

PLANT GROWTH, PHYSIOLOGY, AND WATER STATUS OF SOUR ORANGE SEEDLINGS (*CITRUS AURANTIUM* L.) EXPOSED TO ANTITRANSPIRANTS AND THREE LEVELS OF IRRIGATION

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ABSTRACT

In the current study, the aim is to investigate regulation of water balance especially deteriorating in the heat conditions due to low water uptake and resistance to drought conditions by decreasing water deficit through transpiration in sour orange seedlings under arid or semi-arid conditions. The current study was conducted under greenhouse conditions, at three different water deficit cycles (once per 2 days, four days and eight days). As antitranspirant matters, silicon surfactant (0.01% and 0.02%), Fatty acids (0.2% and 0.4%) and PEG 6000 (polyethylene glycol) (5% and 10%) were applied through spraying the leaves. The effects of the water shortage and antitranspirant treatment on the plant height (cm), stem diameter (mm), leaf and root dry weight (%), total chlorophyll (chlorophyll a+b) ($\text{mg}\cdot\text{g}^{-1}$), carotenoid ($\text{mg}\cdot\text{g}^{-1}$), membrane permeability (%), relative water content (RWC) (%) and lipid peroxidation (MDA) ($\text{nmol}\cdot\text{g}^{-1}$) were studied. The results obtained revealed that increasing irrigation water interval, the plant height decreased to a great extent, when compared to more frequent irrigation. While PEG was found to be more effective in terms of increasing the plant height, fatty acid was found to be more effective in terms of increasing the stem diameter. Membrane permeability increased parallel to increasing drought. Both the antitranspirants and the drought conditions decreased RWC more than the control. While MDA decreased with antitranspirant treatments, it exhibited an important increase in 8-day water deficit cycle.

While different antitranspirants decreased transpiration, the plant growth was not negatively affected from this situation. It was observed that the use of antitranspirants in drought conditions resulted in positive outcomes in the reducing of leaf water loss.

KEYWORDS:

Antitranspirants, leaf water potential, citrus, drought, *Citrus aurantium*

INTRODUCTION

As known well, the extent to which fruit trees are affected from environmental conditions particularly from heat and drought conditions depending on their growth period. Especially when they are young plants, they are highly sensitive to heat stress and drought stress compared to fully grown trees. Therefore, in regions where there is a severe shortage of water, some precautions need to be taken to improve the resistance of young trees to drought stress, which enables the plant to make optimum and efficient use of the water and thus positively affects the growth of the plant.

As known, transpiration in plants has effects on the ascending movement of the water, absorption and transport of mineral nutritional elements and the plant surface temperature. One way of improving plants' resistance to heat is the reduction of transpiration [1]. Some measures have been suggested to protect plants from drought stress. This suggests that to prevent transplant shock by heat and drought it is desirable to prevent leaf dehydration [2]. Foliar application of antitranspirants is a promising tool for regulating transpiration to maintain a positive plant water status [3].

Present study reveals that leaf water content, dry matter content and growth rate of plant were markedly reduced under low water regime. It is therefore suggested that the improvement of growth and plant water status could be done through application of antitranspirants [4]. Antitranspirants have different modes of action [5, 6]. Antitranspirants are chemical compounds to reduce transpiration and maintain high plant water status. The compounds are generally classified under three groups according to their impact mechanisms; film forming mate-

rials, reflecting and physiological antitranspirants [7, 8].

Silicon and fatty acid which are reflecting type and PEG 6000 (polyethylene glycol) are film type antitranspirants. Kaolin clay and Chitosan are reflective materials and are used as antitranspirants [9, 10, 11, 12]. Emulsions of wax, latex, or plastics that dry on the foliage and form thin films can also minimize escape of water from the plant [8]. Silicon (Si) is one of the potential beneficial elements for the growth of plants [13, 14]. It has been reported that silicon increases plant resistance or tolerance in drought stress [15, 16]. Fatty acids, once applied as a liquid foliar spray, form a thin glassy film-coat, which reflects the excess light, which is going to heat up the tissues and thereby reduces the chances of leaf damages. When PEG is used in the rhizosphere area, it limits water transport and thus contributes to the formation of drought conditions [17, 18, 19, 20, 21, 22, 23, 24]. PEG is an acrylic polymer.

It was thought that the effect of PEG on the root will be the same for the plant leaf and so it was used as an antitranspirant in the current study.

