PHYSIOLOGICAL CHANGES OF CULTIVATED EGGPLANT (*SOLANUM MELONGENA* L.) IN RESPONSE TO WATER DEFICIT

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Abstract. The effects of drought on the physiology of eggplants (*Solanum melongena* L. cv., Madona) were studied. Drought stress treatment had been done by irrigating the plants with 80%, 60% and 40% of water needed to reach soil pot capacity, plus normal irrigation (control treatment). The objective of this research was to reveal physiological response of eggplant to different levels of drought stress, as well as, its nutrient. The obtained results showed that, nutrients concentrations of the studied eggplants grown in pots under different irrigation treatments decrease according to water stress levels. Drought stress significantly reduced the amount of total chlorophyll, chlorophyll a, chlorophyll b and total carotenoids resulting in damaged leaves. Carotenoids decreased in response to water stress from 8.65 mg/g FW under control in comparison to 5.84 mg/g FW the fourth water deficit treatment. All water stress treatments increased the accumulation of proline content in plant. Lack of water in plant modified anti-oxidative enzyme activities in order to alleviate the water stress. The results indicate that irrigation treatment with 80% of pot capacity have more efficient water use than 60% and 40%, which reduces drought stress and improves ionic balance of the plants.

Keywords: eggplant, water stress, nutrients, antioxidant enzymes, pigments, proline

Introduction

Eggplant (*Solanum melongena* L.) is a popular nightshade crop plant commonly cultivated in tropical and subtropical farmland. The stunted growth, lack of yield and poor quality of plant product caused by water stress has been well studied by researchers and agriculturists, and many physiological processes involved for this reduction in variable crop plants. Many studies has been carried out in Asia, Africa, Europe and different region on eggplant irrigation (Gaveh et al., 2011; Karam et al., 2011) showing that eggplant can be produced at moderate levels of water stress without severe effect on fruit yield. There is a complex relationship between yield, physiological responses and vegetative growth to different level of water stress, especially in eggplant under semi-arid conditions (Kirnak et al., 2001). Plants experience water stress during intense transpiration rate or when there is limited water supply around their roots. Plants attempt to alleviate the negative effect of drought stress using a set of biochemical and physiological mechanisms.

Concerning water scarcity, Saudi Arabia is an arid high temperature zone and faced with increased competitions for water resources between different sectors (agriculture, industry or domestic consumption). So economic and effective irrigation regimes is an important matter to solve.

High yield of crop production and water savings are some of the benefits in optimizing irrigation water management. Appropriate strategies in agriculture practices are need to be found to get efficient use of limited water in semi arid zones. Abd El-Ghany et al. (2004) stated that the identified varieties that had high and/or moderate

yield potential besides desirable specific adaptation traits are useful for drought stress and could be combined to achieve optimum performance. One important strategy is to better manage water and increase its productivity (Molden, 1997; Molden et al., 2001; Gupta et al., 2016; Tani et al., 2018). Effective irrigation scheduling needs to be designed to ensure the optimal use of limited water. Díaz-Pérez and Eaton (2015) indicated that eggplant may tolerate moderate drought stress, since plants watered at 67% Evapotranspiration level (Etc). A comprehensive knowledge of drought stress effect on plants is critical to enhanced management practices and crops breeding efforts in agriculture under semi arid condition. Many works in this field were developed over the past twenty years, covering topics from plant approaches to control water status under drought, to the physiological and biochemical processes underlying plant response to water deficits (Chaves, 1991; Cornic and Massacci, 1996; Kurniawati, 2014; Buhtz et al., 2017). Kurniawati (2014) found that during the drought treatment, plant growth, biomass, media water content, relative water content, chlorophyll content concentration were reduced while proline and polyamines were increased and reached the high levels on 14 to 21 days after the treatment.

The present research was carried out to study the effect of drought stress on physiological characters of studied eggplants, i.e., leaf chlorophyll, carotenoids, proline and enzyme activity, as well as, some nutrients (K, P, C and N) uptake.

