

The Effects of Water Stress on Cotton Leaf Area and Leaf Morphology

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ABSTRACT

The most important effect of water stress on plants is that it reduces leaf area and leads to changes in leaf morphology. Decreased leaf area results in reduces crop yield through the reduction in photosynthesis. This study investigates the effects of the decrease in leaf area on seed cotton yield, evapotranspiration (ET), water use efficiency (WUE), and leaf geometry in cotton plants under water stress in different growth periods. The cotton plant was divided into three different growth periods (vegetative period (VP), flowering and boll growth period (FB), and boll opening (BO) period), and irrigation water was applied at field capacity level during the periods of full irrigation (T), while non-irrigation was applied during the water stress periods (O). In the experiment, 6 different irrigation strategies were based on: OOO, TTT, OTO, TOO, OTT, and TOT. In each treatment, five leaves were taken from three plants in every replicate during three growth periods, and the leaf area and geometric lengths of each leaf were measured. Seed cotton yield, evapotranspiration, and WUE decreased significantly depending on the severity and duration of the water stress to which the cotton was exposed. Physiologically, cotton leaves under water stress in the first stage of growth tended to increase the leaf lobe numbers while reducing the leaf area. Therefore, there were more leaf lobes numbers measured in OOO than in other treatments. Irrigation in the vegetative growth period was more effective in increasing the leaf area than the other growth periods.

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Su Stresinin Pamuk Yaprak Alanına ve Morfolojisine Etkileri

ÖZET

Su stresinin bitkiler üzerindeki en önemli etkisi yaprak alanını azaltarak yaprak morfolojisinde değişime yol açmasıdır. Yaprak alanının azalması fotosentezdeki azalma yoluyla mahsul veriminin azalmasına neden olur. Bu çalışmada farklı gelişme dönemlerinde susuz bırakılan pamuk bitkisinde yaprak alanındaki azalmanın verim, evapotranspirasyon (ET), su kullanma oranına (WUE) ve yaprak geometrisine etkileri belirlenmeye çalışılmıştır. Pamuk bitkisi 3 farklı gelişme dönemine (vegetatif dönem, çiçeklenme ve koza oluşumu dönemi ve kozaların açılması dönemi) ayrıldı ve tam sulamanın yapıldığı dönemlerde tarla kapasitesi düzeyinde su uygulanırken (T), su stresli dönemlerde sulama suyu uygulanmamıştır (O). Denemede OOO, TTT, OTO, TOO, OTT, TOT konuları olmak üzere 6 farklı sulama stratejisi esas alındı. Her konuda 3 gelişme döneminde her tekerrürdeki 3 bitkiden 5'er yaprak alındı ve her yaprağın yaprak alanı ve geometrik uzunlukları ölçüldü. Pamuğun maruz kaldığı stresin şiddetine ve süresine bağlı olarak verim, evapotranspirasyon ve WUE önemli ölçüde azaldı. Fizyolojik olarak büyümenin ilk evresinde susuz bırakılan pamuk yaprakları alanlarını küçültürken kanat sayılarını artırma eğilimine girmiştir. Bu nedenle yaprak kanat sayısı OOO konusunda diğer konulardan daha fazla ölçüldü. Vegetatif gelişme dönemindeki sulamaların yaprak alanının artmasında gelişme dönemlerinden daha etkili olmuştur.

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INTRODUCTION

Drought stress causes morphological and physiological changes in plants. Varying depending on the duration and severity of the stress, it has effects in physiological (photosynthesis rate, stoma conductivity, leaf turgor loss, etc.), biochemical (accumulation of stress metabolite, increase in antioxidative enzymes, etc.), and molecular levels (synthesis of specific proteins, increased expression of ABA biosynthetic genes, etc.) in plant development. However, drought stress causes morphological changes in the plant by reducing plant height and leaf area (Bañona et al., 2004).

Leaf area has a fundamental role in controlling water use in plants, and it is significantly reduced under water stress. This decrease causes a decrease in the living leaf area where stomatal conductivity occurs (Babu et al., 1983; Correia et al., 2001; Meenakshi, 2005) and photosynthesis (Rucker et al., 1995). Since leaves are the most critical plant organs that use light energy to produce metabolites necessary for plant development during photosynthesis, the amount of light energy they hold is the determinant of plant production (Kanemasu et al., 1985). The change in leaf morphology also plays an essential role in the amount of water consumed by the plant. It was determined that the evapotranspiration values of the cotton plant change depending on the variety and leaf area, and less evapotranspiration occurs in the cotton of the Siokra variety, which has a small leaf area, compared to the other varieties (Can & Ödemiş, 2018). The fact that leaf area is directly related to the photosynthetic activity (Koc & Barutcular, 2000) affects the amount of dry matter, yield, and crop quality (Centritto et al., 2000). Hence, many factors that provide growth and development of the plant can be predicted by determining the leaf area.

