesis, and also responsible for the green color in
48 plants [13]. Chlorophyll *b* helps to increase the absorption band of light to be used in
49 photosynthesis. Under high light intensity, plants can absorb more light energy than actually
50 used in photosynthesis process. The overexcitation of chlorophyll may result in increased
51 formation of reactive oxygen species (ROS) such as chlorophyll triplet and singlet oxygen.
52 Damage caused by singlet oxygen and its reactive products reduce the efficiency of
53 photosynthesis through photoinhibition. Carotenoids are capable to receive the triplet
54 excitation energy of chlorophyll and thus help to prevent the formation of ROS [14]. Studies
55 have shown degradation in photosynthetic pigments concentration in several crops grown
56 under drought conditions, such as cotton, potato (*Solanum tuberosum* L.), wheat (*Triticum*
57 *aestivum* L.), chickpea (*Cicer arietinum* L.), and sunflower (*Helianthus annuus* L.) [15-19].

58 Overproduction of reactive oxygen species (ROS) also damage plant cells irreversibly by
59 degradation of lipids, proteins and nucleic acids [20]. Antioxidant enzymes, such as
60 superoxide dismutase (SOD), catalase (CAT), and ascorbate peroxidase (APX) increase
61 their activity to control the balance between production and scavenging ROS. Reactive
62 oxygen species are eliminated by SOD through a reaction that produces hydrogen peroxide
63 (H₂O₂). Then, the H₂O₂ is converted into oxygen and water by CAT or into water by APX.
64 Research has been reported on antioxidant activity of plants under water-deficit stress, such
65 as maize (*Zea mays* L.) [21], canola (*Brassica napus* L.) [22], quinoa (*Chenopodium quinoa*
66 L.) [23], and potato [16]. Studies on antioxidant responses of cotton plants under drought
67 conditions are still controversial and not well elucidated. Ratnayaka et al. [24] indicated
68 increase in APX activity and no alteration in the activity of SOD or CAT of cotton plants

69 under drought stress. However, studies reported by Deeba et al. [25] suggested higher CAT
70 activity in plants under severe stress and no changes in CAT in plants under mild stress,
71 while SOD was higher only in plants under mild stress and similar activity of SOD in plants
72 under severe stress and control.

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

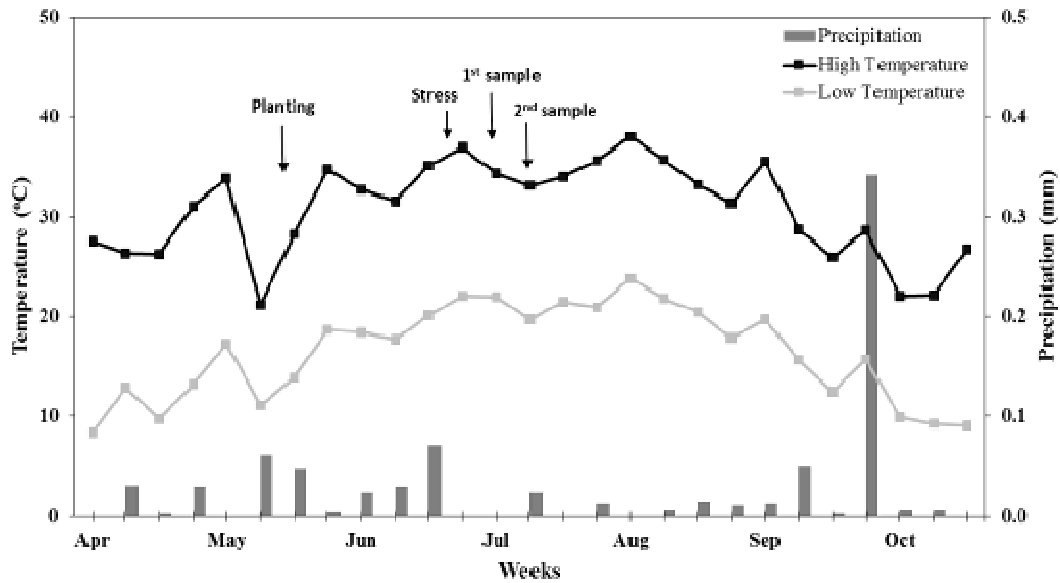
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88 2. MATERIALS AND METHODS

