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ADAPTATION OF COTTON (*Gossypium hirsutum* L.) TO LIMITED WATER CONDITIONS: REVERSIBLE CHANGE IN CANOPY TEMPERATURE

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Abstract

Improving cotton yield under limited water supply needs to deeper understanding of the plant's response and adapting strategies to improve their tolerance. Effects of limited water conditions on ten cotton genotypes (Gossypium hirsutum L.) were examined in a field experiment to evaluate their tolerance level and explore time depending changes in canopy temperature and leaf greenness as indirect determinations of leaf water-status and chlorophyll density. Plant height shortened (15%), dry matter accumulation inhibited (36%), ball number (35%) and eventually lint yield (35%) decreased of all cotton genotypes since irrigation amount decreased 32% under limited watering conditions (LWC). Significant genotypic variation in tolerance level and yielding capacity under LWC were found among genotypes. Leaf tissues accumulated higher proline (stress-related amino acid) to adapt lower water potential conditions while canopy temperature depression (CTD) reversibly decreased and SPAD values were increased. A strong correlation between relative changes in CTD and SPAD values and a significant variation in ability of the cotton genotypes to recover CTD under limited water conditions were found. Our results also suggested that the higher ability to recover CTD of cotton leaves was associated with lower total dry weight reduction and water stress susceptibility under limited water conditions.

Key words*: Cotton, water, canopy temperature depression, SPAD, drought, proline.*

INTRODUCTION

Cotton (*Gossypium hirsutum* L.) is one of the most important cash crops in Turkey since it provides fibre to textile, edible oil to food and animal feed to livestock industries. It is mostly grown South and South-West coastal and South-East part of Turkey under irrigated conditions. Supplementary irrigation is needed because the natural precipitation is not adequate during growing period of cotton in these locations (Turan and Göksoy, 1995). However, increase in frequency of drought events and restricted water resources are considered as main limiting factor for cotton production in Turkey (Tatar, 2016). Therefore, improving water management and increasing water use efficiency under limited watering conditions will inevitably be a major challenge for sustainable cotton production. However, most of the efforts to improve efficient use of water in cotton production system reduce available soil-moisture content which inhibits plant growth (Chastain et al., 2014; Turner et al., 1986), lint yield and productivity (Wells and Stewart, 2010; Pettigrew, 2004; Krieg and Sung, 1986). On the other hand, cotton genotypes differ in their adaptation ability to limited watering conditions (Sezener et al., 2015; Rahman et al., 2008; Penna et al., 1998). The adaptation ability is both considered as a lower reduction in photosynthetic rate thus total dry matter accumulation or lint yield and productivity under restricted irrigation (Megha and Mummigatti, 2017; Singh et al., 2015).

Stomatal limitation hence reduction in leaf internal CO_2 concentration and net photosynthesis are the primary responses of plants under water stress conditions (Chastain et al., 2014; Loka et al., 2011; Cornic and Fresneau, 2002). Lower transpiration mediated by stomatal closer to conserve limited water content in plant tissues dysfunctions so-called cooling system of the leaves (Wiegand and Namken, 1966) and increases leaf temperature (Wanjura et al., 2004; Wiegand and Namken, 1966). Canopy temperature depression (CTD) which is expressed as differences between ambient air and canopy temperature (Ray and Ahmed, 2015; Smith et al., 1986) has been widely implemented to estimate plant water status (Blum et al., 1982; Idso 1982; Jackson et al., 1981; Ehrler, 1972). Many studies previously suggested that there is a strong linear relationship between CTD and soil moisture content, transpiration rate and stomatal conductance (Nagler et al., 2003; Inoue and Moran, 1997; Inoue et al., 1994; Moran et al., 1994; Inoue et al., 1990). Karimizadeh and Mohammadi (2011) also reported that maintaining ability of CTD in wheat genotypes under limited watering conditions was significantly associated with tolerance level of the plants and they suggested CTD measurement as a useful indicator for screening stress tolerance.

Considering genotypic variation on stomatal regulation and evaporative cooling capacity of different species under water limited environments, still limited information exists about time depending changes in leaf level regulation of CTD in cotton plants.

