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ARAŞTIRMA MAKALESİ RESEARCH ARTICLE

Effects of foliar sulfur applications in cotton crop on stomatal conductance under water stress

Pamuk bitkisinde yapraktan kükürt uygulamalarının uzun süreli su stresi koşullarında stoma iletkenliğine etkisi

Derya KAZGÖZ CANDEMİR[1], Berkant ÖDEMİŞ[1]
[1]*Hatay Mustafa Kemal University, Faculty of Agriculture, Department of Biosystems Engineering, Antakya-Hatay, Turkey.*

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Corresponding author: Derya KAZGÖZ CANDEMİR
✉: dryaa.kzgz@gmail.com

Ö Z E T / A B S T R A C T

Aims: This study was performed in 2015-2016 in order to determine how the foliar application of sulfur helped reduce the effects of long-term water stress in Carisma variety cotton plants in different periods of development, namely vegetative growth (VG), flowering and boll development (FB), and boll opening (BO) periods.
Methods and Results: The study was set up in randomized complete block with three replications. Development periods (OOO, TTT, TOO, OTT, OTO, TOT) were assigned to main plots and sulfur doses (S_0: Control, S_1: 150 ml da^{-1}, S_2: 250 ml da^{-1}, S_3: 350 ml da^{-1}) were assigned to sub-plots. Measurements were made on stomatal conductance, as well as evaporatranspiration and yield to evaluate the physiological effects of water stress. The average amounts of irrigation water used in each full irrigation were 127.5 and 138 mm respectively, for the treatment years. As for the evaporatranspiration values, in the first treatment year it was found between 304 and 1012 mm and in the second year from 256 to 1070 mm. Stomatal conductance values ranged from 269 to 1067 mmol m^{-2} s^{-1} in the first year and from 205 to 407 mmol m^{-2} s^{-1} in the second year with the highest stomatal conductance value obtained from full irrigation (TTT) during all growth periods in both years.
Conclusions: The effects of sulfur dosage on stomatal conductance and yield varied due to exposure to long-term water stress at different developmental periods; however, generally speaking, the doses of S_1 and S_2 cause these values to increase.
Significance and Impact of the Study: In the Amik Plain, there was no detailed study to determine how foliar sulfur applications changed the stomatal conductance of cotton when drip-irrigated and subjected to water stress during the different development stages. This research revealed in what dose sulfur should be given to reduce the effects of long-term water stress in Carisma variety cotton plants in Amik plain.

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INTRODUCTION

Agricultural areas suffer from various stresses including climate change, environmental pollution, and land use/cover change. Globally, the cultivated areas are adversely affected by drought stress (26%), over-fertilization (20%), frost conditions (15%), and other stress factors (29%), while the completely stress-free croplands make up only 10% (Blum, 1986; González et al., 2008). Amongst the abiotic stresses, water stress has been considered as a threat for low crop productivity in many regions of the world (Turner, 1997; Sinclair, 2005; Sezener et al., 2015). Therefore, the use of drought-tolerant plants, and the enhancement of the drought tolerance of the crop production play a vital role in food and water securities. Inefficient and ineffective irrigation practices are the primary driver of sustainable agriculture (Kazgoz Candemir and Odemis, 2018), in particular, in the (semi-)arid climate belts (Ozyurt and Akca, 2017).

The water stress impact depends on the stress severity and duration, and the plant development stage and genotype (Loka and Oosterhuis, 2012). Water stress during the flowering and boll stages of the crop development was reported to cause a significant yield loss (Krieg, 1997; Orgaz et al., 1992; Loka, 2012; Loka ans Oosterhuis, 2012). As with other crops, cotton responds to water stress with various physiological strategies among which decreased stomatal conductance, increased leaf temperature (Jones, 1999), weakened photosynthetic capacity (Lawlor and Cornic, 2002), shortened phenological periods (Slafer et al., 2005; Richards, 2006) and reduced leaf area (Walter and Shurr, 2005) are some of the most important ones. Stomatal conductance/resistance is one of the most important mechanisms of the plant physiology since it allows for the gas exchange between the stoma and the atmosphere (Kerepesi and Galiba, 2000; Mansfield and Davies, 1981). Various studies about potassium (Andersen et al., 1992), phosphorus (Sawwan et al., 2000), boron (Odemis and Delice, 2018), and sulfur (Odemis et al., 2017; Kazgoz Candemir and Odemis, 2018) showed that they reduced the plant water stress. The most typical signals of the sulfur deficiency included the paling of young leaves as a result of reduced protein and chlorophyll synthesis, the declined root hydraulic permeability, the decreased stoma openings, and the diminished leaf areas (Dietz, 1989; Marschner, 1995; Jie et al., 2008).