The aim of this work was to determine the effects of drought stresses on growth, physiology and water status of sour orange seedling, and the responses of stress tolerance mediated by foliar application of antitranspirants. For this reason, the applications were performed in the seedling period when plants are the most sensitive towards drought and in summer season when the need for water is the highest.

MATERIALS AND METHODS

Experiments were conducted in a greenhouse at the Mugla City, during May and September 2014. As the plant materials in the study, 7-month sour orange seedlings (*Citrus aurantium* L.) were used. Seedlings were grown in 15-liter plant pots in a greenhouse. Until the applications were initiated, fertilizer and water needs of the plants were provided as homogenous. Plant pot medium consists of soil, peat, pumice and perlite with the ratios of 4:2:1:1 respectively. Each plant was treated with 50 g NPK compound fertilizer (N:18, P₂O₅:18 K₂O:18, B:0.01, Cu:0.01, Fe: 0.05, Mn:0.02, Mo:0.001, Zn:0.02). At the end of trial, nutritional element contents of plant growth media: total N (0.35%), available P (49.3 mg.kg⁻¹), K (204 mg.kg⁻¹), Ca (205 mg.kg⁻¹) and Mg (198 mg.kg⁻¹) were found to be at the sufficient levels.

The greenhouse temperature and humidity values (Hobo® data logger temperature/relative humidity (onset)) were measured by half an hour intervals.

The measurements conducted within the greenhouse between 07.04.2014 and 09.08.2014,

the maximum temperatures were measured to be 40.0-49.1 °C, mean temperatures were measured to be 28.0-35.8 °C and minimum temperatures were measured to be 20.0-30.2 °C. Furthermore, maximum relativity humidity was found to be 59.1-92.8%, mean relative humidity was found to be 22.4-67.4% and minimum humidity was found to be 8.0-35.1%.

The experiment was conducted using a completely randomized design. The experiment was designed as three replicates and each replicate was conducted with two different plant pots. The experiment was conducted at three different water deficit cycles (during transient 2,4 and 8-day water deficit cycles). Thus, with regular irrigation, it was created in limited water conditions.

The applied antitranspirants and their concentrations are given below:

1. Control (water)
2. Silicon Surfactant 1 (% 0.01) (Innogard 309®) (® Dow Corning organic silicon)
3. Silicon Surfactant 2 (% 0.02) (Innogard 309®) (® Dow Corning organic silicon)
4. Fatty Acids 1 (% 0.2) (® Green Miracle) (Butyric acid, Hexanoic acid (Caproid acid))
5. Fatty Acids 2 (%0.4) (® Green Miracle) (Butyric acid, Hexanoic acid (Caproid acid))
6. PEG 1 (% 5) (PEG 6000) (polyethylene glycol)
7. PEG 2 (%10) (PEG 6000) (polyethylene glycol)

The treatments were conducted at 25-day intervals and as three-time leaf spraying. As the possible highest PEG doses were considered to be implemented, a pretesting was conducted by using PEG 6000. 0.5, 1, 2, 5 and 10% concentrations were tried. As no negative effect such as defoliation, paleness, toxic effect etc. was detected, it was decided to use 5% and 10% concentrations in the actual application. Only water was sprayed to the control plants. The first application was conducted on 13 July 2014.

Until one day before the initiation of the application (12 July), all the pots were received equal amount of water. During the period when water need increased as a result of increasing temperature (since 12 July), restricted water treatments were initiated by using different irrigation intervals (once in 2, 4 and 8 days).

Six days after the start of antitranspirant treatments, plant samples were collected for the analysis. Sample collection was performed after the application of water stress. Thus, at the beginning of the application (7th day), in the middle of it (30th day) and at the end of it (55th day) (19 July, 12 August and 08 September, respectively), samples were collected three times. Sample collection was performed one day before irrigation.

In order to determine the antitranspirants effects on seedling growth features, plant height (cm), stem diameter (mm), and leaf and root dry weight (%) were determined. Plant height and stem diame-

ter were measured at the beginning and at the end of the trial. In order to determine leaf and root dry weight (%), leaf samples were taken from the middle of the shoot at the end of the trial. From each of the two plants, an entire leaf was collected and its fresh weight (FW) was recorded. Then, they were dried at 65 °C in stove and their dry weights were determined. For metabolic changes, total chlorophyll (chlorophyll a+b) (mg.g^{-1}), carotenoid (mg.g^{-1}), membrane permeability (%), relative water content (RWC) (mg.g^{-1}) and lipid peroxidation (MDA) (nmol.g^{-1}) values were determined. Total chlorophyll, carotenoid, membrane permeability and RWC values were calculated three times, once at the beginning, once in the middle and once at the end of the experiment, and lipid peroxidation value was calculated twice, once at the beginning and once at the end of trial. In the plants, leaf chlorophyll and carotenoid content was determined according to Strain and Svec [25], membrane permeability was determined according to Lutts et al. [26] and relative water content (RWC) was determined according to Yamasaki and Dillenburg [27]. MDA was analyzed following Cakmak & Horst [28] with some modifications as suggested by Weisany et al. [29].

