

Effect of Water Stress on Growth and Physiology of Tomato (*Lycopersicon esculentum* Mill.)

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Abstract: The effects of drought on physiology of Tomato (*Lycopersicon esculentum* Mill.) cv., Farida grown in pots were studied. Drought stress was done by irrigating the plants with 80, 60 and 40% of water needed to reach soil pot capacity. Nutrients concentrations of the studied tomatoes grown in pots under different irrigation treatments varied according to water stress levels. The effect of water stress treatments on total chlorophyll, chlorophyll a, chlorophyll b and carotenoids resulted in significant increase with treatment 80% water deficit, then significant decrease in accumulation of pigments in response to water stress under 40 and 60% water deficit. All water stress treatments had highly significant effect on increasing accumulation of proline content. Drought stress induced different changes in the anti-oxidative enzyme activities, whereas, Ascorbate peroxidase and Catalase increased significantly with water stress from 0.58 and 140.7 Ug^{-1} FW in control to 4.08 and 414.9 Ug^{-1} FW in T4, respectively. In contrary, superoxide dismutase decreased significantly with water stress from 42.36 in T1, to 31.32, 33.54 and 31.51 in T2, T3 and T4, respectively. The results indicate that irrigation treatment with 80% of pot capacity (T2) was more effective than T3 and T4 from the viewpoint of more efficient water use, which would help to minimize drought damage and keep healthy plants in the same time saving irrigation water by 20%.

Keywords: Tomato, Water stress, Nutrients, Antioxidant enzymes, Pigments, Proline.

INTRODUCTION

Tomato (*Lycopersicon esculentum* Mill.) is one of the most popular and economically important vegetable crops all over the world. Tomato is an important source of antioxidants including, lycopene, phenolics, and vitamin C in human diet. The yield and quality of fresh tomato are governed by both genetic factors and environmental conditions. Among the environmental factors, water is a major component affecting plant growth and in turn, fruit quality of tomatoes. Therefore, optimum irrigation and proper drainage are critical to increase tomato yield and quality (Shao *et al.*, 2014). The reduction in growth, yield and quality by water stress has been well documented, although different physiological processes have been put forward to account for this reduction in different species. Although there have been many studies on the effects of water deficit on yield, comparatively few have addressed the relationship among yield, vegetative growth, and physiological responses to different irrigation regimes (Kirnak *et al.*, 2001). Plants experience water stress either when the water supply to their roots becomes limiting, or when the transpiration rate becomes intense. Plants try to adapt to drought stress with an array of biochemical and physiological interventions. Concerning water scarcity our country is dry, high temperature zone and faced with increased competitions for water resources between different sectors (agriculture, industry or domestic consumption). So, economic use of water in irrigation regimes is an essential matter. Optimizing irrigation water management for the crop production could result in increased productivity and water savings. This goal will be realistic only if appropriate strategies are found for water savings and for more efficient water uses in

agriculture. One important strategy is to better manage water and increase its productivity (Molden, 1997; Molden *et al.*, 2001). It is also necessary to develop new irrigation scheduling approaches, not necessarily based on full crop water requirement, but ones designed to ensure the optimal use of available water. A better understanding of the effects of drought on plants is vital for improved management practices and breeding efforts in agriculture under climate change. Many works were developed over the past couple of decades, covering subjects from plant strategies to control water status under drought, to the physiological and biochemical processes underlying plant response to water deficits (Chaves, 1991; Cornic and Massacci, 1996; Shao *et al.*, 2014). Tomato is regarded as a sensitive crop to water stress, revealing the high correlation between evapotranspiration (ET) and crop production, since ET is a direct measure of crop water loss (Nuruddin *et al.*, 2003). The supply of the required water to the tomato plants is crucial for its growth and economic production, especially in the greenhouse (Aziz *et al.*, 2013)

The present research was carried out to study the effect of drought stress on physiological characters of studied tomato, leaf chlorophyll, carotenoids, proline and enzyme activity, as well as, nutrients uptake.

MATERIALS AND METHODS

An experiment was carried out during the growing season of 2013 at the Agricultural Experimental and Research Station (Dirab), Faculty of Food and Agricultural Sciences – King Saud University, near Riyadh (24°42'N 44°46'E, 600 Alt.). The seeds of Tomato (*Lycopersicon esculentum* Mill.) cv., Farida were planted at nursery in glass house tell seedling

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reach 15 cm height (three foliage leaves) then transported to pots size 30 cm filled with sandy loam (1:1) in the outfield. Plants were fertilized by fifteen grams of compound (Nitrogen, Phosphorus and Potassium) N:P:K 15:15:15 to every pot twice in the life of tomato plants, the first one was before the beginning of flower stage and the second was after three weeks of the first one. Irrigating treatments started on 1st of March

2013 and the harvest were done on 1st of June 2013. Randomized complex block design was used. Soil physical and chemical properties measured according to (Cassel and Nilsen, 1986; Gee and Bander, 1986; Rhoades, 1982). Also, chemical properties of irrigation water were estimated according to (Richards, 1968) as shown in Table (1).