The objective of this study was to determine how foliar sulfur applications affect the stomatal conductance of cotton when drip-irrigated and subjected to water stress during the different development stages.

MATERIALS AND METHODS

The study was performed in the agricultural experimental station of the firm ProGen Seed Company in Hatay in the eastern Mediterranean region of Turkey (36^o 17' 26'' N latitude and 36^o 11' 43'' E longitude). The experimental design was based on the randomized complete block with three replications in full irrigation in 2015 and 2016. The average temperatures recorded during the growing seasons (May, June, July, August, September, and October) in 2015 and 2016 were 26.9°C and 25.9°C, with the total rainfall of 21 mm and 149 mm, respectively. The soil is composed of silt, clay, and loam with no issue of saline-sodium content or drainage. In the soil samples taken to a depth of 30 cm, no sulfur was detected based on the turbidimetric barium method (Fox et al., 1964). Their physical properties are given in Table 1.

Table 1. Soil properties of the experimental area

Depth (cm)	Texture class			FC (%, Pw)	WP (%, Pw)	BD (g cm^{-3})
	Sand (%)	Silt (%)	Clay (%)			
0-30	59.5	15.3	25.2	21.3	13.4	1.66
30-60	57.5	19.3	23.2	24.1	14.2	1.68
60-90	53.5	17.3	29.2	25.0	14.5	1.54
90-120	61.5	15.3	23.2	25.2	14.7	1.49

FC: Field capacity, WP: Wilting point, BD: ;Bulk density

Drip irrigation system was used in the treatments. In the experiment, laterals were used with a dripper gap of 40 cm and placed one in every other row. Irrigation was performed once a week by bringing the existing level of moisture (based on TTT as the reference) to the field capacity. Irrigation water quality was as C_3S_1 (ECw: 1397 (µmhos cm^{-1}). Carisma cotton cultivar used in the treatment was planted and harvested from May 18 to October 14 in 2015 and from June 3 to October 14 in 2016. In its different development periods, cotton was either irrigated at the field capacity (T) or received no irrigation (O) (Table 2). The development periods included vegetative growth (VG), flowering and boll development (FB) and boll opening (BO) (Doorenbos and Kassam, 1979). For each growth stage, there were six rows of cottons, while rows were 15 m in length for each replication. There was no gap between the replications. Space between plant rows was 70 cm and space between plants was 15 cm. There were approximately 100 plants on each row.

Table 2. Water stress treatments applied in different cotton crop developmental stages

Treatments	Emergence water*	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

The soil moisture change was determined through the gravimetric method for a soil depth of 30 cm at the effective root depth of 90 cm. The first irrigation was started after 50% of the available water capacity was consumed. Evapotranspiration rate of the samples was calculated through the *'Soil-Water Budget'* method thus:

$$Et = I + R - Dp - Rf \pm \Delta S \qquad (1)$$

where *Et:* Evapotranspiration (mm); *I:* Amount of supplied irrigation water (mm); *R:* rainfall (mm); *Dp:* deep percolation (mm) (measured based on the samples taken nearly 24 hours after the irrigation of fully irrigated samples at 120 cm depth); *Rf:* the surface runoff (mm); and *ΔS:* the soil moisture content change values (mm 90 cm^{-1}) (James, 1988).