In the analysis of the data, SAS statistical program package was used and comparisons between the means were made by using LSD test [30].

RESULTS

Plant Growth. Though not statistically significant, one of the plant growth features, plant height increased less with the application of antitranspirant substances when compared to the control. The highest change in the plant height was obtained through PEG 2. treatment (13.17 cm). The effect of water deficit cycle on plant height was found to be significant, with decreasing water deficit cycles, higher increase was obtained in plant height. Shoot growth (FW) was significantly suppressed by drought (8 day) stresses compared with wet soil moisture condition (2 day). The higher plant height increase was obtained as a result of 2-day interval watering (13.55 cm) (Table 1).

The interaction between antitranspirant substances and water deficit cycle was found to be significant in terms of height increase and the highest value was obtained for the control plants treated with 2-day water deficit cycle (18.5 cm). In 4-day and 8-day water deficit cycles, greater height increase was obtained with Silicon 2. (silicon's second concentrations) and PEG treatments compared to the control (Table 2).

While the effect of applied antitranspirant substances on the change of stem diameter was found to be non-significant, water deficit cycles caused significant effects. Moreover, the interaction between antitranspirant substances and water deficit cycles was found to be significant.

TABLE 1
The effect of different antitranspirants and water deficit cycles on plant height

Treatments	Plant height (cm)		Plant height change (cm)
	Time (days)		
	7	55	
Antitranspirants (A)			
Control	63.67 a	75.28 a	11.61 ab
Silicon Surfactant 1	59.56 ab	70.44 ab	10.89 ab
Silicon Surfactant 2	63.94 a	74.22 a	10.28 ab
Fatty Acids 1	61.00 ab	70.28 ab	8.22 b
Fatty Acids 2	58.28 b	67.11 b	8.83 ab
PEG 1	61.39 ab	74.56 a	13.17 a
PEG 2	57.50 b	69.11 ab	11.61 ab
Water deficit cycle (I)			
2 days	62.95 a	76.98 a	13.55 a
4 days	62.24 a	72.33 b	10.07 b
8 days	57.10 b	65.41 c	8.36 b
Significance			
A	N.S.	N.S.	N.S.
I	**	***	**
AxI	N.S.	N.S.	*

Means within columns followed by different letters are significantly different according to the t- test (LSD test; $p = 0.05$). N.S: Not statistically significant. The level of significance shown are * $p < 0.05$, ** $p < 0.01$ or *** $p < 0.001$.

TABLE 2
Interaction between plant height change and water deficit cycle

Treatments	Plant height change (cm)			Mean plant height change (cm)
	Water deficit cycle (day)			
	2	4	8	
Antitranspirants (A)				
Control	18.50±8.54	7.83±4.48	8.50±1.32	11.61 ab
Silicon Surfactant 1	18.33±3.69	10.66±2.25	3.67±1.89	10.89 ab
Silicon Surfactant 2	8.83±6.29	12.33±2.52	9.67±2.84	10.28 ab
Fatty Acids 1	9.83±3.88	10.33±0.58	4.50±1.73	8.22 b
Fatty Acids 2	15.17±8.08	4.67±2.93	6.67±3.75	8.83 ab
PEG 1	14.33±2.52	11.67±5.11	13.50±6.26	13.17 a
PEG 2	9.83±9.70	13.00±3.97	12.00±3.04	11.61 ab
Mean water deficit cycle (I)	13.55 a	10.07 b	8.36 b	*

Means values by different letters are significantly different according to the t- test (LSD test; $p = 0.05$). The level of significance shown are * $p < 0.05$.

TABLE 3
The effect of different antitranspirants and water deficit cycles on leaf and root dry weights (%)

Treatments	Leaf dry weight (%)	Root dry weight (%)
Antitranspirants (A)		
Control	35.97 ab	33.70 b
Silicon Surfactant 1	35.22 ab	34.44 b
Silicon Surfactant 2	36.03 a	36.58 ab
Fatty Acids 1	35.72 ab	37.29 ab
Fatty Acids 2	35.34 ab	40.67 a
PEG 1	35.00 ab	35.18 b
PEG 2	34.10 b	36.67 ab
Water deficit cycle (I)		
2 days	34.52 b	35.44 b
4 days	35.82 a	34.17 b
8 days	35.68 ab	39.47 a
Significance		
A	N.S.	*
I	N.S.	**
AxI	N.S.	N.S.