Table (1): Physical and chemical properties of soil and irrigation water.

	Irrigation water	Soil
pH	7.1	8.40
EC (dS/m)	7.0	9.1
Soluble Cations meq/L	Ca ²⁺	35.0
	Mg ²⁺	19.5
	Na ⁺	34.3
	K ⁺	2.0
Soluble Anions meq/L	CO ₃ ²⁻	0.125
	HCO ₃ ⁻	3.5
	Cl ⁻	56.3
	SO ₄ ²⁻	21.9
Saturation point %		27.3
Pot capacity (at 1 bar)		13.6
Wilting point (at 15 bars)		6.8

Irrigation treatments:

Irrigation treatments depended on measuring the deficit of soil moisture contents from the pot capacity, the potential evaporation was calculated using the Pan evaporation as described by (Doorenbos and Pruitt, 1977). Irrigation treatments included three levels of water deficit stress and the control, as follow control T1 (100% pot capacity), T2 (80% pot capacity), T3 (60% pot capacity) and T4 (40% pot capacity) respectively.

Nutrient elements were estimated according to (Bock, 1978). Regarding to Chlorophyll and carotenoid content, the extraction of leaf pigments was performed with 80% acetone, and the absorbance at 663 and 645 nm was measured with an Ultrospec 2100 pro spectrophotometer (Amersham Biosciences). Total carotenoid content, chlorophyll a, chlorophyll b, and total chlorophyll quantities were calculated according to

the method of (Arnon 1949) using the following formula:

$$\text{Total Chl (a and b) (mg/l)} = 20.2 (A_{645}) + 8.02 (A_{663})$$

$$\text{Chl a (mg/l)} = 12.7 (A_{663}) - 2.69 (A_{645})$$

$$\text{Chl b (mg/l)} = 22.9 (A_{645}) - 4.68 (A_{663})$$

$$\text{Car (mg/l)} = A_{480} \times 200$$

The pigment concentration was calculated in mg/g FW of samples.

Extraction and estimation of free proline were conducted according to the procedures described by (Bates et al., 1973). Plant tissues (0.5 g) were homogenized with 5 ml of 3% sulfosalicylic acid and the homogenates were centrifuged at 3000×g for 20 min. In a test tube, 1 ml of the supernatant was mixed with 1 ml acid ninhydrin reagent and 1 ml of glacial acetic acid and incubated in 100°C in water bath for 1 h,

and then the absorbance at 520 nm was determined. Free proline content in sample is estimated by referring to a standard curve made from known concentrations of proline and the results were expressed as $\mu\text{mol proline g}^{-1}$ FW (fresh weight).