The fertilizer treatments were performed in the same way for all the plots at the same dose prevalently used in the region: 20 kg da^{-1} 18-46-0 (DAP) fertilizer before and after sowing. 4 kg da^{-1} pure N fertigation in each of the four irrigations were applied based on the four quarters rule, as was stated by Burt et al. (1995) (S_0). For all the treatments, pure elemental sulfur was applied from the leaves as 150 mL da^{-1} (S_1), 250 ml da^{-1} (S_2), 350 ml da^{-1} (S_3) (Table 3). Sulfur applications were made one time in the middle of each of the development periods, except for the emergence period, and between two irrigations at the early hours of the morning (6:00-6:30) where the wind would not negatively affect the S distribution.

Table 3. Application dates and Sulfur doses applied to treatments

Sulfur doses	First Application	Second application	Third application
S_0	No application		
S_1	150 ml da^{-1}	150 ml da^{-1}	150 ml da^{-1}
S_2	250 ml da^{-1}	250 ml da^{-1}	250 ml da^{-1}
S_3	350 ml da^{-1}	350 ml da^{-1}	350 ml da^{-1}

Stomatal conductance

Stomatal conductance measurements were made 1 day before irrigation, between 11:00-14:00 under the clear sky and on two leaves of two plants marked for the replications of each subject. The device SC-1 (LPS0881) leaf porometer was used for stomatal conductance measurements. Before each measurement in the field, device calibration checks were carried out with the standard calibration papers, and having awaited the stabile weather conditions.

Yield (kg da^{-1}) and statistical analysis

Each experiment plot consisted of six rows. Two rows from the edges, and 0.50 m from the beginning and end of each row were left out due to the edge effect. The remaining area of 13.05 m^2 was harvested and total yield was calculated in kg da^{-1}. The statistical analysis was performed processing the data using Duncan Test in SPSS 18 software package (Bek and Efe, 1988).

RESULT AND DISCUSSION

Irrigation water (mm)

The highest irrigation amount was received by the fully irrigated (TTT) treatment in each of its developmental period (Table 4). In both years, the first irrigations were made when approximately 50% of the available capacity was used up, while the rest was usually applied once a week in the range of 50-60% of available capacity, considering the soil water content with TTT. Irrigation was started on July 10 in the first year and July 14 in the second year and ended on August 27 in both years. The amount of rainfall was 21 mm from the date of planting to harvest in the first year and 149 mm in the second year by the time of harvest. The number of irrigations was 10 and 7 in the first and second years, respectively. The reason for the difference related to the rainfall received during the two irrigation seasons. The average water amount used in each irrigation was 127.5 mm (115-140 mm) and 138 mm (107-169 mm) in the first and second years, respectively. With the same cotton variety in the same region during the same years as this study, the irrigation water amount for each full irrigation was on average not less than 110 mm given the soil water deficit according to Odemis et al., (2017).

Table 4. Irrigation water, evapotranspiration (Et) and yield according to the treatments

Treatments	Irrigation Water (mm)		Evapotranspiration (mm)	
	2015	2016	2015	2016
OOO	91*	149**	304	256
TTT	1136	1078	1012	1070
TOO	350	570	380	639
OTT	877	657	770	636
OTO	478	407	583	425
TOT	749	820	685	787

*: Precipitation+emergence water
**: Precipitation
Precipitation and emergence water are included in all treatments

In both years, the highest and lowest ET values occurred with TTT and OOO, respectively (Table 4). The ET values varied between 304 and 1012 mm in the first year and between 256 and 1070 mm in the second year. With TTT, TOO, OTT, OTO, and TOT, the ET values decreased by 62, 24, 42, and 32% in 2015, and 40, 41, 60, and 26% in 2016. The difference may be attributed to the different growing season length, temperature and rainfall. The ET value was in the range of 299 to 1096 mm in 2015 and 247 to 995 mm in 2016 according to the study by Odemis et al. (2017) about the effects of boron on cotton flowering under the water levels (33%, 66% and 100% of the available capacity) in different doses (0.75 ppm, 300 ppm, and 750 ppm). The ET value varied between 274 and 1045 mm according to the study by Odemis and Delice (2018) and between 921 and 500 mm in Kahramanmaras (Keten et al., 2019).