Cotton is an extremely sensitive plant to water stress. Primarily during flowering, water shortage affects many growth parameters, especially leaf area, and flower shedding increases, while plant height, rooting depth, and canopy width decrease. One of the most obvious visual changes is the formation of redness on the stem from the point of contact with the soil to the top, depending on the level of stress (Ödemiş et al., 2018). The distinctive responses of cotton to water stress make the results of models to be established between stress and parameters affected by stress more significant. Various studies were conducted to reveal the effects of treatments on leaf area or the relationship between leaf area and plant morphological characteristics (Fournioux, 1996; Sala et al., 2015; Abd El-Mageed et al., 2016; Bozkurt &

Keskin, 2018; Pošta & Sala, 2018). Cho et al. (2007) suggested that they developed nonlinear models to estimate the fresh and dry weight of cucumber and individual leaf area using leaf length, leaf width, and SPAD values, and these models had a high correlation coefficient. Sala et al. (2015) estimated the leaf area in the ratio of $R^2=0.987$ (for L) and $R^2=0.995$ (for W) using leaf length (L) and width (W) in their study on 1500 leaves in 5 different apple tree cultivars.

This study examined the amount of irrigation water, evapotranspiration and seed cotton yield in cotton plants exposed to water stress during different growth periods and the morphological changes of leaf area, width, length, leaf lobes numbers, and lobe lengths due to stress.

MATERIALS and METHODS

The experiment was carried out in the randomized blocks of the Carisma variety cotton plants belonging to the *Gossypium hirsutum* L. species based on the split-plot design with 3 replicates in 2015-2016. The region where the experiment area is located (Hatay/Türkiye) reflects the typical climatic character of the Mediterranean region, and the summers are hot and dry, and the winters are warm and rainy. According to long-year climate data, the annual average temperature is 20°C, the coldest month of the year is January with 8.2°C, and the hottest month is August with 29.1°C. Total precipitation during the growing season was measured as 21 mm (2015) and 149 mm (2016). The characteristics of the soils of the research area are given in Table 1.

The cotton plant was divided into three different growth periods (vegetative period (VP), flowering and boll growth period (FB), and boll opening (BO) period) (Doorenbos & Kassam, 1979), and irrigation was applied at field capacity level during the periods of full irrigation (T), while irrigation water was not applied during the water stress periods (O) (Table 2). The cotton plant was planted with a seeder with an interrow spacing of 70 cm and an intrarow spacing of 15 cm. Treatments were formed from 6 rows and 15 meters in length. There was no gap between the replicates. Harvesting was done manually from the remaining 39.2 m² area after leaving out one row from the right and left of each plot and 50 cm from the beginning of the plots.

The soil moisture change was determined by the gravimetric method. The first irrigation started when 50% of the available water capacity was consumed. Irrigation applications were realized using the drip

irrigation method to bring the current soil water content to the field capacity approximately once a

week. Irrigation water quality was identified as C₂S₁. Irrigation water was calculated by using Equation 1.

Table 1. The physical and chemical properties of research area soils

Çizelge 1. Araştırma alanı topraklarına ilişkin bazı fiziksel ve kimyasal özellikler

Depth (cm)	Texture	pH	ECe	CaCO ₃ (%)	Nitrate (%)	Organic mat (%)	Fc (g g ⁻¹)	Pwp (g g ⁻¹)	As (g cm ³)
0-30	SiCL	7.55	644	2.265	1.42	0.33	21.3	13.4	1.66
30-60	SiCL	7.62	560	0.680	1.65	0.34	24.1	14.2	1.68
60-90	SiCL	7.80	429	0.905	2.01	0.38	25.0	14.5	1.54
90-120	SiCL	7.65	400	0.300	2.12	0.37	25.2	14.7	1.49

Fc: Field capacity, Pwp: permanent wilting point, As: bulk density, ECe: Electrical conductivity of soil paste (µmhos cm⁻¹)

Table 2. Water stress treatments applied in different developmental stages

Çizelge 2. Farklı gelişme dönemlerinde uygulanan su stresi konuları

Treatments	Emergence*	Vegetative Growth Period (VG)	Flowering and Boll Development Period (FB)	Boll Opening Period (BO)
OOO	+	-	-	-
OTO	+	-	+	-
TOO	+	+	-	-
OTT	+	-	+	+
TOT	+	+	-	+
TTT	+	+	+	+

(+): Irrigation, (-): Non-irrigation

(T): Irrigation treatments irrigated at field capacity level, (O): Non-irrigation treatments

*: In the first year, 70 mm water was given for equal emergence, while there was no need to irrigate in the second year due to precipitation.

$$d = ((PWFC \cdot PWA) \times As \times D) / 100 \quad (1)$$

Where; d: Soil moisture content in depth (mm); PWFC: Field capacity (%); PWA: Moisture content of each layer (%); As: Bulk density (g cm⁻³); D: Later depth (mm). Volume of water to be applied to each plot was calculated by Equation 2.

$$I = (d \times A \times P) / Ea \quad (2)$$

Where; I: Total irrigation water amount (L); d: Soil moisture content in depth (mm); A: Plot size (m²); P: Wetted area (%), According to the (Yıldırım, 2008) P was taken as 35%; Ea: Irrigation efficiency (%).