89 2.1 Study Sites, Plant Material, and Sampling Protocol

90 Field experiments were conducted at the Quaker Avenue Research Farm of Texas Tech
91 University in Lubbock, TX (N 33°59'93", W 101°90'72 ") and at the Lon Mann Cotton
92 Research Station of University of Arkansas in Marianna, AR (N 34°43'50", W 90°45'34") in
93 2012 and 2013, respectively. Seeds of one commercial cotton cultivar, ST 5288B2F
94 (Stoneville, Bayer CropScience, Lubbock TX) were sown on May 23, 2012 (Lubbock, TX)
95 and three, DP 0912 B2RF (Delta and Pine Land, Monsanto Company, St. Louis MO), PHY
96 499 WRF (PhytoGen, Dow AgroSciences, Indianapolis IN), and ST 5288B2F (Stoneville,
97 Bayer CropScience, Lubbock TX) on May 8, 2013 (Marianna, AR) at a 0.96 m inter-row
98 spacing and at a rate of 11 seeds m⁻¹ row. The soil series in Lubbock is an Amarillo-Acuff
99 sandy clay loam (Fine-loamy, mixed, superactive, thermic Aridic Paleustalfs), and the soil
100 series in Marianna is a Memphis silt loam (fine-silty, mixed, active, thermic Typic
101 Hapludalfs). Fertilization was performed according to soil tests prior to planting and
102 recommended rates for cotton at both sites. Herbicide and pesticide applications were also
103 applied according to Texas A&M AgriLife extension recommendations at Lubbock and
104 University of Arkansas Cooperative Extension Service recommendations at Marianna.
105 Mepiquat chloride was applied as needed to control vegetative growth and all plots within a
106 location received identical applications. Irrigation was performed as necessary using a
107 subsurface drip system at Lubbock according to an on-site GRW 100 weather station
108 (Campbell Sci.) and furrow system at Marianna according to University of Arkansas
109 Cooperative Extension Service recommendations until the appearance of floral buds
110 (squaring stage). When plants reached the pinhead square stage, on June 25 at Lubbock
111 and June 11 at Marianna, water was withheld from the water-deficit stress treatment for
112 fourteen days at both sites. To further characterize site conditions, the weekly average
113 maximum and minimum temperatures, and weekly average precipitation of each location
114 throughout the season along with the sample dates are presented in Figures 1 and 2.

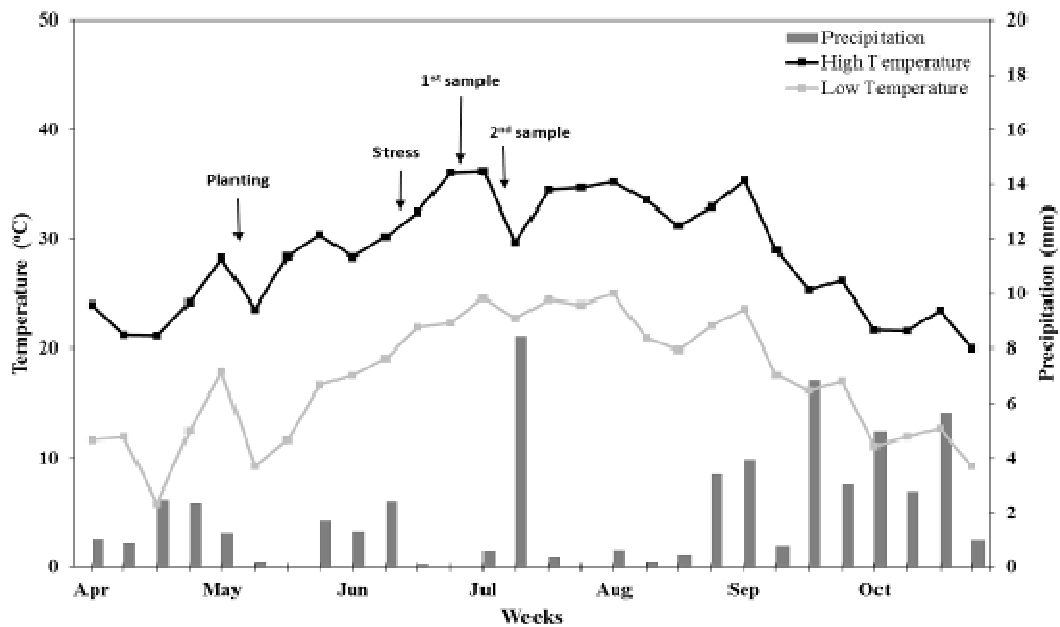
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117 **Fig. 1. The average weekly precipitation (mm), and average weekly high and low**
 118 **temperatures (°C) for Lubbock, TX from April to October, 2012. Arrows indicate**
 119 **planting date (on May 23), onset of the water-deficit stress (on June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