Relation of stomatal conductance and the irrigation water

Higher stomatal conductance means open stomata with high transpiration rate. In both years, stomatal conductance was affected by the *amount of irrigation water applied during the development period (DP)* and by the interaction of the *sulfur dose and irrigation water amount* in these periods *(DP*SD)* (*$p<0.001$*), whereas *sulfur doses (SD)* did not affect stomatal conductance (Table 5).

Table 5. Analysis of variance table for stomatal conductance

Year	Source of variation	df	SS	MS	F
2015	DP	5	14432512.45	2886502.49	190.23***
	SD	3	124527.08	41509.03	2.74ns
	DP*SD	15	728941.92	48596.13	3.20***
	Error	252	3823715.72	15173.48	
2016	DP	5	1340429.68	268085.94	101.31***
	SD	3	7448.38	2482.79	0.94ns
	DP*SD	15	101564.41	6770.96	2.56***
	Error	240	635117.70	2646.32	
2015-2016	DP	5	12170143	2434029	268.58***
	SD	3	70918.88	23639.63	2.61*
	Year	10	15958621	1595862	176.09***
	DP*SD	15	546222.8	36414.85	4.02***
	DP*Year	50	24585057	491701.1	54.26***
	SD*Year	28	1120546	40019.51	4.42***
	DP*SD*Year	140	3277045	23407.46	2.58***
	Error	492	4458833	9062.67	

DP: development period, SD: sulfur doses, df: degree of freedom, SS: Sum of square, MS: Mean Squares, *** p <0.001, ** p <0.01, *p <0.05 significant, ns: not significant

Average stomatal conductance varied between 269 and 1067 mmol m^{-2} s^{-1} in the first year and between 205 to 407 mmol m^{-2} s^{-1} in the second year. In the first year, all the subjects significantly differed from one another, while a significant difference between TOO and OTT, and OTO and TOT was detected in the second year. In both years, TTT with the highest stomatal conductance significantly differed from the rest (Table 6). The decreased irrigation water decreased the stomatal conductance. In the first year, the decrease in the irrigation water from 1136 to 91 mm decreased the stomatal conductance by 69%. In the second year, the decrease from 1078 to 149 mm decreased the stomatal conductance by 50%. The highest average stomatal conductance was found with TTT in both years. The stomatal conductance fell with TOO, OTT, OTO, and TOT by 73, 37, 75, and 43% in 2015 and 29, 28, 37, and 34% in 2016, respectively (Table 6). The decreased irrigation water prolonged the duration of stress and decreased the stomatal conductance. These results are in agreement with Loka and Oosterhuis (2014); Odemis et al. (2017); Can (2017). Whether the stress was prolonged as with OOO or short-lived as with TOT or OTT determines the yield. The pronounced stress responses of cotton at the different developmental stages also showed how the S dosage applied affected the stomatal conductance.

Table 6. Stomatal conductance (Sc) (mmol m^{-2} s^{-1}) and yield (kg da^{-1}) according to the treatments

Applications		2015		2016	
		Yield	SC	Yield	SC
OOO		252	332	168	205
TTT		533	1066	564	406
TOO		236	286	272	290
OTT		451	670	337	294
OTO		287	269	322	256
TOT		305	610	356	269
Development Period (DP)		***	***	***	***
S_0		316	521	301	405
S_1		348	546	352	395
S_2		361	554	344	395
S_3		352	533	348	392
Sulfur Dose (SD)		**Ns**	**ns**	***	**ns**
OOO	S_0	186	297	149	231
	S_1	237	353	150	191
	S_2	288	351	189	197
	S_3	296	326	183	199
TTT	S_0	480	1127	500	430
	S_1	547	1057	581	387
	S_2	560	1021	586	405
	S_3	544	1060	587	403
TOO	S_0	203	246	258	261
	S_1	253	363	298	312
	S_2	244	314	277	324
	S_3	244	221	255	263
OTT	S_0	459	616	328	287
	S_1	443	641	351	281
	S_2	466	723	333	312
	S_3	437	699	337	296
OTO	S_0	303	227	259	244
	S_1	280	302	338	253
	S_2	285	283	340	242
	S_3	280	265	350	286
TOT	S_0	263	615	314	278
	S_1	326	561	396	273
	S_2	322	634	337	275
	S_3	308	628	378	249
DP x SD		**ns**	***	**ns**	***