Means within columns followed by different letters are significantly different according to the t- test (LSD test; $p = 0.05$). N.S: Not statistically significant. The level of significance shown are * $p < 0.05$ or ** $p < 0.01$.

TABLE 4
The effect of different antitranspirants and water deficit cycles on leaf total chlorophyll and carotenoid values

Treatments	Total chlorophyll (mg.g ⁻¹)			Carotenoid (mg.g ⁻¹)		
	Time (days)			Time (days)		
	7	30	55	7	30	55
Antitranspirants (A)						
Control	3.18 c	2.93	3.60 a	0.19 b	0.18 b	0.25 a
Silicon Surfactant 1	3.67 ab	3.22	3.55 ab	0.25 ab	0.20 ab	0.24 ab
Silicon Surfactant 2	3.55 abc	3.40	3.30 abc	0.24 ab	0.24 a	0.20 bc
Fatty Acids 1	3.74 ab	3.07	3.21 abc	0.26 ab	0.21 ab	0.20 bc
Fatty Acids 2	3.93 a	3.10	3.18 bc	0.30 a	0.19 ab	0.18 c
PEG 1	3.45 bc	3.28	3.11 c	0.22 b	0.21 ab	0.19 bc
PEG 2	3.46 abc	3.29	3.23 abc	0.23 ab	0.20 ab	0.20 bc
Water deficit cycle (I)						
2 days	3.60	3.31	3.35	0.24	0.22	0.21
4 days	3.56	3.06	3.32	0.25	0.19	0.21
8 days	3.56	3.18	3.26	0.23	0.20	0.20
Significance						
A	N.S.	N.S.	N.S.	N.S.	N.S.	N.S.
I	N.S.	N.S.	N.S.	N.S.	N.S.	N.S.
AxI	N.S.	N.S.	N.S.	N.S.	N.S.	N.S.

Means within columns followed by different letters are significantly different according to the t- test (LSD test; $p = 0.05$). N.S: Not statistically significant.

TABLE 5
The effect of different antitranspirants and water deficit cycles on membrane permeability, relative water content (RWC) and lipid peroxidation (MDA)

Treatments	Membrane permeability (%)			RWC (%)			MDA (nmol.g ⁻¹ FW)	
	Time (days)						7	55
	7	30	55	7	30	55		
Antitranspirants (A)								
Control	26.71	22.24 ab	19.88 ab	63.05 a	68.01 a	78.28	2.27 b	3.38 a
Silicon Surfactant 1	23.38	21.89 ab	16.85 b	62.92 a	66.04 ab	80.94	2.15 b	3.10 ab
Silicon Surfactant 2	28.30	23.36 a	21.84 ab	63.77 a	64.03 abc	77.11	2.47 b	3.08 ab
Fatty Acids 1	28.75	19.82 bc	24.50 a	61.55 ab	57.82 d	77.45	2.34 b	2.85 ab
Fatty Acids 2	22.66	20.14 bc	18.10 ab	62.61 a	62.23 bcd	78.71	2.05 b	2.83 ab
PEG 1	22.47	22.57 ab	20.62 ab	57.94 b	63.57 abc	80.63	2.02 b	2.79 b
PEG 2	27.22	18.92 c	20.59 ab	57.33 b	60.29 cd	78.73	2.96 a	2.78 b
Water deficit cycle (I)								
2 days	21.76 b	19.64 b	19.85	61.51	65.73 a	80.37 a	2.11 b	2.91 b
4 days	27.02 a	22.04 a	19.10	60.53	61.19 b	76.11 b	2.16 b	2.72 b
8 days	28.15 a	22.16 a	22.08	61.89	62.93 ab	80.03 a	2.70 a	3.29 a
Significance								
A	N.S.	*	N.S.	*	**	N.S.	**	N.S.
I	*	**	N.S.	N.S.	*	**	***	**
AxI	N.S.	***	N.S.	N.S.	N.S.	*	N.S.	N.S.

Means within columns followed by different letters are significantly different according to the t- test (LSD test; p = 0.05).

N.S: Not statistically significant. The level of significance shown are *p < 0.05, **p < 0.01 or ***p < 0.001.