Materials and Methods

Within the experimental researches to evaluate many of solanaceae plants at the Agricultural Experimental and Research Station (24°42'N 44°46'E, 600 Alt.) in Dirab region, Saudi Arabia, during the spring growing season (March to June), it is worth mentioning like research Almohisen (2015). Seeds had been planted at nursery in greenhouse until seedlings have three foliage leaves and reached 15 cm shoot height, then were transported to 30 cm pots size (30 cm top diameter, 25 cm base diameter and 30 cm pot height) after filled with sandy loam (1:1) media in the outfield. Irrigating treatment started on 1st of March and the harvest were done on 1st of June. All agronomy practices and plant protection had been done according to the agricultural region. Randomized complex block design with three replications were used. Soil physical and chemical properties measured according to Cassel and Nielsen (1986), Klute et al. (1986) and Rhoades (1982). Also, chemical properties of irrigation water were estimated according to Bock (1984) as shown in *Table 1*.

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 Richards (1969). Irrigation treatments included 4 levels of water deficit stress that were control T1 (100% pot capacity), T2 (80% pot capacity), T3 (60% pot capacity) and T4 (40% pot capacity).

Physiological parameters

Elements were estimated according to Doorenbos (1975). 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:

$$A_{663} = 82.04 \text{ Chl}_a + 9.27 \text{ Chl}_b \quad (\text{Eq.1})$$

$$A_{645} = 16.75 \text{ Chl}_a + 45.6 \text{ Chl}_b \quad (\text{Eq.2})$$

where A is the density values at the respective wavelengths as obtained on the spectrophotometer and Chl_a, Chl_b are grams per liter of chlorophyll a and b, respectively. From the solving previous equations we will obtain the following:

$$\text{Chl a (mg/l)} = 12.7 (A_{663}) - 2.69 (A_{645}) \quad (\text{Eq.3})$$

$$\text{Chl b (mg/l)} = 22.9 (A_{645}) - 4.68 (A_{663}) \quad (\text{Eq.4})$$

$$\text{Total Chl (a and b) (mg/l)} = 20.2 (A_{645}) + 8.02 (A_{663}) \quad (\text{Eq.5})$$

Then carotenoid content can be calculated as following:

$$\text{Car (mg/l)} = A_{480} \times 200 \quad (\text{Eq.6})$$

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

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⁺	3.6	35.0
	Mg ²⁺	0.5	19.5
	Na ⁺	3.2	34.3
	K ⁺	0.114	2.0
Soluble Anions meq/L	CO ₃ ⁻²	0	0.125
	HCO ₃ ⁻	1.0	3.5
	Cl ⁻	4.7	56.3
	SO ₄ ⁻²	1.71	21.9
	Saturation point %		27.3
	Pot capacity (at 1 bar)		13.6
	Wilting point (at 15 bar)		6.8

Extraction and determination of proline were directed according to the techniques 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. One ml of the supernatant was mixed with 1 ml acid ninhydrin reagent and 1 ml of glacial acetic acid in a test tube and incubated in 100°C in water bath for 1 h, and then the absorbance value at 520 nm was noted. Free proline content in sample is determined by referring to a proline standard curve of known concentrations and the results were expressed as μmol 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 pH 7.0 potassium phosphate buffer, 1 mM 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 impede the photochemical decrease of nitrobluetetrazolium salt (NBT). The SOD activity was measured at 560 nm according to the technique 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 μ M NBT, 10 mM L- methionine, 0.66 mM disodium EDTA, 0.0033 mM riboflavin and 50 μ l of supernatant. Reaction was underway under fluorescent light for 15 minutes by adding 10 μ l of riboflavin solution. One unit of antioxidant 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 1 min with a spectrophotometer. The reaction mixture contained 50 μ l of 28 mM guaiacol, 900 μ l of 50 mM potassium phosphate buffer (pH 6.0), 50 μ l of 5 mM H_2O_2 , and 10 μ l 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 (1974). The disappearance of H_2O_2 were determined at 240 nm wavelength in a mixture containing 50 mM sodium phosphate buffer (pH 7.0), 20 mM H_2O_2 and 5 μ l of supernatant. The decrease in the absorption was followed for 1 min 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) content was determined according to the means of Wang et al. (1991). The mixture contained in a test tube, 50 mM pH 7.0 potassium phosphate buffer, 0.5 mM ascorbic acid, 0.1 mM hydrogen peroxide, and 20 μ l of enzyme extract were added 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 counted as 1 mmol ml^{-1} ascorbate oxidized min^{-1} .