In the first year, the lowest stomatal conductance was obtained with OOO and OTO when the subject did not receive any S dosage (S_0). The highest stomatal conductance was obtained with the S_1 dose, while the stomatal conductance declined with the elevated S dose. With the doses of S_1, S_2, and S_3, the stomatal conductance increased by 19, 18 and 10% for OOO and 33, 25 and 17% for OTO, respectively. As far as TTT, irrigated to the field capacity at all the developmental stages, was concerned, the stomatal conductance increased by 6, 9 and 6% in 2015 and decreased by 10, 6 and 6% in 2016 with S_1, S_2, and S_3, respectively. These results showed that the S application did not increase the maximum stomatal conductance when the soil had sufficient moisture. When the soil water content was sufficient, the foliar S applications prevented the further opening of stomata. On the contrary, potential gas exchange rate might be reduced due to the prohibitive layer S formed on the leaf surface. Nevertheless, the S application increased the stomatal conductance. As for TOO, fully irrigated in the vegetative period, S_1 and S_2 increased the stomatal conductance by 48 and 28% in 2015 and 20 and 24% in 2016, respectively. It is of great significance that these increases occurred in leaves that had to endure early aging due to stress in the flowering, and boll development and opening periods.

In both years, a significant relation for the amount of irrigation water was found with ET and stomatal conductance (p < 0.01). One unit increase in irrigation water and ET increased the stomatal conductance by 0.74 and 1.07 mmol m^{-2} s^{-1} in the first year and 0.19 and 0.21 mmol m^{-2} s^{-1} in the second year (Figure 1).

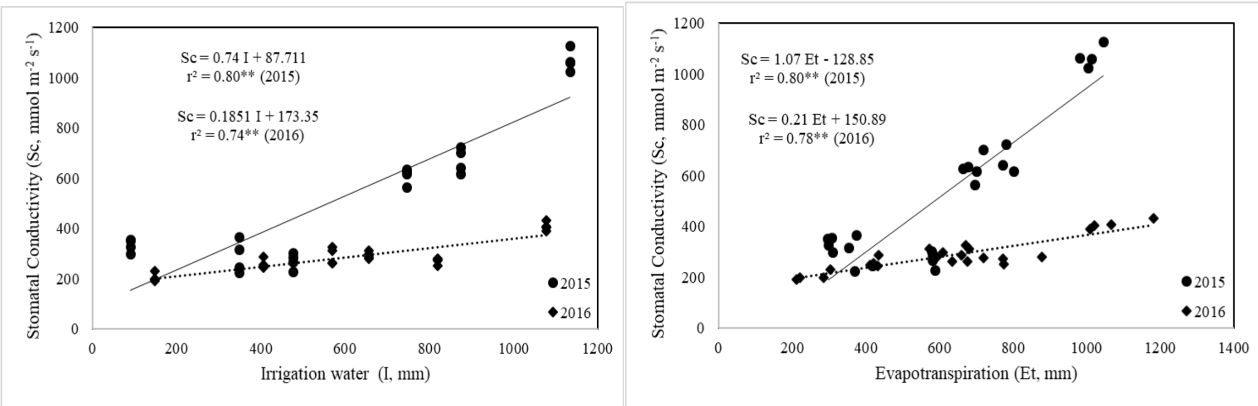

Figure 1. The relationship between irrigation water and evaporatranspiration and stomatal conductance

In the first year, the highest stomatal conductance was observed with TOO at a dose of 150 ml da^{-1} where the yield was also higher (Table 6). The doses after 150 ml da^{-1} did not make any difference in the yield and decreased the stomatal conductance, a stress in the plant. A similar effect was observed with OTT in 2015, with the highest yield obtained at 250 ml da^{-1} and the highest stomatal conductance, both of which dropped with the increased dose. As for OTO in 2016, the increased S dose increased both stomatal conductance and yield, with the highest ones obtained at 350 ml da^{-1} for both. Considering each developmental period separately, the duration and intensity of the stress that the plant was exposed during the developmental period are the main driver of the change in the stomatal conductance. The effect of the S application on the increased stomatal conductance varied depending on its dosage and the level of stress.