TABLE 6
Interaction between membrane permeability and water deficit cycle (30th day)

Treatments	Membrane permeability (%)			Mean membrane permeability (%)
	Water deficit cycle (day)			
Antitranspirants (A)	2	4	8	
Control	20.66±0.51	25.21±1.79	20.84±3.90	22.24 ab
Silicon Surfactant 1	22.31±1.49	22.66±4.21	20.70±4.48	21.89 ab
Silicon Surfactant 2	19.36±0.35	27.41±8.33	23.32±2.41	23.36 a
Fatty Acids 1	21.73±1.73	18.63±0.82	19.12±0.23	19.82 bc
Fatty Acids 2	19.60±0.62	14.86±1.54	25.96±1.58	20.14 bc
PEG 1	15.52±1.41	27.87±4.62	24.33±3.67	22.57 ab
PEG 2	18.29±1.74	17.64±2.01	20.84±2.97	18.92 c
Mean water deficit cycle (I)	19.64 b	22.04 a	22.16 a	***

Means values by different letters are significantly different according to the t- test (LSD test; p = 0.05).

The level of significance shown are *p < 0.05.

TABLE 7
Interaction between RWC and water deficit cycle (55th day)

Treatments	RWC (%)			RWC mean (%)
	Water deficit cycle (day)			
Antitranspirants (A)	2	4	8	
Control	76.40±2.29	80.17±4.02	78.28±1.84	78.28
Silicon Surfactant 1	78.96±0.54	82.24±3.54	81.61±3.69	80.94
Silicon Surfactant 2	81.03±5.97	69.15±3.30	81.15±2.75	77.11
Fatty Acids 1	76.63±7.45	74.80±10.73	80.93±0.55	77.45
Fatty Acids 2	81.90±3.20	75.50±1.15	78.73±2.08	78.71
PEG 1	82.80±4.64	76.66±1.16	82.44±3.60	80.63
PEG 2	84.89±4.24	74.23±0.48	77.07±4.63	78.73
Mean water deficit cycle (I)	80.37 a	76.11 b	80.03 a	*

Means values by different letters are significantly different according to the t- test (LSD test; p = 0.05).

The level of significance shown are *p < 0.05.

Leaf dry weight (%) was found to be non-significant; on the other hand, root dry weight (%) was found to be considerably higher in antitranspirant treatments compared to the control. In fatty acid 2. concentrations, the highest value was determined to be (40.67%) and relative to the control, by nearly 21% more increase was observed. Eight-day water deficit cycle resulted in higher root dry weight ratio (39.47%) than the other two water deficit cycles. Less watering increased root dry weight (%) to a considerable extent (Table 3).

Plant Physiology and Water Status. Total chlorophyll and carotenoid were not significantly affected either from antitranspirant treatments or from different water deficit cycles. On the other hand, the values obtained on 19 July and 12 August (7th and 30th days) as a result antitranspirant treatments were found to be higher compared to the control. In the 19 July (7th day) treatments, while fatty acids increased by %24 more than the control, in the 12 August (30th day) treatment, Silicon Surfactant 2 increased by 16% more than the control. In general, with increasing water deficit cycle, though small, a decrease was observed in chlorophyll and carotenoid contents (Table 4).

Though antitranspirant substances affected membrane permeability in different manners, this effect was in general found to be statistically non-significant. However, increasing drought conditions led to a significant increase in membrane permeability. RWC; on the other hand, recorded considerably lower values in the face of antitranspirants and increasing level of drought compared to the control. In the treatments, 10% PEG 7th day recorded 9% and 30th day recorded 11% lower value than the control. As a result of the treatment with antitranspirant substances, lipid peroxidation showed considerably lower values in general when compared to the control. Drought increase caused a considerable increase in MDA (Table 5).

The interaction between antitranspirant substances and water deficit cycle was found to be statistically significant in the middle of the treatment (30th day) in terms of membrane permeability (Table 6). The highest value was obtained with 4-day water deficit cycle in 5% PEG treatment (27.87%). With 2-day water deficit cycle, Silicon 0.01% and Fatty acid 0.2% showed higher values than the control but the other treatments showed lower values. In four-day water deficit cycle, Silicon 0.02% and PEG 5% were found to be higher compared to the control. In eight-day water deficit cycle, Silicon 0.02%, Fatty acid 0.4% and PEG 5% were found to be higher compared to the control.

For RWC level, interaction between antitranspirant substances and water deficit cycle was found to be statistically significant at the end of the treatment (55th day) (Table 7).

