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# Effect of the Root Zone Temperature and Salt Stress on Plant Growth, Main Branches and some other Chemical Characteristics of Tomato Fruit

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**Abstract:** In order to study the impact of salt stress (0, 1.5, 3 and 6) ds.m$^{-1}$ in nutrient's solution on tomato plant (*Solanum lycopersicum* L. cv. memory) at different root zone temperature [low (20°C), medium (25°C) and high (30°C)], an experiment was carried at Department of Horticultural, Ferdowsi University of Mashhad, Islamic Republic of Iran. The result showed that low and high root zone heating decreased leaf area, total sugar and phenol content compared to root zone temperature 25°C (optimum), while main branches number, pH, E.C. and anthocyanin of fruit increased at high root zone temperature compared to low root zone temperature. Flavonoid increased under the root zone temperature of 20°C in comparison with temperatures 25 and 30°C, and stem diameter was not affected by root zone heating. Furthermore, salt stress at the level of 3 ds.m$^{-1}$ increased stem diameter, total sugar, pH and EC of fruit, leaf area and phenol content, whereas salt stress at a high level (6 ds.m$^{-1}$) increased flavonoid content. Besides, anthocyanin content decreased in control and salt stress at 6 ds.m$^{-1}$ when compared to salt stress at 3 ds.m$^{-1}$.

**Key words:** Root zone temperature, Tomato fruit, Salt stress.

## Introduction

The temperature of the root-zone influences the growth and chemical composition of many plants (Yan*et al*., 2013). It was previously shown that using deep flow technique (DFT) hydroponics root-zone temperatures modulate the production of sugar and polyphenols in carrots and red leaf lettuce (Sakamoto & Suzuki, 2015a). While, in strawberry plants, Kim *et al.* (2009) reported that heating of cultivation media increased the development of flower bud initiation during the low temperature season, and finally enhanced fruit yield. However, the application of temperature stress in root zone resulted in an alteration in the production of

some secondary metabolites under greenhouse conditions (Chadirin *et al.*, 2011).

Salt stress effects on plant growth are associated with osmotic and ionic stresses and to specific ion toxicity, which is manifested at physiological, biochemical and molecular levels (Munns & Tester, 2008; Gupta & Huang, 2014). Reactions of tomato to salinity within its salt-tolerance range under natural conditions have been extensively investigated but only a few researchers have studied the effect of higher salt concentrations on tomato responses (Maggio *et al*., 2014). Subjecting the plants to 'shock treatments' at very high NaCl concentrations, well above those they encounter in the field, may cause adaptation mechanisms that cannot be observed at low or moderate levels of salinity. Moreover, there are only a few papers reporting the effects of both salinity and drought on the same plant material(Giannakoula & Ilias, 2013). Salt stress causes unfavourable effects on both plant growth and fruit enlargement in tomatoes. But in contrast it has been reported that salinity at a moderate level can improve fruit quality by affecting the levels of soluble solids, such as sugars and acids, as well as the pH value; and all these are important factors in quality evaluations of fruit sold in markets. This phenomenon has been attributed to a "concentration effect" that results from the suppression of fruit enlargement in soluble solids plants exposed to salt stress. However, during the past decade, increasing evidence has indicated that changes in assimilatory metabolism and the translocation of assimilates into the fruit are likely to be the reasons for the increase in soluble solids and other components(Saito & Matsukura, 2015). In African snake tomato, increasing the root-zone temperature increased the contents of phenols, ascorbic acid, and chlorophylls in the leaves (Adebooye*et al.*, 2010).

The aim of thecurrent study was to investigate the effect of optimum level of EC in nutrient solution and root-zone temperature on flavonoid content nm/g(FW), total sugars, fruit phenol content mg/100 g (FW), EC of fruits, pH of fruits, anthocyanin n/mg (FW), leaf area ($cm^2$), average number of branches and stem diameter of tomato grown hydroponically under greenhouse conditions.

## Materials & Methods

### Culture of Seed and Seedling translocation

This study was conducted in the Department of Horticulture, Ferdowsi University, Mashhad, Islamic Republic of Iran. Seeds of F1 tomato, cultivar (Memorycultivar) were sowing in 2×2×2 cm sponge cubes containing vermiculite on first of January 2016,under greenhouse conditions. The half-strengthnutrient solution was used for feeding tomato seedlings. 30 days after the culture, the fully-grown seedlings were transferred to a hydroponic system with a distance of 25 cm apart. Plant density was 4.0 plants /$m^2$.A special plastic container with the height and length of 30 and 50 cm, respectively, was prepared for performing transactions on plants. Inside the container, a very transparent plastic cylinder with the height and diameter of 25 and 20 cm, respectively, was placed so root zone temperature can be adjusted well. The cylinder was then filled with water. The electrical heater using for aquarium was placed in water for setting root zone temperature. Finally, and in order to prevent or reduce water loss through evaporation process, the plastic cylinder was closed by a special cover. Thereafter, the plastic container was filled with perlite and coco peat at the ratio of 50:50. 48 hours after warming the water inside the cylinder plastic, temperature transferred to the growth medium by heat exchange. Root zone temperature in the

growth medium was measured by using a digital thermometer. It was found that temperatures in the agricultural medium were similar to the hot water in the plastic cylinder. Root zone temperature was set at three levels of 20, 25 and 30 ± 2°C.