121



122

123 **Fig. 2. The average weekly precipitation (mm), and average weekly high and low**
 124 **temperatures (°C) for Marianna, AR from April to October, 2013. Arrows indicate**

125 **planting date (on May 8), onset of the water-deficit stress (on June 11), and first and**
126 **second sample dates (on June 18 and June 25, respectively)**

127

128 For the Lubbock site, field measurements of stomatal conductance were performed seven
129 and fourteen days after the onset of the stress and samples for laboratory determinations of
130 activities of antioxidant enzymes (SOD, CAT, and APX) were collected fourteen days after
131 the onset of the stress. For Marianna, field measurements of stomatal conductance and
132 chlorophyll *a* fluorescence, and samples for laboratory determinations of concentrations of
133 pigments (chlorophyll *a*, chlorophyll *b*, and carotenoids) were taken seven and fourteen days
134 after the onset of the stress. Both field and laboratory measurements were performed on the
135 fully-expanded main-stem leaves from the fourth node below the apical meristem from the
136 two middle rows of each plot at Lubbock and Marianna.

137

138 **2.2 Measurements**

139 **2.2.1 Stomatal Conductance**

140 Stomatal conductance ($\text{mmol H}_2\text{O m}^{-2}\text{s}^{-1}$) was measured between 1100 and 1400 h
141 in five leaves per plot and two readings per leaf due to the small surface area of the cuvette
142 (6.35 mm^2) on each sample date using a steady-state leaf porometer (SC-
143 1 Leaf Porometer, Decagon, Pullman, WA) on the abaxial surface of fully expanded leaves.

144

145 **2.2.2 Chlorophyll *a* Fluorescence**

146 Actual quantum yield of electron transport through photosystem II (Φ_{PSII}) was
147 measured *in situ* in five leaves per plot under natural field irradiance between 1200 and 1400
148 h using a portable fluorometer Model OS1-FL (Opti-Sciences, Hudson, NH). Steady-state
149 fluorescence prior to a saturation pulse was measured to obtain F_t , followed by induction of
150 maximum fluorescence on the adaxial surface of the leaves with a saturating white light
151 pulse for 0.95 s for estimation of F'_m when all reaction centers are closed due to infinite light
152 intensity. Φ_{PSII} was obtained according to the equation $\Phi_{\text{PSII}} = (F'_m - F_t) / F'_m$ [27]. Electron
153 transport rate (ETR) through photosystem II was obtained by calculation according to the
154 equation $\text{ETR} = \Phi_{\text{PSII}} \times \text{PAR} \times 0.5 \times 0.84$, where PAR is the absorbed light (in $\mu\text{mol photon}$
155 $\text{m}^{-2}\text{s}^{-1}$) at the leaf surface, 0.5 is a factor on the partitioning of energy between PSII and PSI
156 and 0.84 is a common leaf absorbance coefficient for C_3 plants.

157

158 **2.2.3 Pigments Concentration**

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

165 **2.2.4 Activities of Enzymes**

166 Leaves were collected from each plot and immediately frozen for further laboratory
167 determination of activities of enzymes. Superoxide dismutase (SOD) activity was obtained
168 by identification of the enzyme's ability to inhibit photochemical reduction of
169 nitrobluetetrazolium with one unit of SOD, indicating the amount of enzyme required to
170 inhibit 50% of the rate of nitrobluetetrazolium reduction measured at 560 nm according to
171 Giannopolitis and Ries [29]. Catalase (CAT) activity was determined by the variation in H₂O₂
172 absorption in an interval of 60 seconds using a spectrophotometer at 240 nm, according to
173 Peixoto et al. [30]. A molar extinction coefficient of 39.4 mM⁻¹ cm⁻¹ was used for CAT activity
174 determination. Ascorbate peroxidase (APX) activity was determined by measuring the
175 negative variation of H₂O₂ absorption in a spectrophotometer at 290 nm, according to
176 Koshiba [31]. A molar extinction coefficient of 2.8 mM⁻¹ cm⁻¹ was used for the calculations.
177 The protein concentration (μg μL⁻¹) was considered in the calculations for the specific activity
178 of the three enzymes.