In this interaction, the highest value (84.89%) was obtained with 2-day water deficit cycle in 10% PEG treatment. In 2-day water deficit cycle, antitranspirant treatments increased RWC level more than the control. In four-day water deficit cycles, while Silicon 0.01% was found to be higher than the control, the other antitranspirant treatments showed lower values. In 8-day water deficit cycle, except PEG 10%, all the other antitranspirants were found to be higher values compared to the control.

DISCUSSION

The interaction between antitranspirant substances and water deficit cycle was found to be significant in terms of height increase; the highest value was obtained in the control plants treated with 2-day water cycle (18.50 cm). With 4 and 8-day water deficit cycles, larger height increase was obtained in Silicon 2. concentration and PEG treatments compared to the control. With increasing water deficit cycles, the plant height decreased to a great extent when compared to more frequent watering. In terms of plant height, substances used as antitranspirants did not negatively affect the plant growth. Similarly, in a study where Transfilm and Vapor Gard were used as antitranspirants, plant height was not affected significantly. They act as a physical barrier to water lost from leaves [3].

While among the antitranspirants, PEG was found to be more effective than the others in terms of plant height increase, in terms of stem diameter development, Fatty acid was found to be more effective. Root dry weight increased more by Fatty Acid 2 dose at the antitranspirant treatments and in drought conditions it has increased according to the control group. This shows that depending on the decrease in water deficit cycles, the ratio of water in the plant decreases. Similar findings were reported by Wu and Xia [31].

Though the changes in leaf chlorophyll and carotenoid values are statistically non-significant, in drought conditions lower values were obtained compared to the control. On the other hand, at the beginning and in the middle of the trial, with the effect of antitranspirants, higher values were obtained compared to the control but at the end of the treatment lower values were obtained. This shows that substances used as antitranspirants in drought conditions did not affect chlorophyll and carotenoid values negatively. Goreta et al., [3] also found that film-forming antitranspirants during water deficit stress did not significantly affect physiological parameters of pepper seedlings. In another study it was observed that as irrigation intervals were increased, the leaf chlorophyll content decreased [32]. In another study, it was determined that varieties of leaf of pomegranate total chlorophyll increased by the amount of irrigation [33]. The interaction be-

tween antitranspirant substances and water deficit cycle was found to be statistically significant in the middle of the experiment period (30th day) in terms of membrane permeability. Membrane permeability increased depending on drought increase. In a similar study, it was determined that kaolinite applied to Ruby red grapefruit trees as antitranspirant by foliar increased photosynthesis and water use efficiency [10]. In another study the results showed that stomatal conductance and transpiration were reduced, while AT (antitranspirant) impaired photosynthesis at standard. Drought had a minor impact on chlorophyll fluorescence. AT minimized the reductions in leaf water potential. AT could significantly improve drought tolerance in sweet pepper plants [34].

Plant water relations were determined by measurements of relative water content (RWC %). Both the applied antitranspirants and drought conditions decreased RWC more than the control. RWC level at the end of interaction between antitranspirant substances and water deficit cycle (55th day), were found to be statistically significant. In 2-day water deficit cycle, antitranspirant treatments increased RWC level more than the control. In 4-day water deficit cycle, while Silicon 0.01% was found to be higher compared to the control, the other antitranspirant treatments showed lower values. In 8-day water deficit cycle, except PEG 2, all the antitranspirants were found to be higher than the control. However, in a study, no improvement in RWC or reduction of cell membrane injury was found with the application of film-forming materials [3]. Drought stress caused a decrease in leaf water potential and stomatal conductance [35]. In another similar study reported that relative water content was significantly affected with the irrigation treatment irrespective of antitranspirant treatments. Reportedly in the works, the turgidity of leaves was much higher in normal irrigation throughout the growth period over limited water supply [4]. In the current study, while MDA decreased as a result of antitranspirant treatments, it exhibited a considerable increase in 8-day water deficit cycle.

As known well, as an indicator of oxidative stress, MDA levels are of great importance. Antioxidant enzymes play a role in drought resistance [16]. Therefore, reduction of MDA level by the applied antitranspirants can be evaluated as a positive valuable result.

Davenport et al. [5] reported similar results of increased water status by antitranspirants which might be due to reduced transpiration and increased stomatal resistance affecting membrane system and influx of water.

CONCLUSION

While the applied different antitranspirant substances decreased evapotranspiration, plant growth was not negatively affected. This shows that these substances can be used as antitranspirants in the citrus cultivation on the sour orange seedling by reducing the evapotranspiration in arid regions.