Metabolic malfunctioning, disorder of carbohydrate metabolism (Loka and Oosterhuis, 2012) as well as damages in photosynthetic tissues (Shahenshah and Isoda et al., 2010; Bilger and Björkman, 1990; Björkman and Demming-Adams, 1994; Inamullah and Isoda, 2005) are the secondary water stress induced response of plants. Cotton plants as most of the other plant species accumulate proline during secondary phase of the stress (Verbruggen and Herman, 2008) due to its crucial role in osmoregulation, structural protection (Yi et al., 2016) and ROS scavenging (Smirnoff and Cumbes, 1989). Changes in green color of leaves are the most apparent and visual symptom of differentiation in chlorophyll content and density under stress conditions (Conaty et al., 2008; Thongbai et al., 2001; Boquet et al., 1999). Chlorophyll pigments mostly degrade and generally leaves become light-green under limited water conditions (Tatar et al., 2016; Hejnak et al., 2015; Sarani et al., 2014) though a number of the studies report increasing chlorophyll content (Martinez and Guiamet, 2004; Ahmad et al., 2013) which imply reducing leaf area and increasing pigment density. Most of the water stress related studies determine chlorophyll concentration of stress-induced leaves at the end of treatments (Pandey et al., 2003; Patil et al., 2011) and misses the changes during adaptation period. Therefore, non-destructive determination of chlorophyll concentration such as SPAD measurement gives more detail about leaf level adaptation to water limited conditions.

The aim of the study was to (i) determine tolerance level and yield potential of ten cotton genotypes under limited water conditions, (ii) asses leaf level adaptation of cotton under restricted irrigation considering CTD and chlorophyll concentration (iii) and evaluate correlations between the adapting ability of the leaves and the tolerance levels of the genotypes.

MATERIALS AND METHODS

This study was conducted in 2014/15 at the experimental site of Ege University, Faculty of Agriculture, Department of Field Crops, Izmir, Turkey (38°27’6”, 27°13’32”E). The soil type of experimental field was clay loam, mild alkaline and moderate calcic. Air temperature (°C) and relative humidity (%) were recorded by a gauge (Tinytag Plus 2®) every 10 minutes and rain amounts (mm) were measured by pluviometer during the growing period of cotton (Figure 1).

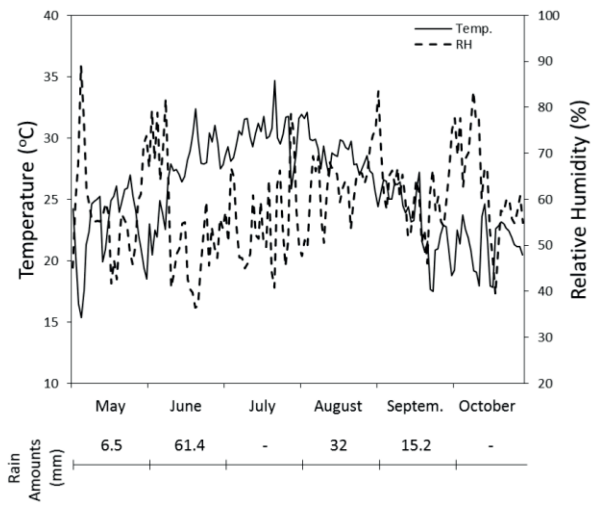

Figure 1. Average temperature (°C), relative humidity (%) and rain amounts (mm) recorded during growing period of cotton in the experimental site

The experiment was comprised of two different irrigations and ten cotton genotypes with three replications in the Randomized Block Design. The plot dimensions were 2.8 x 3 m and the experiment was consisted of 60 plots. Drip irrigation system was settled to the all plots and irrigation amounts were recorded by water flow meter. Row space and sowing distance in lines were 0.7 m and 0.20-0.25 m, respectively. Initially, 100 kg/ha of basal nitrogen as composite fertilizer (15-15-15) and 100 kg/ha as ammonium nitrate (33%) at the beginning of flowering stage were applied. The cotton plants were maintained for dispelling the negative effect of weeds and insects. Commercial insecticide containing malathion were sprayed to the plants to avoid *Aphis* spp. and *Empoasca* spp.