179

180 **2.3 Statistical Analysis**

181 The experiments were arranged in a strip plot design with water treatments running across
182 all blocks in strips at Lubbock and a strip split plot design with water treatments as the main
183 unit running across all blocks in strips in a randomized complete block design and the
184 cultivars were randomly assigned in the sub unit for each whole plot in each block at
185 Marianna, with five replications for the two sites. Data were subjected to analysis of variance
186 using JMP Pro 11 (SAS Institute, Cary, NC). The treatments water regime, cultivar, and
187 sample date were treated as fixed effects. The blocks and the block x treatments interaction
188 were treated as random effects. Tukey's HSD test ($P = .05$) was used to separate treatment
189 combination mean performance. Differences between main factors for a variable were
190 indicated in tables and, when interaction between the factors was observed, a graph was
191 plotted. Sigma Plot 12.5 (Systat Software Inc., San Jose, CA) was used to plot the graphs.

192

193 **3. RESULTS AND DISCUSSION**

194 **3.1 Stomatal Conductance**

195 Significant effect of cultivar or interaction between the factors (cultivar, water regime, and
196 sample date) was not observed for leaf stomatal conductance of cotton plants from Marianna
197 site (Table 1). However, stomatal conductance was significantly affected by water regime (P
198 = .01) and sample date ($P = .01$) separately. When stomatal conductance results were
199 combined from all cultivars and sample dates, the rates were significantly lower for the
200 water-deficit stressed plants than the well-watered control (Table 1). For the effect of sample
201 date, leaves collected on July 25 had significantly higher stomatal conductance rates than
202 the leaves collected on July 18 (Table 1).

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

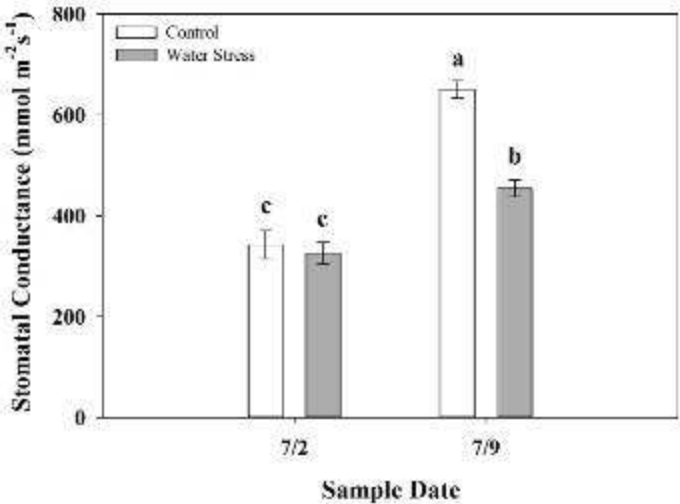
Source of variation	g_s mmol m ⁻² s ⁻¹	Φ_{PSII}	ETR μmol electrons m ⁻² s ⁻¹	Chl a	Chl b	Carotenoids
					μg cm ⁻²	
Cultivar						
DP 0912	667	0.401 a	237	8.43 b	2.11 b	2.63 b
PHY 499	697	0.360 b	216	8.96 a	2.35 a	2.67 ab
ST 5288	645	0.381 ab	230	8.97 a	2.38 a	2.74 a
Water Regime						
Control	848 a [†]	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

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

[§] 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).

215



216

217 **Fig. 3. Effect of the interaction of sample date (July 2, one week after the onset of the**
218 **water-deficit stress, and July 9, two weeks after the onset of the water-deficit stress)**
219 **and water regime (well-watered control and water-deficit stress during the early floral**
220 **bud development) on stomatal conductance (mmol m⁻²s⁻¹) in cotton leaves cv. ST**
221 **5288B2F at Lubbock, TX**