Statistical procedures

Data obtained were subjected to statistical Analysis Of Variance (ANOVA), according to Gomez and Gomez (1984). Averages of the main effects of stress water irrigation were associated using the revised Least Significant Difference test (LSD) method at 0.05 probability level. Computations and statistical analysis were done using Statistical Analysis System computerized package program (SAS).

Results and discussion

The statistical analysis of variance for the resulting data showed that, the four water stress levels had significant differences on the all physiological studied traits except nitrogen percentage in regard to the examined genotype eggplant (Table 2). Studied plants content of Potassium (K), Phosphorous (P), Carbon (C) and Nitrogen (N) were measured. Results show that highest average of potassium (14.30 mg/g DW) was obtained from irrigation treatment with 80% of pot capacity (T2), while the lowest

(6.13 mg/g DW) was recorded by the irrigation treatment with 60% of pot capacity (T3) without any significant differences with (T4) 40% of pot capacity (6.23 mg/g DW). There was significant difference in Phosphorous content between treatments, highest was in T2 by (54.9 mg/g DW), while the lowest was (19.53 mg/g DW). Similar trend was obtained with carbon content, where the highest value (37.61 mg/g DW) was obtained by (T2) and the lowest value (31.54 mg/g DW) was obtained by (T4). The water stress treatments resulted in no significant (at $P < 0.05$) regarding to nitrogen percentage in all treatments. Eggplant total yield affected by leaf N and decreased at the lowest irrigation rate 33% Etc (Díaz-Pérez and Eaton, 2015). In general, T2 shows the highest values of mineral content NPK. Farghali and El-Sharkawl (1988) found that total K^+ content in Cotton seedlings roots and aerial parts increased progressively with decreasing water stress, particularly under light condition. Data are agree with Kirnak et al. (2001) whose, found that nutrient concentrations in mature leaves of eggplants grown in pots under different irrigation treatments decrease according to water stress levels. In addition, Almohisen (2015) found that similar trend with Carbon, Potassium, Phosphorous content and Nitrogen percentage in tomato.

The effect of water stress treatments on total chlorophyll, chlorophyll a, chlorophyll b and total carotenoids resulted in significant decrease (at $P < 0.05$) in accumulation of pigments. Total chlorophyll was (1.17, 0.70, 0.92, and 0.76 mg/g FW) in T1, T2, T3 and T4 of water stress, respectively. Kirnak et al. (2001) found that total chlorophyll content in water stress treatment was lowered by 55% compared to control treatment. Regarding to chlorophyll a data obtained was (0.80, 0.36, 0.36 and 0.30 mg/g FW) for the four water deficit treatments; T1, T2, T3 and T4, respectively. The highest value of chlorophyll b was 0.38 mg/g FW in both T1 and T4, on the other side, T3 gave the lowest value (0.38 mg/g FW). Chlorophyll indexes have been used as subsidiary estimators of chlorophyll content and leaf nitrogen concentrations (Liu et al., 2006). Carotenoids decreased dramatically in response to water stress from 8.65 mg/g FW in T1 (control treatment) to 5.84 mg/g FW in the fourth treatment (the most stress).