The evapotranspiration of the treatments was determined according to the "Soil-Water Budget" method (James, 1988), the water use efficiency (WUE) was determined according to Howell et al., (1984).

The fertilizer treatments were performed equally to

all plots with 20 kg da⁻¹ of 18-46-0 (DAP) fertilizer before sowing and 4 kg da⁻¹ pure nitrogen fertigation method in each of the first four irrigations after sowing (Burt et al., 1995).

Five leaves were taken from each replicates in each growth period in determining the leaf area and leaf geometry (five leaves were taken one day before irrigation from each replicates), and these leaves were drawn on sketch papers and their geometrical structures were determined. Leaf lobe lengths were identified with the help of a digital caliper (Dasqua 2310-7105 Digital Caliper (IP54 Protected)), while leaf area was determined by an electronic planimeter (Ushikata X-PLAN 380 f.c. planimeter). The distance between the two furthest points of the leaf is defined as 'height' and the widest part as 'width,' while the other lengths are called 'lobes' (Figure 1).

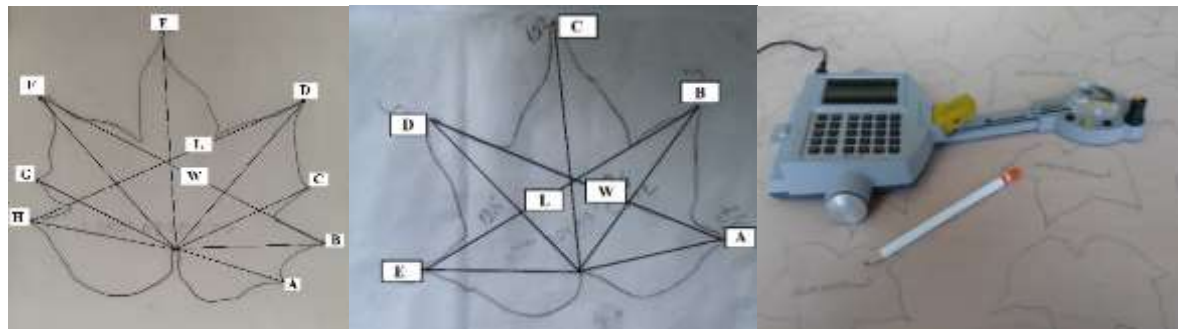


Figure 1. Width (W), length (L), lobe (A-E)-(A-H), and area measurements of cotton leaf

Şekil 1. Pamuk yaprağına ait en (W), boy (L), kanat (A-E)-(A-H) ve alan ölçümleri

RESULTS and DISCUSSION

In this research, the amount of irrigation water, evapotranspiration, seed cotton yield, and water use efficiency (WUE) changed depending on the treatments and years. The highest and lowest evapotranspiration were measured in TTT and OOO, respectively. The fact that the experimental area was windy during the irrigation season caused evaporation and evapotranspiration (ET) to be measured more than expected compared to other parts of the plain. Therefore, ET measured on TTT

treatment in both years (1046 mm in the first year, 1182 mm in the second year) and was higher than ET measured in cotton cultivation areas in the region (Table 3). Evapotranspiration in cotton was determined between 449-615 mm (Ertek & Kanber, 2001), 985-1103 mm (Baştuğ & Tekinel, 1989), and 778 mm-594 mm ranges (Howell et al., 1984) in Çukurova conditions. ET was measured as 1096-995 mm between 2015 and 2016 in the experiment area (Ödemiş et al., 2018).

Table 3. Changes of the irrigation water, evapotranspiration, seed cotton yield and WUE

Çizelge 3. Deneme konularının sulama suyu, bitki su tüketimi (ET), verim ve su kullanım etkinliği (WUE) değerlerinin yıllara ve konulara bağlı değişimleri

Treat.	Irrig. Water (mm)			ET* (mm)			Seed cotton yield (kg da ⁻¹)			WUE** (kg m ⁻³)		
	2015	2016	Mean	2015	2016	Mean	2015	2016	Mean	2015	2016	Mean
OOO	90	149	120	311	303	307	185.5±31.43	148.6±17.68	167±18.23	0.60	0.49	0.54
TTT	1135	1078	1106	1046	1182	1114	480.1±31.43	499.8±17.68	489.9±18.23	0.46	0.42	0.44
TOO	349	570	459	419	676	547	203.3±31.43	258.1±17.68	230.7±18.23	0.49	0.38	0.44
OTT	876	657	767	803	661	732	458.7±31.43	328.4±21.66	393.6±20.39	0.57	0.50	0.53
OTO	477	407	442	590	433	512	303.3±31.43	259.3±30.63	281.3±25.79	0.51	0.60	0.55
TOT	748	820	784	701	879	790	263±31.43	313.5±21.66	288.3±20.39	0.37	0.36	0.36