In both years, a significant relation (p<0.01) was found between stomatal conductance and yield. One unit increase in the stomatal conductance increased the yield by 0.35 kg da^{-1} in the first year and 1.69 kg da^{-1} in the second year (Figure 2).

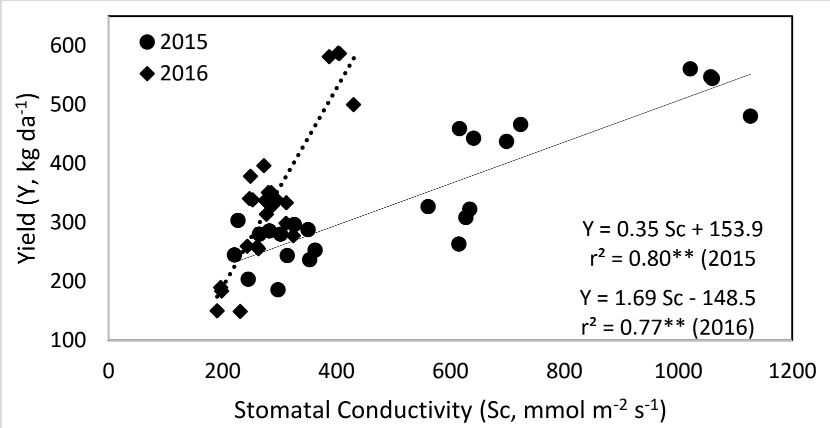

Figure 2. The relationship between stomatal conductance and yield

The length of the non-irrigated duration in the developmental periods (stress duration and severity), and the S doses are the reason why the stomatal conductance varied temporally (Figure 3-4).

For the first year, with the long-term stress (OOO), the S_1, S_2 and S_3 doses had a relatively positive effect on the stomatal conductance when compared to the S_0 dose, with the S_2 dose as the biggest contributor (Figure 3). In the second year, the S doses had a negative effect, and thus, decreased the stomatal conductance. Even though the initial soil moisture values were close in both years, the big difference observed in the stomatal conductance was attributed to the difference in temperature, vapor pressure, rainfall amount and wind speed. When the stomatal conductance was below 200 mmol m^{-2} s^{-1}, no external intervention for the stress relief helped to increase the conductance.

In both years, during the flowering-boll development and boll opening periods (TOO), all the S doses were sufficient for the stress relief. However, with S_3 (the highest S dose), the stress was prolonged. Nevertheless, S_3 increased the stomatal conductance with OTT.