As PEG used to create drought conditions exhibited an effect similar to that of silicon and fatty acid, this substance can be used as antitranspirant. As a result, in future research higher concentrations of PEG should be tested.

REFERENCES

- [1] Kacar, B., Katkat, V. and Ozturk, S. (2010) Bitki Fizyolojisi. Nobel yayın dağıtım. 140-147.
- [2] Shinohara, T. and Leskovar, D.I. (2014) Effects of ABA, antitranspirants, heat and drought stress on plant growth, physiology and water status of artichoke transplants. *Scientia Horticulturae*. 165, 225–234.
- [3] Goreta, S, Leskovar, D.I. and Jifon J.L. (2007) Gas Exchange, Water Status, and Growth of Pepper Seedlings Exposed to Transient Water Deficit Stress are Differentially Altered by Antitranspirants. *Journal of the American Society Horticultural Science*. 132(5), 603-610.
- [4] Misra, A.K., Das, B.K., Datta, J.K. and De, G.C. (2009) Effect of Antitranspirants on Water Status and Growth Pattern of Mulberry (*Morus alba* L.) Under Two Levels of Irrigation. *Indian Journal Agricultural Research*. 43(4), 307 – 310.
- [5] Davenport, D.C., Hagan, R.M. and Martin, P.E. (1969) Antitranspirants: Uses and effects on plant life. *California Turfgrass Culture*. 19, 25–27.
- [6] Gale, J. and Hagan, R.M. (1966) Plant antitranspirants. *Annual Review of Plant Physiology*. 17, 269–282.
- [7] Moftah, A.E., and Al-Humaid, A.R.I. (2005) Effects of antitranspirants on water relations and photosynthetic rate of cultivated tropical plant (*Polianthes tuberosa* L.). *Polish Journal of Ecology*. 53(20), 165–175.
- [8] Nitzsche, P., Berkowitz, G.A. and Rabin, J. (1991) Development of a seedling-applied antitranspirant formulation to enhance water status, growth, and yield of transplanted bell pepper. *Journal of the American Society Horticultural Science*. 116(3), 405–411.
- [9] Bittelli, M., Flury, M., Campbell, G.S. and Nichols, E.J. (2001) Reduction of transpiration through foliar application of chitosan. *Agricultural and Forest Meteorology*. 107, 167–175.