*ET: Evapotranspiration, **WUE: Water Use Efficiency

The irrigation strategy applied during the growth periods caused the ET to change in different treatments. Although the average ET values in TOO and OTO treatments, which were irrigated in only one of the three growth periods, were different based on the years, they were found to be at the same level on average. Similarly, in OTT and TOT treatments irrigated in two of the three growth periods, the ET value of the TOT treatment that was not irrigated during the flowering period was measured higher. On the other hand, higher crop yield was obtained in OTT. This indicates that the contribution of ET in the vegetative period to seed cotton yield is not as effective as in the flowering period. Moreover, evapotranspiration shows significant differences during growth periods. Tekinel and Kanber (1989) found out that the daily water consumption of cotton is 1-2 mm from emergence to square, 2-4 mm from square to the first flower, 3-8 mm from the beginning of flower to the first boll opening, and 8-14 mm from the first boll opening to the last effective flowering. It is known that the cotton plant is more sensitive to water during the flowering period than other periods (Karami et al., 1980). Although young leaves are more sensitive to photosynthesis in the vegetative period, stress during the peak of flowering (fruit set) weakens fruit set and increases flower shedding. Therefore, in our study, the highest seed cotton yield after TTT treatment was obtained from the OTT treatment irrigated during flowering and boll formation (393.6 kg da⁻¹) (Table 3). Although Krieg (1997) reported that water stress from the square to the time of the first flower cause a great decrease in seed cotton

yield, the fact that the soil moisture did not decrease much with the effect of winter precipitation in our study caused the stress to be at a lower level than expected. The effect of stress during the flowering period was also clearly observed in WUE. The lowest WUE was measured for TOT in both years (Table 3). The WUE value was calculated higher in the treatments irrigated during the flowering period. The low calculation of WUE in the second year in the treatment of non-irrigated OOO was thought to be due to the low contribution of excessive precipitation to the seed cotton yield in the period between the last irrigation and harvest. However, many variables such as radiation load, temperature, humidity, ambient CO₂ concentration, soil type and structure, soil water availability, nutrition, and genetic makeup affect the change of WUE (Reich et al., 1985; Reddy et al., 1995; Loveys et al., 2004).

Leaf Morphological Features

Leaves have an important role in plant functions and adaptation to environmental conditions. Changes in their morphological or anatomical features may occur due to their response to environmental conditions. Although mainly composed of epidermis, stomata, and mesophyll, they exhibit marked differences in area, thickness, and shape among different species due to phylogenetic relationships and adaptation to particular environments. Some studies investigated how morphological features such as leaf area vary between different ecosystems and adapt to environmental factors (Tian et al., 2016). Our research suggested that cotton leaves showed

morphologically different responses (leaf lobe number, leaf area, leaf width, leaf length, and leaf lobe length) to water stress in different irrigation strategies.

Leaf Lobe Number

It was determined that the lobes indicated by A, B, C, D, and E were common on the leaves of all treatments, while the lobes of F, G, H were lost or not formed at all in some treatments. Therefore, F, G, and H lobes were excluded in the regression relationships regarding the number of lobes. Physiologically, cotton leaves under water stress in the first stage of growth tended to increase the number of lobes while reducing their area. Therefore, the number of lobes was measured the highest in the OOO treatment (average 6.07 units) and the lowest in the fully irrigated TTT treatment (average 5.40 units) (Table 4). In the vegetative period, the number of lobes was higher in the treatments that were non-irrigated (OTT and OTO). Fewer lobes (especially in the first year) were determined in the TTT, fully irrigated each period. Four major leaf shape alleles exist in tetraploid cotton, including normal, sub-okra, okra, and super-okra. Besides, it was found that leaf shape has

consistent effects on boll rot resistance, earliness, flowering rate, chemical spray penetration, lint trash, and seed cotton yield. Nevertheless, different studies reported inconsistent effects on various insect resistances, photosynthetic rate, water use efficiency, and fiber quality (Andres et al., 2016).

Leaf Area

Leaf area decreased as water stress increased. Leaf area decreased by 40% in the first year and 22% in the second year compared to the fully irrigated treatment. Among the treatments exposed to water stress periodically, leaf area was determined the highest in TOT and lowest in OTO (Table 4). It was observed that irrigation during the vegetative period plays a significant role in increasing the leaf area. The data on TOO proves this situation. Even in the TOO treatment irrigated only in the VG period, leaf area was found to be higher than the OTT treatment irrigated in the FB and BO periods. The leaf area assessment is of higher importance for plant development. It is considered that approximately 95% of light is intercepted above an LAI of 3.