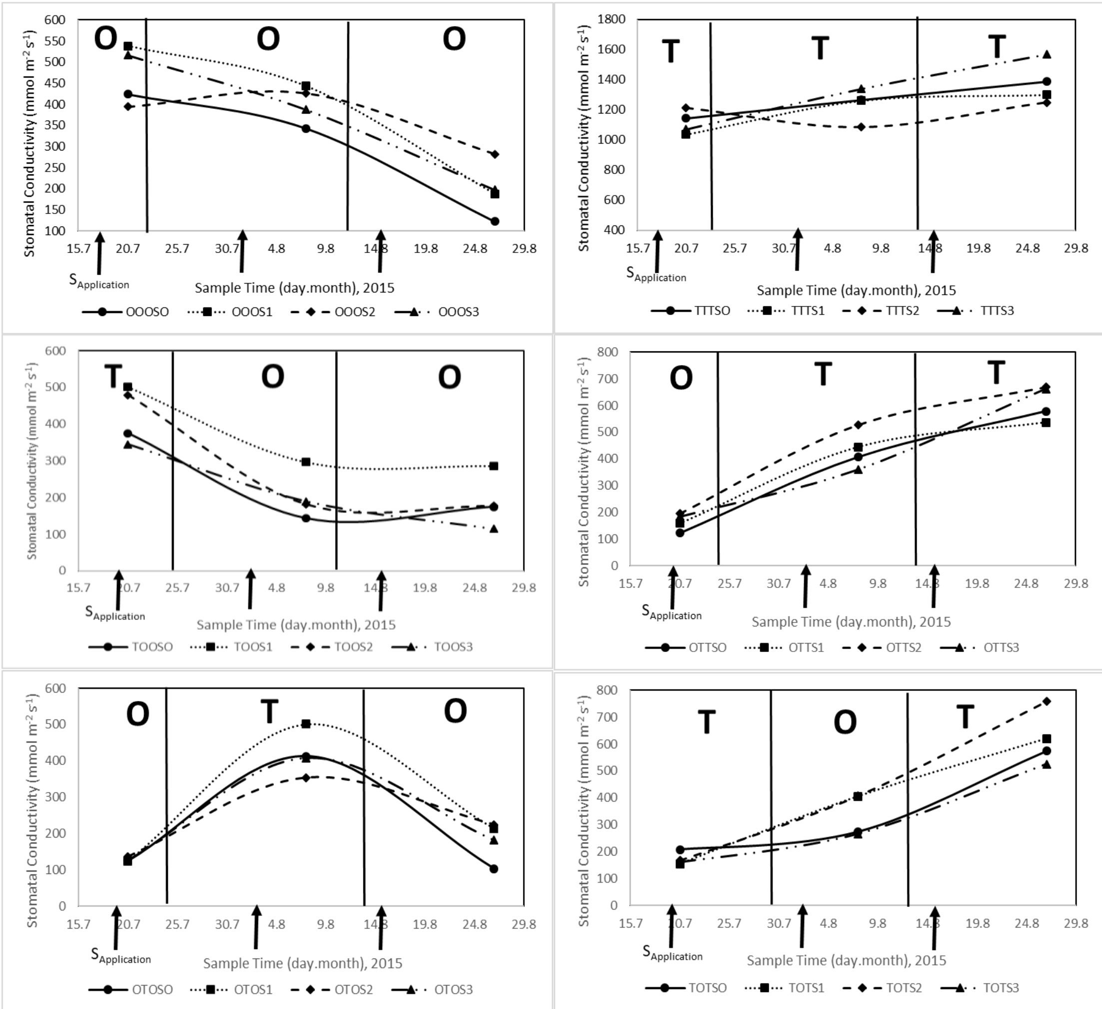

Figure 3. The effect of applied sulfur doses on stomatal conductance, 2015

In the first year, the S doses had no effect on the vegetative growth with OTO. However, S_3 increased the stomatal conductance to enable the plant to survive without stress until the harvest in the second year. In the second year, the lack of irrigation during the flowering and boll formation periods (2nd period) with TOT caused a stress and reduced the stomatal conductance (Figure 4). However, this expected effect was not observed in the first year, and the stomatal conductance rose at the time of stress thanks to S_1 and S_2 (Figure 3).

Overall, the foliar S applications (S_1: 150 ml da^{-1}; and S_2: 250 ml da^{-1} sufficiently alleviated the stress in the cotton under the prolonged stress, whereas S_3 further stressed the plant and decreased its stomatal conductance.