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

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

225

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

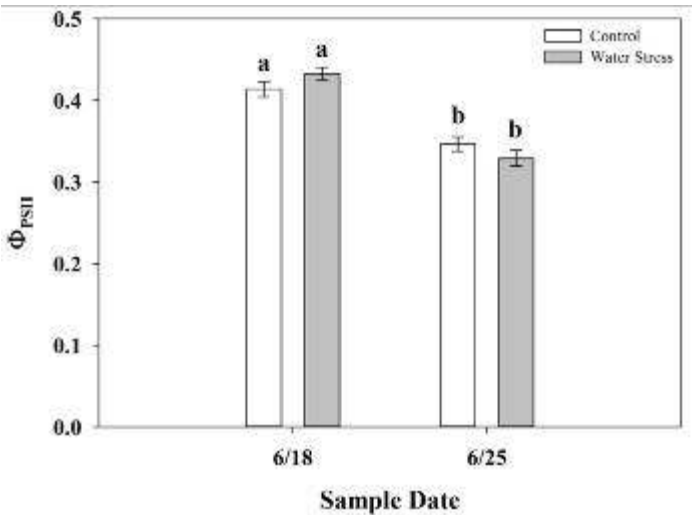
231 Leaf age also has an effect on stomatal conductance rates. Young leaves usually have
232 higher concentrations of abscisic acid (ABA) in guard cells inducing stomatal closure and
233 therefore increasing resistance to transpiration [36]. With the maturity of the leaves, a
234 decline in ABA concentration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

237

238 **3.2 Photosynthetic Efficiency**

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

251



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

257 *All values are means $\pm$ standard error ($n = 5$)*
 258 *Columns not sharing a common letter are significantly different according to Tukey's*
 259 *HDS test at $P = .05$*
 260

261

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

272 physiological responses of cotton plants under drought conditions during the early floral bud
273 development have not been reported. Additionally, studies on soybean, wheat and sorghum
274 also showed reduction in the photosynthetic efficiency of plants grown under drought stress
275 [38-40].

276

277 **3.3 Concentrations of Pigments**

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

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

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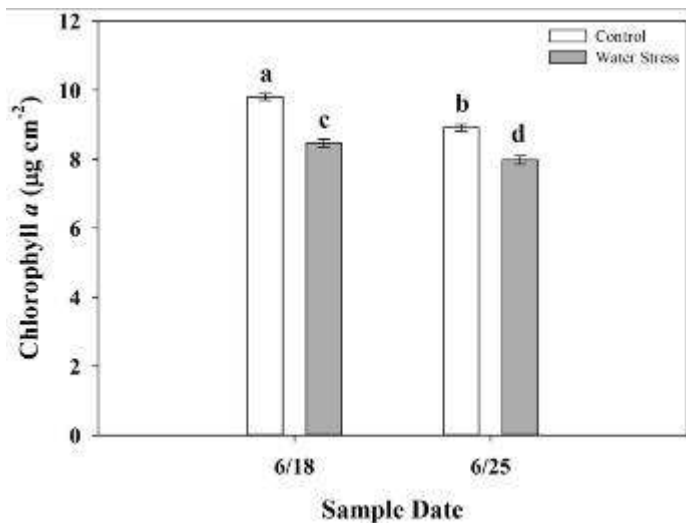


Fig. 5. Effect of the interaction of sample date (June 18, one week after the onset of the water-deficit stress, and June 25, two weeks after the onset of the water-deficit stress) and water regime (well-watered control and water-deficit stress during the early floral bud development) on chlorophyll 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.

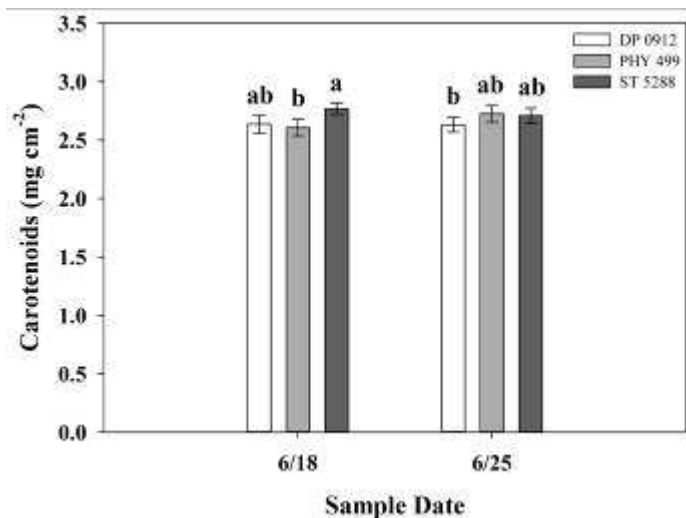


Fig. 6. Effect of the interaction of sample date (June 18, one week after the onset of the water-deficit stress, and June 25, two weeks after the onset of the water-deficit stress) and cultivar (DP 0912, PHY 499, and ST 5288) on carotenoids concentration ($\mu\text{g cm}^{-2}$) in cotton leaves at Marianna,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