For extraction and analysis of antioxidant enzymes, leaf samples (0.5 g fresh weight, FW) were homogenized in 3 ml extraction buffer (0.1 M potassium phosphate buffer (pH 7.0), 1mM EDTA, 0.05% Triton X-100) in a pre-chilled pestle and mortar, centrifuged at 15,000 g for 20 min at 4°C. The supernatant was used for the estimation antioxidant enzyme activities. Superoxide dismutase activity (SOD EC 1.15.1.1) activity was estimated by the ability of this enzyme to inhibit the photochemical reduction of nitro blue tetrazolium salt (NBT). The SOD activity was measured at 560 nm according to the method of (Beyer and Fridovich, 1987) and the assays were carried out at 25°C. The final assay volume of 3 ml contained 50 mM Tris-HCl buffer (pH 7.8), 33 μM NBT, 10 mM L-methionine, 0.66 mM disodium EDTA, 0.0033 mM riboflavin and 50 μl of supernatant. Reaction was started under fluorescent light for 15 minutes by adding 10 μl of riboflavin solution. One unit of SOD was defined as the amount of enzyme activity that inhibited the photo reduction of NBT to blue formazan by 50%. The SOD activity of each extract was expressed as Ug^{-1} FW. Peroxidase (POX) activity was estimated by the method of (Sakharov and Ardila, 1999). Peroxidase enzyme activity was determined by the oxidation of guaiacol in the presence of H_2O_2 . The increase in absorbance was recorded at 470 nm for 1min with a spectrophotometer. The reaction mixture contained 50 μl of 28 mM guaiacol, 900 μl of 50 mM potassium phosphate buffer (pH 6.0), 50 μl of 5 mM H_2O_2 , and 10 μl of crude extract. POX activity of the extract was expressed as the POX Ug^{-1} FW. Catalase (CAT EC 1.11.1.6) activity was measured spectrophotometrically according to the method of (Aebi, 1983) The disappearance of H_2O_2 at 240 nm in a reaction mixture containing 50 mM sodium phosphate buffer (pH 7.0), 20 mM H_2O_2 and 5 μl of supernatant. The decrease in the absorption was followed for 1min at 240 nm, and 1 mmol H_2O_2 oxidized $\text{ml}^{-1} \text{min}^{-1}$ was defined as 1 U of CAT activity. Ascorbate peroxidase (APX EC 1.11.1.11) activity was measured according to the methods of (Wang *et al.*, 1991). The reaction mixture contained 50 mM potassium phosphate buffer (pH 7.0), 0.5 mM ascorbic acid, 0.1 mM hydrogen peroxide, and 20 μl of enzyme extract in a total volume of 1 ml. The concentration of oxidized ascorbate was calculated by the decrease in absorbance at 290 nm. The absorption coefficient was $2.8 \text{ mM}^{-1} \text{ cm}^{-1}$. One unit of APX was defined as $1 \text{ mmol ml}^{-1} \text{ ascorbate oxidized min}^{-1}$.

Data obtained were subjected to statistical Analysis Of Variance (ANOVA), according to (Gomez and Gomez, 1984). Averages of the main effects were compared using the revised Least Significant Difference test (LSD) at 0.05 level of probability. Computations and statistical analysis were done using SAS.

RESULTS AND DISCUSSION

Studied plants content of Potassium (K), Phosphorous (P) and Nitrogen (N) were measured, Table (2). Results show that highest average of potassium (11.37 and 11.33 mg/g DW) was obtained from irrigation treatment with 60% and 100% of pot capacity (T3 and T1 treatments), while the lowest (03.14 mg/g DW) was recorded by the irrigation treatment with 40% of pot capacity (T4). (Farghali *et al.*, 1988) found that total K^+ content in Cotton seedlings roots and aerial parts increased progressively with decreasing water stress, particularly under light condition. There was significant difference in phosphorous content between treatments, and the highest was in T4 (58.37 mg/g DW), while the lowest was in T3 (28.77 mg/g DW). Similar trend was obtained with carbon content and nitrogen percentage. The effect of water stress treatments on total chlorophyll, chlorophyll a, chlorophyll b and carotenoids resulted in significant (at $P \leq 0.05$) starting with increase in accumulation of pigments with treatment two (irrigation with 80% of pot capacity) then tend to decrease as response to increasing water stress. Total chlorophyll was (0.84, 1.71, 0.56, and 0.50 mg/g FW) in T1, T2, T3 and T4 respectively. Regarding to chlorophyll a data obtained were (0.60, 1.18, 0.41 and 0.94 mg/g FW) for T1, T2, T3 and T4 respectively. While, chlorophyll b was 0.23, 0.53, 0.15 and 0.21 mg/g FW for T1, T2, T3 and T4, respectively. (Kirnak *et al.*, 2001) found that total chlorophyll content in water stress treatment was reduced by 55% compared to control treatment. Carotenoids decreased in response to water stress from 0.53 mg/g FW in T2 to 0.15 and 0.21 mg/g FW in the T3 and T4 treatments.

The data of analysis of variance showed that, all water stress treatments had highly significant effect on increasing accumulation of proline content. Data showed that proline was 4.36, 7.14, 10.33 and 8.36 $\mu\text{mol g}^{-1}$ FW in T1, T2, T3 and T4 respectively. In response to water stress, proline accumulation generally occurs in the cytosol where it plays significant role in cytoplasmic osmotic adjustment (Anjum *et al.*, 2012). In this study, higher level of proline accumulation in T2 over T3 and T4 enabled the water-stressed plants to maintain low water potentials.