Totally 10 genotypes of *Gossypium hirsutum* L. (ST 468, ARCOT 008, ST 373, ST 498, GAIA, MAY P 06, ARCOT 009, FLASH, GLORIA and PG 2018) which is well adapted to coastal part of Turkey were used in the experiment. Two watering treatments were applied after homogenous plant stands were obtained (4-5 leaves stage). Totally 454 mm of water were received by irrigation in well watering (WW) plots whereas 310 mm in limited watering (LW) treatment.

Youngest fully developed leaves were collected 30 days after treatments for proline analysis. According to Bates et al. (1973), 50 mg of oven dried leaf samples were extracted with 10 ml sulfosalicylic acid solution in mortar and then filtered. Then, 2 ml ninhydrin solution and 2 ml glacial acetic acid were added to extracted sample and boiled at 100°C in water-bath. Then the reaction of the mixture was stopped by ice-bath. When the mixture was reached to room temperature, 3 ml toluene was added to the mixture. Upper layer of the solution was used to determine absorbance values by using spectrophotometer (Carry 50®).

Canopy temperature (CT) was monitored using with infrared thermometer after onset of the treatments. Measurements were held 7 times and 5 CT data were obtained from each plot in every measurement. Then canopy temperature depression (CTD) was calculated according to Ayeneh et al. (2002) by using following equation:

$$CTD = T_A - T_C$$

where:

CTD - canopy temperature depression; T_A - instant air temperature (°C); T_C canopy temperature (°C).

SPAD value were determined using with (Konica Minolta – SPAD-502 Plus) parallel to CTD measurements (7 times after onset of the treatments) The measurements were held in youngest fully developed leaves of randomly selected 5 plants in each plots.

After removing border rows of all plots, plant height, total dry weight and boll number of randomly sampled 10 plants from each plot were determined before harvest. Then all plots were harvested by hand-picking two times during October. The harvested products were separated into lint and seed by rollergin type machine. Cotton lint and seeds were weighed separately and lint percentages were calculated.

Data were subjected to analysis of variance for each parameter. All data were analyzed by using standard ANOVA techniques of Statistica software. The mean values of each parameter were compared according to LSD test described by Steel and Torrie (1980).

RESULTS AND DISCUSSIONS

Plant height, total dry weight, boll number, lint yield of selected 10 cotton genotypes significantly decreased and proline content increased under limited water conditions whereas lint percentage did not remarkably change (Table 1). The highest decrease in total dry weight were found in FLASH (50.0%) while lowest decreases in ARCOT 008 (17.2%) and PG 2018 (21.3%). Reduction in lint yield was also lower in PG 2018 (15.6%) and GAIA (15.4%) under limited watering relative to well-watering conditions. Similar to total dry weight reduction, FLASH had the most drastic decrease in lint yield (48.9%). Limiting water led 1.54 fold increase in proline accumulation of the leaves in average (Table 1). However, proline content increased in all cotton genotypes though no significant genotypic variation was found.

Average of SPAD values for all growing period markedly increased (14.2%) under limited water conditions (Figure 2). Relative increase in SPAD value was more remarkable in MAY P 06 (18.6%) while ARCOT 008 had lowest increase (11.1%).

Canopy temperatures in average were 5.9°C lower than ambient air (canopy temperature depression - CTD) in well-watered (WW) conditions whereas cooling capacity of the leaves under limited water (LW) conditions was 4.3°C (Figure 2). Higher relative increase in CTD under LW conditions was recorded in ST373 and lower in ACROT008.

The quadratic functions of relative change in CTD and SPAD values for each cotton genotypes during growth period were figured and demonstrated as discriminant of polynomial (Δ) which indicates recovery of plants (Figure 3). The highest Δ (8.62 [r = 0.98]) was found in PG 2018 while MAY P 06 had the lowest Δ value (2.74 [r = 0.87]) in relative change of CTD functions. On the other hand, considering relative change in SPAD values under LW conditions MAY P 06 had higher Δ (1.24 [r = 0.92]) whereas GAIA had lower Δ (0.40 [r = 0.81]).