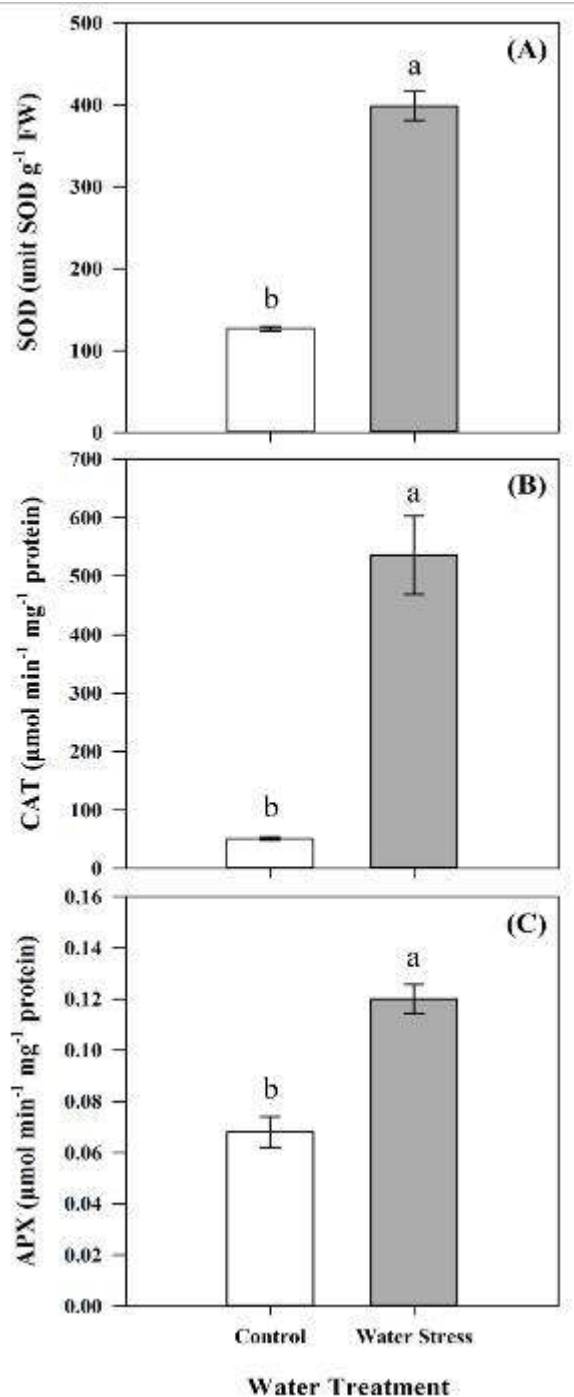


Fig. 7. Effect of water regime (well-watered control and water-deficit stress during the early floral bud development) on activities of the enzymes superoxide dismutase (SOD, unit SOD g⁻¹ FW) (A), catalase (CAT, μmol min⁻¹ mg⁻¹ protein) (B), and ascorbate peroxidase (APX, μmol min⁻¹ mg⁻¹ protein) (C) 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 HSD test at P = .05

371 4. CONCLUSION

372 Leaf stomatal conductance and pigments concentration of all cotton cultivars were sensitive
373 to water-deficit stress at the squaring development; however, photosynthetic efficiency was
374 not responsive to the stress, maintaining similar rates on stressed and non-stressed plants.
375 Actual quantum activity of PSII seemed to be insensitive to water-deficit stress, preventing
376 impairment of leaf photosynthesis. Increased antioxidant enzymes activity of water-deficit
377 stressed plants appeared to be associated with scavenger of free radicals as a mechanism
378 to withstand the stress period. ST 5288 and PHY 499 seemed to have improved tolerance to
379 water-deficit conditions than DP 0912. The knowledge of cultivars with enhanced drought
380 stress tolerance helps producers to select the most appropriate cultivar for their location.
381 However, further research is needed on physiological traits related to drought at the
382 reproductive development of cotton to evidence tolerance of these cultivars.

383

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