Table 4. Changes in leaf morphological characteristics by treatment
 Çizelge 4. Yaprak morfolojik özelliklerinin konulara bağlı değişimleri

GP	Lobe N (units)			Area (cm ²)			Width (cm)		
	2015	2016	Mean	2015	2016	Mean	2015	2016	Mean
OOO	5.89±0.25a	6.25±0.32a	6.07±0.21a	5914±357.92a	7180±551.15a	6547±366.42a	11.64±0.32a	11.61±0.40a	11.62±0.27a
TTT	5.33±0.29a	5.46±0.33a	5.40±0.23a	9847±405.84c	9201±575.66a	9524±395.77c	14.72±0.36c	13.95±0.44c	14.33±0.30c
TOO	5.67±0.25a	5.42±0.32a	5.54±0.21a	7981±357.92b	7800±551.15a	7890±366.42b	13.44±0.32b	13.14±0.40bc	13.29±0.27c
OTT	5.72±0.25a	6.04±0.35a	5.88±0.22a	6737±357.92a	8323±603.76a	7530±385.22a	12.19±0.32a	13.07±0.44bc	12.63±0.29b
OTO	5.78±0.25a	6.08±0.35a	5.93±0.22a	5976±357.92a	7610±603.76a	6793±385.22a	11.68±0.32a	12.42±0.44ab	12.05±0.29a
TOT	5.56±0.27a	5.29±0.33a	5.42±0.21a	9080±379.63c	8335±575.66a	8708±385.22c	14.18±0.34bc	13.91±0.42bc	14.04±0.29c

GP	Lenght (cm)			A (cm)			B (cm)		
	2015	2016	Mean	2015	2016	Mean	2015	2016	Mean
OOO	11.56±0.33a	11.14±0.46a	11.35±0.31a	4.98±0.20a	5.69±0.25a	5.34±0.17a	7.12±0.26a	7.47±0.27a	7.30±0.20a
TTT	14.80±0.38c	13.59±0.48c	14.19±0.34c	6.66±0.23d	7.21±0.26d	6.94±0.19c	9.58±0.30d	9.14±0.29d	9.36±0.21d
TOO	13.45±0.33b	12.62±0.46bc	13.04±0.31c	6.59±0.20cd	6.74±0.25cd	6.67±0.17bc	8.86±0.26cd	8.96±0.27bc	8.91±0.20cd
OTT	11.93±0.33a	12.54±0.50abc	12.23±0.33b	5.84±0.20bc	6.59±0.27bc	6.22±0.18b	8.34±0.26bc	9.04±0.30cd	8.69±0.21bc
OTO	11.63±0.33a	11.97±0.50ab	11.80±0.33a	5.46±0.20ab	5.95±0.27ab	5.70±0.18a	7.78±0.26ab	7.98±0.30ab	7.88±0.21ab
TOT	13.98±0.35bc	12.36±0.48abc	13.17±0.33c	6.61±0.21cd	6.80±0.26cd	6.73±0.18bc	9.37±0.28d	9.13±0.29d	9.25±0.21d

GP	C (cm)			D (cm)			E (cm)		
	2015	2016	Mean	2015	2016	Mean	2015	2016	Mean
OOO	8.69±0.29a	8.28±0.31a	8.48±0.23a	8.00±0.28a	7.84±0.33a	7.92±0.23a	5.66±0.25a	6.13±0.37a	5.89±0.24a
TTT	11.24±0.33c	10.35±0.33c	10.80±0.25c	9.71±0.31b	9.32±0.35b	9.52±0.25c	7.38±0.29b	7.28±0.41b	7.33±0.26b
TOO	9.87±0.29b	9.53±0.31bc	9.70±0.23bc	8.59±0.28ab	8.75±0.35ab	8.67±0.23bc	6.47±0.25b	6.87±0.37ab	6.67±0.24b
OTT	9.24±0.29ab	9.29±0.34bc	9.27±0.24bc	8.28±0.28a	8.98±0.39b	8.63±0.25bc	5.89±0.25a	6.62±0.41ab	6.25±0.25a
OTO	8.93±0.29a	9.02±0.34ab	8.97±0.24ab	8.01±0.28a	8.34±0.37ab	8.18±0.24ab	5.85±0.25a	6.20±0.39ab	6.02±0.24a
TOT	10.88±0.31c	9.57±0.33bc	10.22±0.24bc	9.44±0.29b	9.10±0.35ab	9.27±0.24c	6.93±0.29b	7.07±0.37b	7.00±0.25b

This indicates how efficiently intercepted light can be modified into sugar. On the other hand, photosynthesis requires water and carbon dioxide. Because gas exchanges are of primary interest, the rates of stomatal conductivity and carbon dioxide assimilation are also significant indicators of the efficiency of modification of light into sugar. The radiation use efficiency (RUE) of cotton is calculated

as the division of its total dry biomass by its sum of intercepted light (Loison, 2019).

Leaf Width and Lenght

Leaf width and length were similarly affected by water stress during the growth periods. The mean values for leaf width and length are listed as TTT,

TOT, TOO, OTT, OTO, and OOO, from largest to smallest. The fact that the values in TTT and TOT treatments are at approximately the same level reflects that the water stress during the flowering and boll formation period did not cause a significant decrease in leaf width and length. On the other hand, lack of irrigation in the vegetative period (OTT and OTO) caused leaf length and width as much as the almost non-irrigated treatment (OOO) (Table 5). Studies reveal that water stress leads to an increase in specific leaf weight (Wilson et al., 1987), while it causes a decrease in leaf size (Pettigrew, 2004a). Water stress also reduces the formation of new leaves, resulting in a reduction in overall plant leaf area. Since the effect of stress is less severe on the main stem leaves, less leaf development is seen on both the main stem and the sympodial branches

(Krieg & Sung, 1986).