Table 1. Plant height (cm), total dry weight (kg/m^2), boll number (number/plant), lint yield (kg/ha), lint percentage (%) and proline content (mg/g) of 10 cotton genotypes grown under well-watered (WW) and limited watering (LW) conditions

Cultivars	Plant Height (cm)			Total Dry Weight (kg/m^2)			Boll Number (number/plant)			Lint Yield (kg/ha)			Lint Percentage (%)			Proline (mg/g)		
	WW	LW	AVG	WW	LW	AVG	WW	LW	AVG	WW	LW	AVG	WW	LW	AVG	WW	LW	AVG
ST 468	78.5	66.5	**72.5**	0.97	0.55	**0.76**	22.3	15.2	**18.7**	1463	885	**1174**	43.1	44.2	**43.7**	1.45	2.33	**1.89**
ARCOT 008	77.2	73.2	**75.2**	0.87	0.72	**0.79**	22.0	14.4	**18.2**	1164	840	**1002**	40.1	40.8	**40.4**	1.15	1.77	**1.46**
ST 373	90.2	73.2	**81.7**	1.06	0.66	**0.86**	19.3	13.8	**16.6**	1606	1048	**1327**	40.7	41.7	**41.2**	1.02	2.34	**1.68**
ST 498	85.9	72.6	**79.3**	1.11	0.64	**0.87**	16.3	13.6	**15.0**	1659	955	**1307**	42.5	44.0	**43.2**	1.85	2.11	**1.98**
GAIA	82.3	68.9	**75.6**	0.95	0.69	**0.82**	22.1	12.2	**17.2**	1417	1202	**1310**	41.9	43.3	**42.6**	1.41	2.42	**1.91**
MAY P 06	86.6	68.9	**77.7**	1.05	0.67	**0.86**	26.1	15.0	**20.6**	1684	1110	**1397**	42.9	43.7	**43.3**	1.14	2.73	**1.94**
ARCOT 009	80.1	63.7	**73.5**	0.89	0.54	**0.75**	17.8	12.5	**15.7**	1247	680	**964**	44.0	41.2	**42.9**	1.69	1.92	**1.78**
FLASH	84.2	68.7	**76.5**	1.12	0.56	**0.84**	20.1	12.4	**16.3**	1643	837	**1240**	41.3	43.0	**42.2**	1.30	1.96	**1.63**
GLORIA	86.7	75.9	**81.3**	1.12	0.71	**0.92**	22.8	13.1	**18.0**	1629	952	**1290**	42.9	42.9	**42.9**	1.32	2.02	**1.67**
PG 2018	86.1	82.1	**84.1**	0.94	0.74	**0.84**	20.3	11.6	**15.9**	1217	1032	**1124**	43.4	43.7	**43.6**	2.02	2.03	**2.02**
AVG	**83.8**	**71.3**	**77.6**	**1.01**	**0.65**	**0.83**	**20.9**	**13.5**	**17.2**	**1473**	**954**	**1213**	**42.3**	**42.8**	**42.6**	**1.41**	**2.17**	**1.79**