Regarding the proline content, as mentioned in the first paragraph of results and discussion, the analysis of variance data showed that, the three water stress treatments (40, 60, 80%) had significant effect on increasing proline accumulation compared to control. Data showed that proline was 3.63, 6.24, 5.42 and 5.42 $\mu\text{mol g}^{-1}$ FW in T1, T2, T3 and T4, respectively. In response to water stress, proline accumulation usually occurs in the cytosol which plays a significant role in adjustment of cytoplasmic osmotic (Anjum et al., 2012; Almohisen, 2015). In this study, the higher levels of proline accumulation in T4 followed by T3 then T2 enabled the water-stressed plants to maintain low water potentials. Data in Table 2 show the effect of four water stress treatment on four enzymes content in eggplants. Peroxidase was 15.77 Ug^{-1} FW in control treatment (T1, 0% water deficit), in treatment number two (T2, 20% water deficit) Peroxidase was 2.64 Ug^{-1} FW, while it was 36.76 and 12.45 Ug^{-1} FW in (T3, 40% water deficit) and (T4, 60% water deficit), respectively. Ascorbate peroxidase and Catalase decreased significantly with water stress from 1.76 and 430.15 Ug^{-1} FW in control treatment (T1) to 0.54 and 109.45 Ug^{-1} FW in T4 (the high level from water scarcity), respectively. On the other hand, superoxide dismutase values varied significantly, where the accumulation of superoxide dismutase was 21.66, 28.49, 14.56 and 27.37 Ug^{-1} FW in T1, T2, T3 and T4, respectively. On the other hand, superoxide dismutase values varied significantly, where the accumulation of superoxide dismutase was 21.66, 28.49, 14.56 and 27.37 Ug^{-1} FW in T1, T2, T3 and T4, respectively.

Table 2. Some physiological characters of studied genotype eggplant in response to water deficit

Chapter	K	P	C	N%	Total Chlorophyll	Chlorophyll a	Chlorophyll b	Carotenoids	Proline	Peroxidase	Ascorbate peroxidase	Catalase	Superoxide
Treatments	(mg/g DW)				(mg/g FW)	(mg/g FW)	(mg/g FW)	(mg/g FW)	($\mu\text{mol g}^{-1}$ FW)	(Ug^{-1} FW)	(Ug^{-1} FW)	(Ug^{-1} FW)	(Ug^{-1} FW)
T1	6.90	23.70	36.71	1.68	1.17	0.80	0.38	8.65	3.63	15.77	1.76	430.15	21.66
T2	14.30	54.90	37.61	1.75	0.70	0.36	0.33	8.76	6.24	2.64	1.13	383.46	28.49
T3	6.13	29.33	35.71	1.73	0.92	0.63	0.28	6.85	5.42	36.76	0.63	239.26	14.56
T4	6.23	19.53	31.54	1.32	0.76	0.30	0.38	5.84	8.11	12.45	0.54	109.45	27.37
F test	*	*	*	ns	*	*	*	*	*	*	*	*	*
L.S.D at 0.5%	0.353	0.602	1.858	--	0.127	0.005	0.024	0.118	0.152	0.359	0.107	1.696	0.650

T1: 100%, T2: 80%, T3: 60%, T4: 40%, of pot capacity *: significant at 5% level of probability and ns: not significant

Drought stress brought different variations in the anti-oxidative enzymes activities (Anjum et al., 2012). Moreover, it has been reported that peroxidase, and catalase activities showed an increased or stabled in the early level of drought and then decreased with progression of water stress. It is conspicuous that, higher levels of antioxidants are interrelated to tolerance of plant toward water stress (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.

These significant differences between all values for leaf chlorophyll, carotenoids, proline, enzyme activity and nutrients elements as impact to water stress treatments that may be reflecting the role of these components as a biological regulator in plant (El-Saidy et al., 2011; Ahanger et al., 2016), and may be due to the roll of each one in metabolism system in plant, plus the partial inhibition co-with vitamins of a few interaction in reactive oxygen species production (Ratnakar and Rai, 2013; Ahanger et al., 2017).

Conclusion

In Considering the previous results as a whole and in regard to the examined genotype, it can be concluded that, there are different physiological changes for all studied tratis exeptonly one (N contant) in response to drought stress treatments. The results indicate that irrigation treatment with 80% of pot capacity (T2) more effectively than 60% of pot capacity (T3) and 40% of pot capacity (T4) from the standpoint of more efficient water use. Which would help to diminish drought injury, retain healthy plants to achieve optimum performance and adaptation under drought stress and saving irrigation water usage by 20%.

Change history: The online version of this paper was modified on 27th April 2020. In the following sentence of the Abstract the last percentage data was mistyped (it was corrected from 20% to 40%): “Drought stress treatment had been done by irrigating the plants with 80%, 60% and 40% of water needed to reach soil pot capacity, plus normal irrigation (control treatment).”

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