Leaf Lobe Length

The effect of water stress on the lobe length was found to be significant in the experiment (Table 5). The lobe lengths increased over time on the dates of measurement until becoming stable. In general, five lobes were measured on all leaves (A, B, C, D, and E), while the other three lobes (F, G, and H) did not form on some leaves. Average lobe lengths were measured at the highest value in full irrigation (TTT) and lowest in non-irrigation (OOO). In lobes A, B, C, D, and E, lobe lengths from the highest to the lowest were measured for TOT, TOO, OTT, and OTO, respectively.

Table 5. Regression coefficients (r^2) between leaf area and leaf morphological characteristics
 Çizelge 5. Yaprak alanı ile yaprak morfolojik özellikleri arasındaki regresyon katsayıları (r^2)

Treat.	Leaf Width (cm)	Leaf Length (cm)	A (cm)	B (cm)	C (cm)	D (cm)	E (cm)
OOO	0.87** (n=9)	0.87** (n=9)	0.54 ns (n=9)	0.62ns (n=9)	0.62ns (n=9)	0.31ns (n=9)	0.25ns (n=9)
OOO	0.55* (n=12)	0.55* (n=12)	0.15ns (n=12)	0.16ns (n=12)	0.25ns (n=12)	0.27ns (n=12)	0.13ns (n=9)
OOO	0.55** (n=21)	0.55** (n=21)	0.26ns (n=21)	0.21ns (n=21)	0.19ns (n=21)	0.17ns (n=21)	0.01ns (n=18)
TTT	0.91** (n=7)	0.94** (n=7)	0.93** (n=7)	0.94** (n=7)	0.94** (n=7)	0.71* (n=7)	0.71* (n=7)
TTT	0.75* (n=11)	0.78* (n=11)	0.68* (n=11)	0.78* (n=11)	0.74** (n=11)	0.68* (n=11)	0.79** (n=9)
TTT	0.79** (n=18)	0.76** (n=18)	0.75** (n=18)	0.75** (n=18)	0.75** (n=18)	0.66** (n=18)	0.58** (n=16)
TOO	0.90** (n=9)	0.87** (n=9)	0.58ns (n=9)	0.55ns (n=9)	0.54ns (n=9)	0.57ns (n=9)	0.56ns (n=9)
TOO	0.60* (n=12)	0.62** (n=12)	0.52ns (n=12)	0.49ns (n=12)	0.51ns (n=12)	0.53ns (n=12)	0.53ns (n=12)
TOO	0.62** (n=21)	0.60** (n=21)	0.41ns (n=21)	0.40ns (n=21)	0.36ns (n=21)	0.40ns (n=20)	0.39ns (n=20)
OTT	0.86** (n=9)	0.62* (n=9)	0.64* (n=9)	0.63* (n=9)	0.65* (n=9)	0.91** (n=9)	0.63* (n=9)
OTT	0.85** (n=10)	0.63* (n=10)	0.65* (n=10)	0.63* (n=10)	0.67* (n=10)	0.68* (n=10)	0.62* (n=10)
OTT	0.84** (n=19)	0.61** (n=19)	0.52* (n=19)	0.58** (n=19)	0.58** (n=19)	0.74** (n=19)	0.55* (n=19)
OTO	0.66* (n=9)	0.67* (n=9)	0.64* (n=9)	0.63* (n=9)	0.65* (n=9)	0.63* (n=9)	0.65* (n=9)
OTO	0.75** (n=10)	0.67* (n=10)	0.76** (n=10)	0.88** (n=10)	0.80** (n=10)	0.61* (n=10)	0.69* (n=10)
OTO	0.67** (n=19)	0.47* (n=19)	0.64** (n=19)	0.69** (n=19)	0.46* (n=19)	0.46* (n=19)	0.44* (n=19)
TOT	0.67* (n=8)	0.68* (n=8)	0.59ns (n=8)	0.64ns (n=8)	0.60ns (n=8)	0.56ns (n=8)	0.53ns (n=7)
TOT	0.91** (n=11)	0.87** (n=11)	0.56ns (n=11)	0.52ns (n=11)	0.46ns (n=11)	0.56ns (n=11)	0.50ns (n=11)
TOT	0.71** (n=19)	0.85** (n=19)	0.43ns (n=19)	0.37ns (n=19)	0.36ns (n=19)	0.42ns (n=19)	0.42ns (n=18)

Relationships Between Leaf Area, Seed cotton yield and Water Use

The leaf area measurement is important in determining the plant's response to environmental conditions and predicting the development of the vegetative parts of the plant. In addition to studies investigating the relationship between the change in leaf area and seed cotton yield factors (leaf dry weight, vegetative components (stems and leaves), dry weight, and plant height) (Ghaderi & Soltani, 2007), there are also studies using the morphological features of the leaf to estimate the single leaf area (Fournioux, 1996; Sala et al., 2015; Pošta & Sala, 2018). Ghaderi and Soltani (2007) expressed that plant height is not a good determinant of leaf area, but dry leaf weight (LDW) or stem+leaf dry weight (VDW) can be used to predict leaf area. However, besides potential evaporation (E_o), leaf area index (LAI) is an important variable in determining evaporation from the soil surface on the first day after irrigation (Al-Khafaf, 1978). Marani et al. (1985) stated that the increased stress due to the decrease in the amount of irrigation water decreased the leaf expansion and leaf area, as well as decreased photosynthetic rate by increasing leaf senescence.