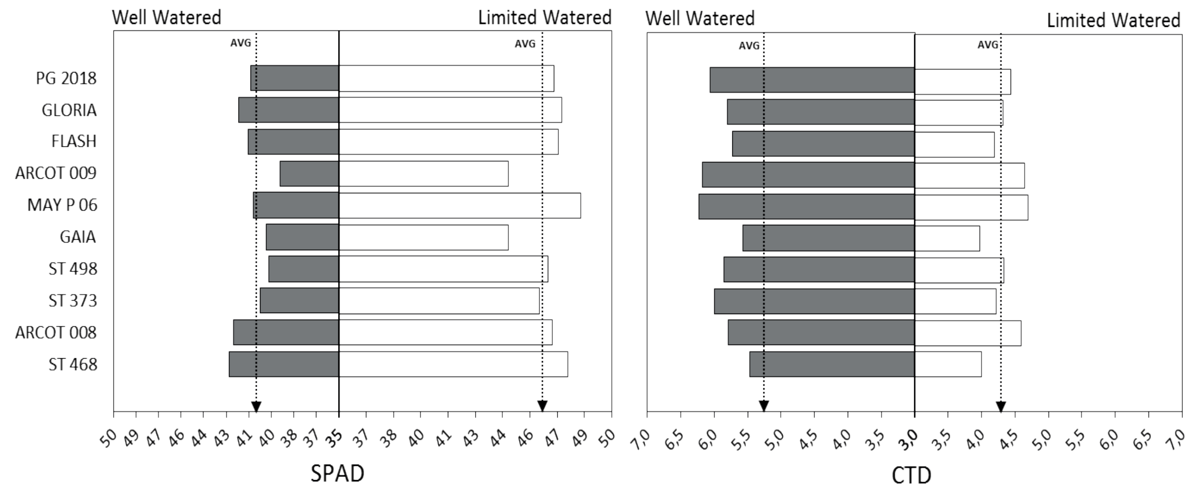

Figure 2. Average values of canopy temperature depression (CTD) and SPAD (totally 7 measurements during adaptation) of ten cotton genotypes under well-watered and limited-watered conditions. Black pointed lines demonstrate average (AVG) values of SPAD and CTD

Correlation between the relative increase in CTD and SPAD values of ten cotton genotypes was demonstrated in Figure 4. The figure indicated that higher relative increase in CTD value was significantly associated with relative increase in SPAD value (r = 0.67).

Relations between recovering ability (Δ) of CTD with relative reduction in Total Dry Weight (TDW) under LW conditions and Stress Susceptibility Index (SSI) are introduced in Figure 5.

Correlation analysis revealed that higher ability of the cotton leaves to recover CTD significantly (r = 0.78) associated with lower total dry weight reduction under limited water conditions. Moreover, stress susceptibility index of the cotton genotypes decreased due to increasing ability of plants to CTD recovery.