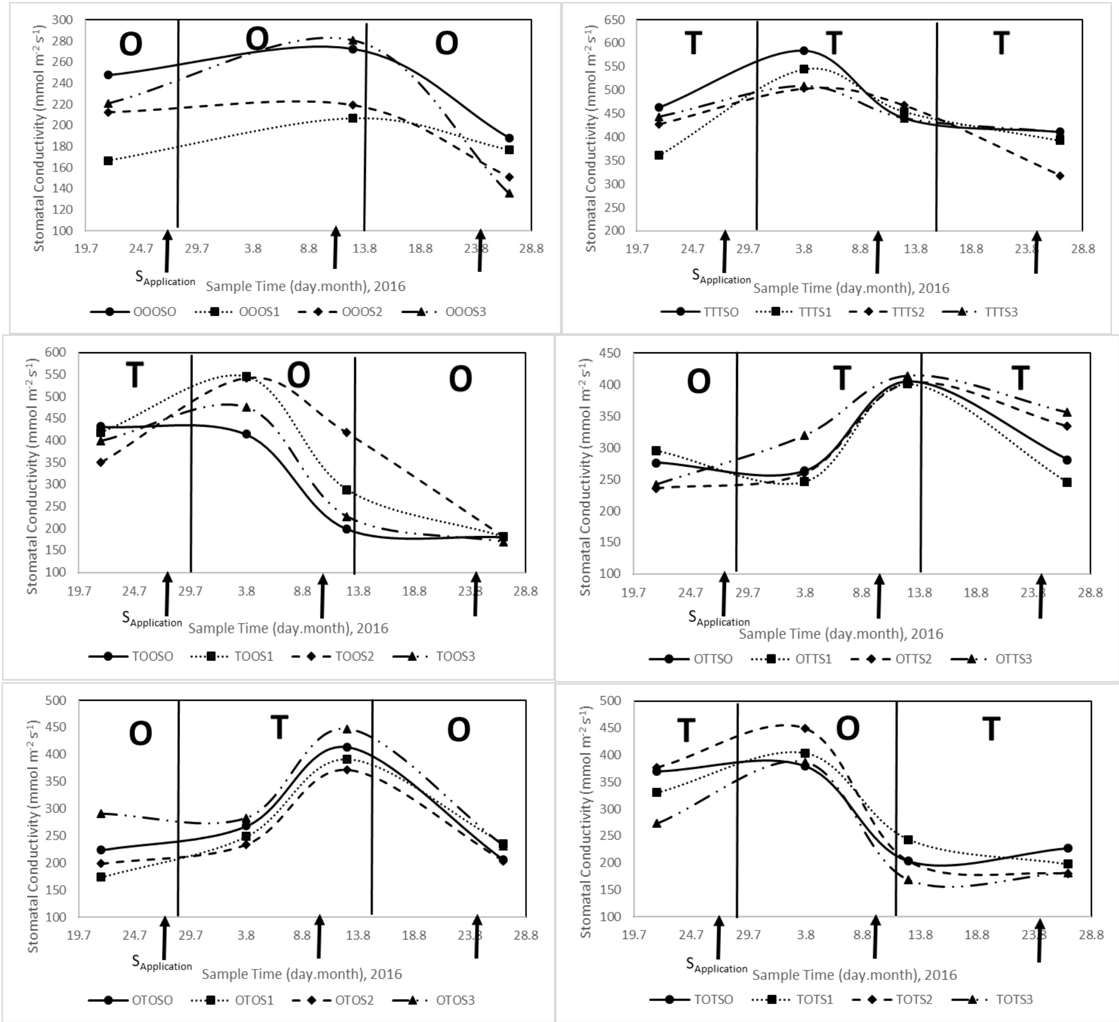

Figure 4. The effect of applied sulfur doses on stomatal conductance, 2016

CONCLUSION

The water stress sensitivity of the cotton changed according to its developmental stage. How to enhance its resistance to the soil water deficiency (water stress) during the developmental periods was not explored. In this experimental study, an irrigation strategy was designed to quantify the effect of the stress duration and severity on the stomatal conductance as well as of the foliar S application on the increased stomatal conductance for the different developmental stages of the cotton grown in the Mediterranean region. S_1 and S_2 doses had a more positive contribution on the stomatal conductance. The plant under the sufficient soil water content (TTT) could not open its stoma any further as a result of the foliar S application. On the contrary, the restrictive S layer on the leaf surface reduced the potential stomatal conductance rate. In conclusion, whatever the expected result may be, the S application with the irrigation at the field capacity level is not considered necessary.

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CONFLICT OF INTEREST

The authors declare no conflict of interest for this study.

AUTHOR’S CONTRIBUTIONS

The contribution of the authors is equal.

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