In our study, insignificant regression relationships in the first year and significant in the second year were found between leaf area and irrigation water amount, evapotranspiration, water use efficiency, and seed cotton yield (Figure 2-3-4-5). The average irrigation water amount and evapotranspiration values of the two years were effective in increasing the leaf area. The response of the leaves to the water stress during the growing periods was different. Leaf area was reduced by only 4.6% in the OTT treatment (767 mm), which was applied 67% more irrigation water than in the TOO treatment (459 mm). Similarly, the leaf area was found to be only 29% more in the TOT treatment (784 mm), which applied 56% more water than in the OTO treatment (442 mm). These data demonstrate that irrigation water applications during the vegetative growth and flowering periods are effective in increasing the leaf area, and the plant is more sensitive to water during the vegetative period.

In the relationship between ET-leaf area, irrigation water showed similar characteristics to the relationship between -ET (Figure 3). Based on the OOO treatment, the increase rates in the ET and leaf area are 363%-145% in TTT, 178%-121% in TOO, 238%-115% in OTT, 167%-104% in OTO, and 257%-133% in TOT. Based on this finding, the ET values of the treatments irrigated only during the vegetative growth and flowering periods caused an increase in leaf area by 121% and 104%, respectively. Compared to the OTT and TOT treatments irrigated in the two growth periods, the water consumption amounts in

the TOO and OTO treatments were found to be more effective on the leaf area.

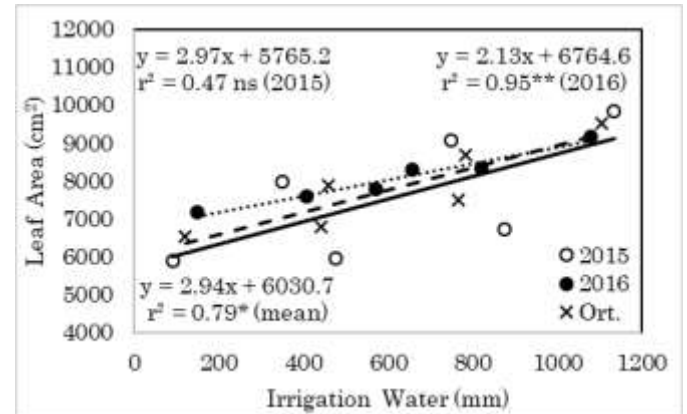


Figure 2. Relationships between leaf area and irrigation water

Şekil 2. Yaprak alanı ile sulama suyu arasındaki ilişkiler

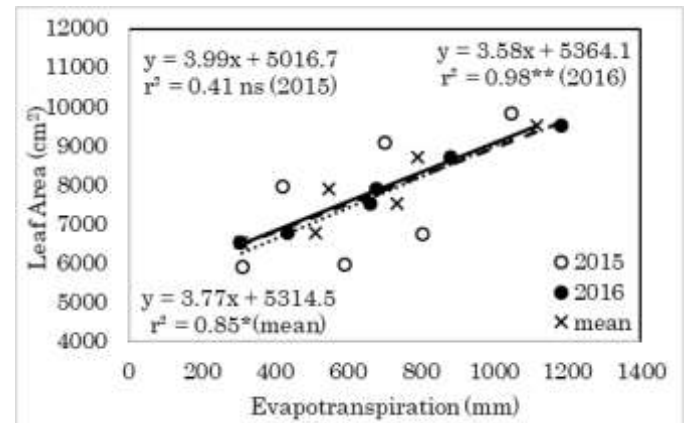


Figure 3. Relationships between leaf area and evapotranspiration

Şekil 3. Yaprak alanı ile bitki su tüketimi arasındaki ilişkiler

Water stress caused a significant regression relationship between average seed cotton yield and leaf area only in the second year (Figure 4). Compared to the non-irrigation treatment (OOO), the seed cotton yield increased by 293% in the fully irrigated treatment (TTT), while the leaf area increased by 145%. Besides, while the leaf area increased by 115% in the OTT (flowering and boll opening period), seed cotton yield increased by 236%. When OTT and TOT treatments are compared, it is seen that the flowering and boll formation period are determinative on seed cotton yield. However, it was observed that water stress in the mentioned period led to a significant decrease in WUE. No significant relationship was detected between WUE and leaf area (Figure 5). Krieg (1997) stated that water stress reduces the number and area of leaves, resulting in decreased photosynthesis and seed cotton yield. He

also pointed out that regarding the water supply that affects seed cotton yield components, the period from the square to the first flower is the most critical period of development. Drought sensitivity was highest at the peak flowering period when water stress resulted in the highest seed cotton yield reduction. The drop in seed cotton yield caused by water stress is mostly due to a fall in the number of bolls (Pettigrew, 2004b). Water stress before flowering lowers the number of fruiting sites.