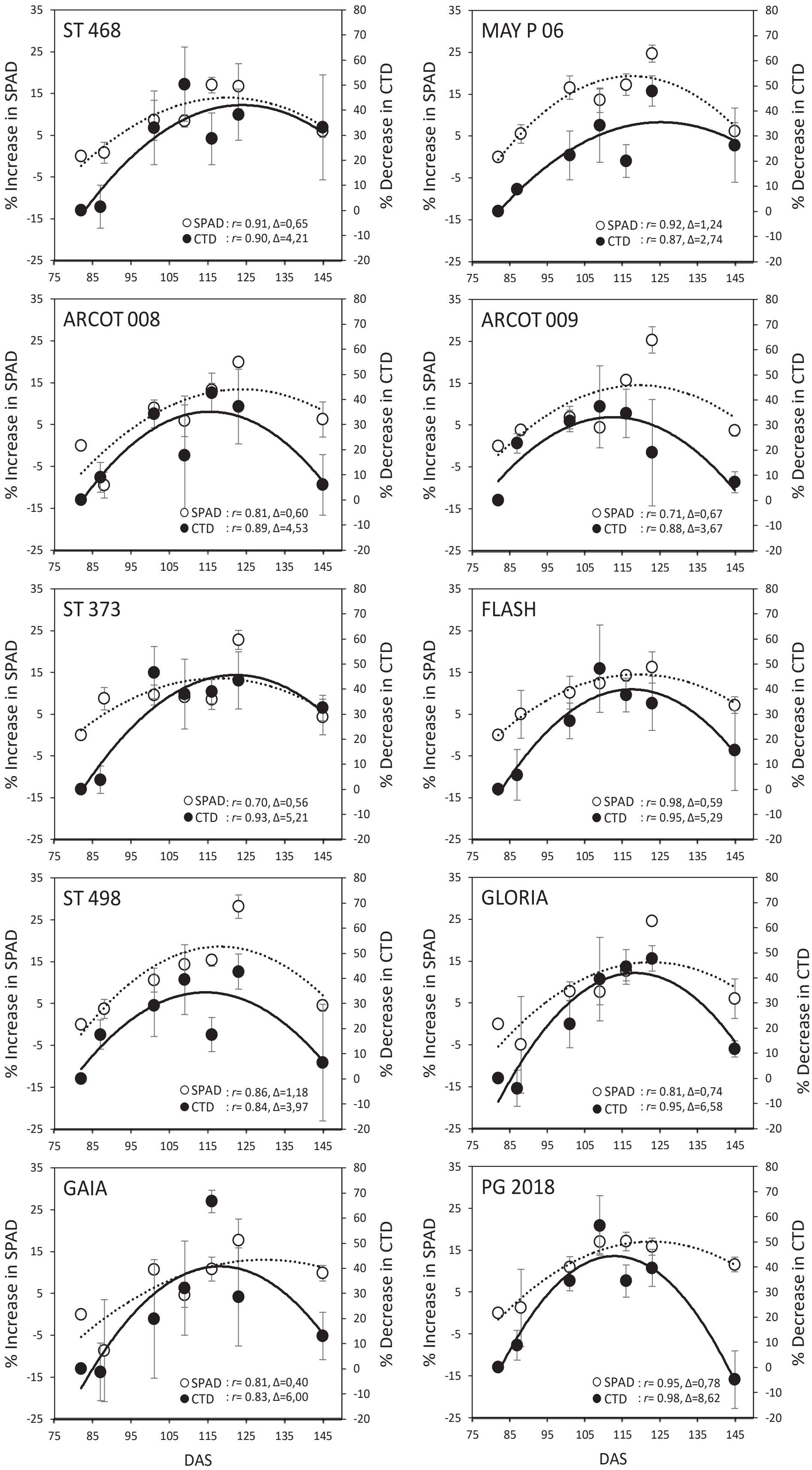

Figure 3. Changes in SPAD and canopy temperature depression (CTD) values under limited-watered condition relative to well-watered conditions of ten cotton genotypes during adaptation period. Delta (Δ) indicates discriminant of quadratic polynomial functions of SPAD and CTD values referring recovering ability of the cotton genotypes (DAS: day after sowing)

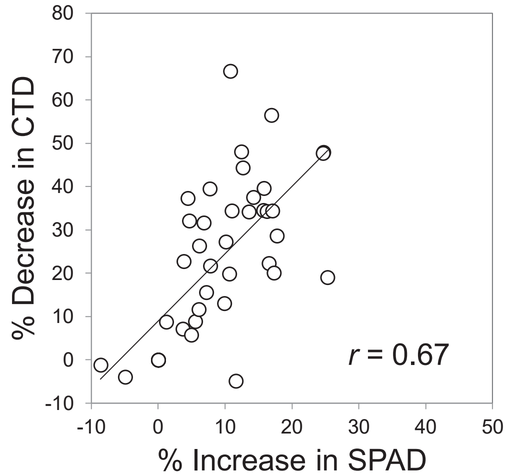

Figure 4. Correlation between relative changes in SPAD and canopy temperature depression (CTD) values under limited-watered condition relative to well-watered conditions of ten cotton genotypes during adaptation to limited watering conditions

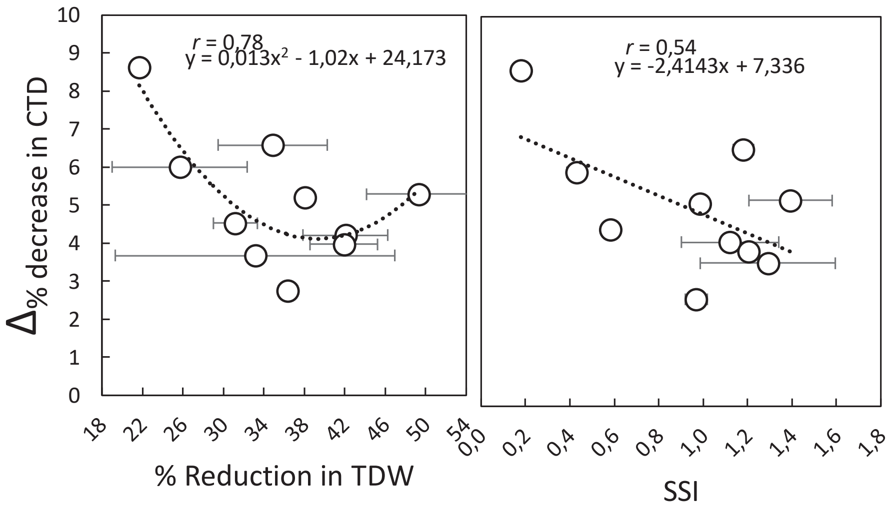

Figure 5. Correlation between relative changes in SPAD and canopy temperature depression (CTD) values under limited-watered condition relative to well-watered conditions of ten cotton genotypes during adaptation to limited watering conditions