- [10] Jifon, J.L. and Syvertsen, J.P. (2003) Kaolin particle film applications can increase photosynthesis and water use efficiency of 'Ruby Red' grapefruit leaves. *Journal of the American Society Horticultural Science*. 128(1), 107–112.
- [11] Iriti, M., Picchi, V., Rossoni, M., Gomarasca, S., Ludwig, N., Gargano, M. and Faoro, F. (2009) Chitosan antitranspirant activity is due to abscisic acid-dependent stomatal closure. *Environmental and Experimental Botany*. 66(3), 493–500.
- [12] Glenn, D.M. (2012) The Mechanisms of Plant Stress Mitigation by Kaolin-based Particle Films and Applications in Horticultural and Agricultural Crops. *Hortscience*. 47(6), 710–711.
- [13] Marschner, H. (1995) *Mineral Nutrition of Higher Plants*. London: Academic Press. 889p.
- [14] Epstein, E. (1999) Silicon. *Annual Review of Plant Physiology and Plant Molecular Biology*. 50, 641–664.
- [15] Gong, H., Chen, K., Chen, G., Wang, S. and Zhang, C. (2003) Effects of silicon on growth of wheat under drought. *Journal of Plant Nutrition*. 26, 1055–1063.
- [16] Gong, H., Zhu, X., Chen K., Wang S. and Zhang, C. (2005) Silicon alleviates oxidative damage of wheat plants in pots under drought. *Plant Science*. 169, 313–321.
- [17] Fernandez-Conde, M.E., De La Haba, P., Gonzales-Fontes, A. and Maldonado, J.M. (1998) Effects of Drought (Water Stress) on Growth and Photosynthetic Capacity of Cotton (*Gossypium hirsutum* L.). 5th Internet World Congress for Biomedical Sciences, Canada, December, 7–16.
- [18] Turkan, I., Bor, M., Ozdemir, F. and Koca, H. (2005) Differential Responses of Lipid Peroxidation and Antioxidants in the Leaves of Drought-Tolerant *P. acutifolius* Gray and Drought-Sensitive *P. vulgaris* L. Subjected to Polyethylene Glycol Mediates Water Stress. *Plant Science*. 168, 223–231.
- [19] Zgalli, H., Steppe, K. and Lemeur, R. (2005) Photosynthetic, Physiological and Biochemical Responses of Tomato Plants to Polyethylene Glycol-Induced Water Deficit. *Journal of Integrative Plant Biology (Formerly Acta Botanica Sinica)*. 47(12), 1470–1478.
- [20] Zgalli, H., Steppe, K. and Lemeur, R. (2006) Effects of different levels of water stress on leaf water potential, stomatal resistance, protein and chlorophyll content and certain anti-oxidative enzymes in tomato plants. *Journal of Integrative Plant Biology*. 48(6), 679–685.
- [21] El-Tayeb, M.A. (2006) Differential Responses of Pigments, Lipid Per-oxidation, Organic Solutes, Catalase and Per-oxidase Activity in the Leaves of Two *Vicia faba* L. Cultivars to Drought. *International Journal Agriculture and Biology*. 8(1), 116–122.
- [22] Kulkarni, M. and Deshpande, U. (2007) In Vitro screening of tomato genotypes for drought resistance using polyethylene glycol. *African Journal of Biotechnology*. 6(6), 691–696.
- [23] Marcinska, I., Czyczylo-Mysza, I., Skrzypek, E., Grzesiak, M.T., Janowiak, F., Filek, M., Dziurka, M., Dziurka, K., Waligorski, P., Juzon, K., Cyganek, K. and Grzesiak, S. (2013) Alleviation of Osmotic Stress Effects by Exogenous Application of Salicylic or Abscisic Acid on Wheat Seedlings. *International Journal of Molecular Science*. 14, 13171–13193.
- [24] Zekri, M. (1991) Effects of Peg- Induced Water Stress on Two Citrus Cultivars. *Journal of Plant Nutrition*. 14(1), 59–74.
- [25] Strain, H.H. and Svec, W.A. (1966) Extraction, separation, estimation and isolation of chlorophylls. In *the chlorophylls*. (Eds.: Vernon, L.P. and Seely, G.R.). Academic Press, N.Y., 21–66.
- [26] Lutts, S., Kinet J.M. and Bouharmont, J. (1996) NaCl-induced senescence in leaves of rice (*Oryza sativa* L.) cultivars differing in salinity resistance. *Annals of Botany*. 78, 389–398.
- [27] Yamasaki, S. and Dillenburg, L.R. (1999) Measurements of leaf relative water content in *Araucaria angustifolia*. *Revista Brasileira de Fisiologia Vegetal*. 11, 69–75.
- [28] Cakmak, I. and Horst, W.J. (1991) Effect of aluminium on lipid peroxidation, superoxide dismutase, catalase, and peroxidase activities in root tips of soybean (*Glycine max*). *Physiologia Plantarum*. 83, 463–468.
- [29] Weisany, W., Sohrabi, Y., Heidari, G., Siosemardeh, A. and Ghassemi-Golezani, K. (2012) Changes in antioxidant enzymes activity and plant performance by salinity stress and zinc application in soybean (*Glycine max* L.). *Plant Omics Journal*. 5, 60–67.
- [30] SAS Institute, (1989) *Inc.SAS/STAT user's guide: Version 6.0 Ed.*, SAS Institute Inc., Cary, NC.
- [31] Wu, Q.S. and Xia, R.X. (2005) Arbuscular mycorrhizal fungi influence growth, osmotic adjustment and photosynthesis of citrus under well-watered and water stress conditions. *Journal of Plant Physiology*. 163(2006), 417–425.
- [32] Demirtas, M.N. and Kirnak, H. (2009) Effects of different irrigation systems and intervals on physiological parameters in apricot. *Yuzuncu Year University Journal of Agricultural Science*. 19(2), 79–83.

- [33] Bahaulddin, A. and Hepaksoy, S. (2011) The Effect of Irrigation Doses on the Leaf Content of Different Pomegranate Varieties. MSc in Department of Horticulture Thesis. 67p.
- [34] Del Amor, F.M., Cuadra-Crespo, P., Walker, D.J., Camara, J.M. and Madrid, R. (2010) Effect of foliar application of antitranspirant on photosynthesis and water relations of pepper plants under different levels of CO₂ and water stress. *Journal of Plant Physiology*. 167(15), 1232-1239.
- [35] Guler N.S., Saglam, A., Demiralay M. and Kadioglu A. (2012) Apoplastic and symplastic solute concentrations contribute to osmotic adjustment in bean genotypes during drought stress. *Turkish Journal of Biology*. 36(2), 151-160.

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