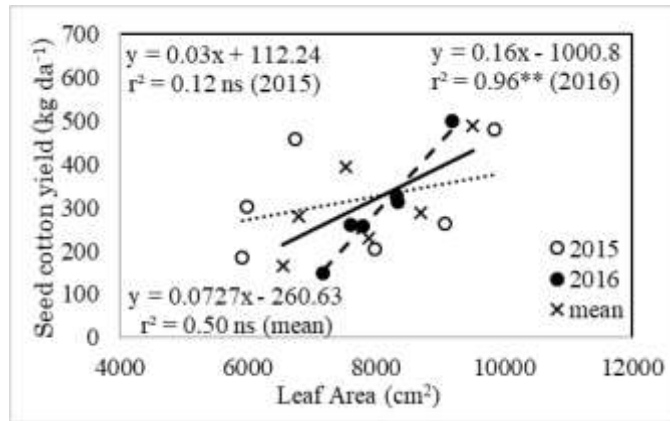


Figure 4. Relationships between leaf area and seed cotton yield

Şekil 4. Yaprak alanı ile verim arasındaki ilişkiler

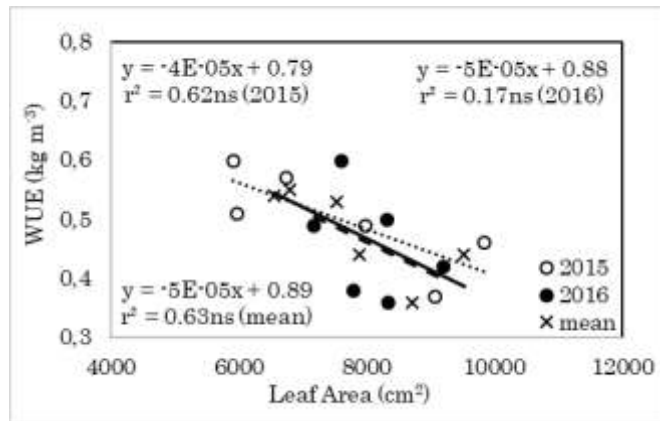


Figure 5. Relationships between leaf area and WUE

Şekil 5. Yaprak alanı ile su kullanım etkinliği arasındaki ilişkiler

Relationships Between Leaf Area and Other Morphological Parameters

The number of leaf lobes varied between five and eight depending on the treatments. Therefore, in the regression relationships between leaf area and leaf lobe lengths, only the lobes (A, B, C, D, and E) common in all treatments were taken as the basis. Lobe lengths varied according to years and irrigation. In the regression analysis, the leaf area increase did not cause a significant change in lobe lengths in the OOO, TOO, and TOT treatments. As seen in TOO and TOT treatments, non-irrigation during the flowering period did not increase the lobe lengths. Additionally,

it was observed that the increase in leaf area in the treatments above was caused by irrigation, especially in the vegetative period. As the leaf area increased, a lower regression coefficient was determined between leaf lobe length and leaf area.

CONCLUSION

Many studies investigate the effects of water stress on seed cotton yield, evapotranspiration, and WUE that the cotton plant is exposed to during its growth (Howell et al., 1984; Pettigrew, 2004a; Ödemiş et al., 2018; Can & Ödemiş, 2018; Kazgöz-Candemir & Ödemiş, 2018). However, there was no study examining the correlation of these parameters with leaf area and morphological features. Hence, our study demonstrated that leaf area created significant correlation relationships with seed cotton yield, irrigation water amount, and evapotranspiration (especially in the second year of the study). However, no correlation was determined between leaf area and WUE. In the vegetative period, under stress conditions, the leaf first increased the number of lobes, while the decrease in stress and the increase in leaf width and length caused the disappearance of non-specific (small) lobes. During the flowering period, the leaf width and length became stable and reached the maximum level, and the leaf area reached the highest level. However, after the flowering period, it was observed that some lobes could maintain their length while re-stress reduced leaf area. It can be suggested that the duration and severity of the stress in that period are more effective than the development periods in the change in leaf morphology. This is more evident in the leaf area. Whether the leaf area is in the flowering period or the boll formation period, it could increase its growth under stress-free conditions until the bolls were formed. However, the most significant increase occurred with the effect of irrigation during the vegetative growth period. As the leaf area increased, a lower coefficient of regression between leaf lobe length and leaf area was determined. It was observed that irrigation during the flowering period was more effective in increasing the lobe lengths.

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Author's Contributions

The contribution of the authors is equal.

Statement of Conflict of Interest

Authors have declared no conflict of interest.

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