Cotton is mostly grown under irrigated conditions in Turkey since the rainfall amount is not sufficient during growing period. According to the future predictions for the cotton growing areas, water resources will be drastically restricted due to climate change within the present century (Tatar, 2016). Therefore, increasing water use efficiency and adaptation to limited watering conditions of cotton is expected to be primary issue for sustainable cotton production. However, limited water conditions often inhibit cotton growth and productivity (Chastain et al., 2014; Pettigrew, 2004; Krieg and Sung, 1986). Our results also indicated that plant height shortened, dry matter accumulation inhibited, ball number and eventually lint yield decreased since irrigation amount decreased 32%. However, genotypic variation was found in productivity of selected cotton genotypes responses to limited water conditions. GAIA could be defined as high-yielding cotton genotype (1.202 kg/ha) under limited-watering conditions comparison to other selected genotypes. Beside the yield performance, PG 2018 was the most tolerant and FLASH was the most sensitive cotton genotypes considering the relative changes in dry matter production and lint yield under limited watering conditions. Ullah et al. (2017) stated that the emphasis should not be only on stress tolerant cotton varieties but also on stability of yield. In order to distinguish this fundamental fact, GAIA might be suggested for limited watering

production systems while PG 2018 and FLASH could be referred as contrasting genotypes (tolerant and sensitive respectively) in terms of their responses to limited watering conditions.
SPAD measurement is widely used to estimate chlorophyll content of the leaves (Brito et al., 2011; Istipliler et al., 2016; Tatar et al., 2016). Although SPAD units commonly decreased at the end of the water stress treatments in many studies (Fanizza et al., 1991; Saravia et al., 2016), initial increases in SPAD have been also reported in maize (Martinez and Guiamet, 2004), artichoke (Paungbut et al., 2017) and wheat (Tatar et al., 2016). Increase in SPAD value as an initial response of cotton plants to water limited conditions then lasting decrease could be perceived as leaf level regulation to reduce transpiring area in the present study. We assumed that the reduction in leaf area led an increase in chlorophyll density thus SPAD value during earlier phase of limited watering conditions. Arunyanark et al. (2008) also reported an increase in photosynthetic pigment density in peanut plants under drought conditions. We suggested that the ability of the cotton genotypes reducing leaf area and hence the transpiring surface to conserve water under limited conditions depend largely on the elasticity of the leaf tissues.
The linear relationship between canopy temperature depression (CTD) with soil moisture content, transpiration rate and stomatal conductance have been previously reported in several studies (Nagler et al., 2003; Inoue and Moran, 1997; Inoue et al., 1994; Moran et al., 1994; Inoue et al., 1990). CTD has been also approved as an indicator of plant water status (Karimizadeh and Mohammadi, 2011; Blum et al., 1982; Idso 1982; Jackson et al., 1981; Ehrler, 1972) and used to determine cooling ability of leaves as screening drought responses of plants (Karimizadeh and Mohammadi, 2011; Pinter et al., 1990; Hatfield et al., 1987). The relative decrease then the increase in CTD values of the cotton genotypes under limited water conditions indicated that a reversible adaptation plays a role to maintain transpiration and cooling leaves in the present study. The strong correlation between relative changes in CTD and SPAD values of the cotton genotypes under limited water conditions revealed that leaves became smaller and warmer during initial phase of water limitation then they adapted until a certain level. On the other hand, we demonstrated that there was a significant variation in ability of the cotton genotypes to recover CTD. We may also suggest that the higher ability to recover CTD of cotton leaves is associated with lower total dry weight reduction and water stress susceptibility under limited water conditions.

CONCLUSIONS

Genotypic variation was found in productivity of selected cotton varieties responses to limited water conditions. GAIA could be defined as high-yielding cotton genotype under limited-watering conditions comparison to other selected genotypes. Beside the yield performance, PG2018 was the most tolerant and FLASH was the most sensitive cotton genotypes considering the relative changes in dry matter production and lint yield under limited watering conditions. A strong correlation between relative changes in CTD and SPAD values and a significant variation in ability of the cotton genotypes to recover CTD under limited water conditions were found. Our results also suggest that the higher ability to recover CTD of cotton leaves was associated with lower total dry weight reduction and water stress susceptibility under limited water conditions.

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