Data in Table (2) show the effect of three water stress treatments on four enzymes content in studied tomatoes plants. Peroxidase was 46.54 Ug^{-1} FW in control treatment (T1), in treatment two (T2, 20% water deficit) Peroxidase was 53.82 Ug^{-1} FW, while it decreased dramatically (8.61 and 5.26 Ug^{-1} FW) in T3, (40% water deficit) and T4 (60% water deficit). Ascorbate peroxidase and Catalase increased significantly with water stress from 0.58 and 140.7 Ug^{-1} FW in control to 4.08 and 414.9 Ug^{-1} FW in T4 respectively. In contrary, superoxide dismutase decreased significantly with water stress from 42.36 in T1, to 31.32, 33.54 and 31.51 in T2, T3 and T4 respectively. Drought stress induced different changes in the anti-oxidative enzyme activities (Anjum *et al.*, 2012). Moreover, it has been reported that peroxidase,

and catalase activities showed an increase or maintenance in the early phase of drought and then a decrease with progression of water stress. It is conspicuous that higher levels of antioxidants are related to plant drought tolerance (Tahi *et al.*, 2008). Superoxide dismutase is considered to form the first line of protection against reactive oxygen species (ROS), which catalyzed the superoxide radical (O_2^-) to O_2 and H_2O_2 which are further quenched by diverse antioxidant enzymes.

From the results, it can be concluded that there are different physiological changes in response to drought stress treatments. The results indicate that irrigation treatment with 80% of pot capacity (T2) was more effective than T3 and T4 from the viewpoint of more efficient water use, which would help to minimize drought damage and keep healthy plants in the same time saving irrigation water by 20%.

Table (2): Some physiological characters of studied tomato in response to water deficit.

Treatments	K	P	C	N (%)	Total Chlorophyll (mg/g FW)	Chlorophyll a (mg/g FW)	Chlorophyll b (mg/g FW)	Carotenoids (mg/g FW)	Proline ($\mu\text{mol g}^{-1}\text{FW}$)	Peroxidase (Ug^{-1}FW)	Ascorbate peroxidase (Ug^{-1}FW)	Catalase (Ug^{-1}FW)	Superoxide dismutase (Ug^{-1}FW)
	(mg/g DW)												
T1	11.33	44.50	37.33	5.44	0.84	0.60	0.23	6.03	04.36	46.54	0.58	140.7	42.36
T2	03.47	36.20	33.57	5.36	1.71	1.18	0.53	8.97	07.14	53.82	0.69	119.2	31.32
T3	11.37	28.77	35.23	3.69	0.56	0.41	0.15	5.94	10.33	08.61	1.27	126.8	33.54
T4	03.13	58.37	39.73	5.75	0.50	0.29	0.21	7.03	08.36	05.26	4.08	414.9	31.51
L.S.D. at 0.5%	0.410	0.440	1.555	1.44	0.089	0.276	0.090	0.088	0.209	0.220	0.166	1.06	0.618

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تأثير الإجهاد المائي على فسيولوجي ونمو نبات الطماطم

إبراهيم بن عايد المحيسن

قسم الأحياء - كلية العلوم والدراسات الإنسانية بالقويعة - جامعة شقراء

تمت دراسة أثر الجفاف علي بعض الصفات الفسيولوجية لنبات الطماطم، صنف فريدة المنزرعة في أصص. وقد تم إجهاد الجفاف عن طريق ري النباتات بـ ٨٠٪ و ٦٠٪ و ٤٠٪ من المياه اللازمة للوصول إلى السعة القصوي للوعاء. وجد تباين في تركيزات العناصر المغذية في النباتات محل الدراسة وفق معاملات الري المختلفة وفقا لمستويات الإجهاد المائي. أثرت معاملات الإجهاد المائي على الكلوروفيل الكلي، كلوروفيل أ، كلوروفيل ب والكاروتينات، وأحدثت في البداية زيادة معنوية مع المعاملة الثانية (٢٠٪ نقص في مياه الري عن سعة الوعاء)، أعقبه انخفاض كبير في تراكم الصبغات تبعاً لزيادة الإجهاد المائي في المعاملات الثالثة والرابعة (٤٠٪ و ٦٠٪ نقص في مياه الري عن سعة الوعاء). كان لكل معاملات الإجهاد المائي تأثير كبير للغاية على زيادة محتوى النباتات المعاملة من البرولين. الإجهاد الناتج عن الجفاف سبب تغييرات مختلفة في أنشطة الإنزيمات المضادة للأكسدة. وتشير النتائج إلى أن معاملة الري بـ ٨٠٪ من السعة الحقلية للوعاء (T2) أكثر فعالية من T3 و T4 من وجهة نظر كفاءة استخدام المياه. التي من شأنها أن تساعد على تقليل الضرر الناتج عن الجفاف والحفاظ على النباتات بحالة جيدة وفي نفس الوقت توفير مياه الري بنسبة ٢٠٪.