**Measurement**

The greenhouse was heated by the hotwater system. The values related to E.C., pH, and volume of the inflow nutrient solution were recorded daily using a handheld EC and pH meters, while the values related to influx nutrient solution were recorded weekly. During the experiment, care was taken to control pests and diseases. The data of solar radiation and humidity outside greenhouse were obtained from weather institutions of Mashhad.Within the greenhouse, there were four experimental blocks, each containing 12 experiments.EC and RZT treatments were randomly distributed within each block.

**Measurment of pH and EC of fruit (fuit juice)**

For measuring the pH and E.C. of fruit juice, 2 g of fruit pulp was taken and then the extraction was performed. The extract was then diluted by using distilled water in theproportion of 1:10 (fruit juice:distilled water). Finally, pH and E.C. were measured by using pH meter and handheld EC meter, respectively.

**Measurment of total soluble sugars**

For measurement of soluble solids, 500 mg fruit sample was first weighted, and then the extract was extracted during two steps by using 10 ml of 95% ethanol and centrifuged at 3500 rpm for 15 min. Next, 3 ml of Anthrone reagent was added to the samples. Finally, after applying a10-min temperature of hot water, the rate of light absorbance was measured by using spectrophotometer at 430 nm.(DuBois *et al*., 1956; Al Hassan*et al.*,2015)**.**

**Measuring the amount of anthocyanin in fruit**

In order to measure the amount of anthocyanin's in fruits, the method described by Wagner & Wüthrich (1979) was used. To do so, 0.1 g of fresh plant tissue was crushed in a mortar with 10 ml of methanol acid (pure methanol and pure chlorhydric acid in the volume ratio of 1:99), and the extract obtained was placed in the dark in the fridge for 24 hours. Then, the supernatant was carefully separated, and the absorption rate was measured by using spectrophotometer at the wavelength of 512 nm. The concentration was calculated by using the following formula and taking into account the extinction coefficient equal to 33,000 cm/molar.

$$A= \varepsilon bc$$

Where A:is absorption, b : is the width of the cell in spectrophotometer device, and c:is the concentration of solution(Wagner & Wüthrich, 1979).

**Measurement of total phenol in fruit**

To measure total phenol, first, 100 mg of plant sample was extracted with 10 ml of solvent (methanol or ethanol) and the obtained solution was diluted by using the same solvent at a ratio of 1 to 100. Then,1.2 ml of 7.5% Sodium Carbonate and 1.5 ml of 10% Folin Ciocalteu were added to 300 µl of the diluted extract. After being placed in the dark for 30 min, the samples were measured at the wavelength of 765 nm. The standard curve of this trait was plotted at concentrations of 0, 3, 5, 8, 10 and 12 mg per litre (Leja *et al*., 2013).

**Measurement of total flavonoids of fruit**

The aluminium chloride colorimetric method was used for quantifying the amount of total flavonoid. To do so, 0.1 g of plant tissue was extracted by using10 mm methanol. 0.5 mm of the obtained extract was brought up to the volume of5 mm by using distilled water. In the next step,0.3 mm of 5% NaNo2 and, after 5 minutes,0.6 mm of 10% AlCl3 were added to the obtained solution. Finally, 2 mm 1-molar NaOH and 2 mm distilled water was added to the final solution, and the absorption rate was measured at the wavelength of 510 nm. Samples concentration was obtained by using quercetin standard curve (Toor & Savage, 2005).

**Leaf area.** To measure leaf area, 5 leaves were randomly selected from each plant at the end of the experiment, and the assessment was performed by using Area Measurement System-Conveyor Belt Unit in the Department of Agronomy, Faculty of Agriculture, Ferdowsi University. Stem diameter was also measured by caliper at the end of the experiment.

**Stem diameter**: Recorded stem diameter by a caliper. And it recorded three areas from plant stem and took the final rate.

**Branches number**: Refers to the mean number of branches produced by sampled plants and was calculated by dividing the total number of branches counted from the sampled plants to the number of sampled plants to get mean branches number per plant.

## Results

**The Main effect of root zone heating**

Based on the results, root zone heating had a significant effect on leaf area so that temperature25$^{o}$C increased leaf area and temperatures (20 and 30) $^{o}$C decreased the index (Table 1). Stem diameter did not show significant changes in different levels of temperature (Table 1). Main branch number decreased in root zone temperatures of 20 and 25◦C compared to the root zone temperature of 30◦C (Table 1). Regarding total sugars and phenol content in fruits, there were affected differences among different levels of temperatures. The aforementioned parameters decreased in both root zone temperatures of 20 and 30$^{o}$C when compared to optimum root zone heating of 25$^{o}$C (Table 1). Moreover, Anthocyanin did not showed any significant effect in compared with flavonoids content of fruits. Where, greater flavonoids level were obtained under 20 degree of heating zone (Table 1). In addition, E.C. and pH in fruits increased in root zone heating of 30$^{o}$C compared to the optimum temperature of 25$^{o}$C (Table 1). A greater level of anthocyanin was found in root zone heating of 30$^{o}$C in comparison with root zone heating of 20◦C (Table 1).

**The Main effect of salt stress.**

With respect to the effect of salt stress on leaf area, a significant increase was observed in both salt stress levels of 3 and 6 ds.m$^{-1}$ compared to the control (Table 2). Stem diameter increased in the salt stress level of 3 ds.m$^{-1}$ compared to the treatment containing salt stress at 6 ds.m$^{-1}$level;however,the change was not significant(Table 2).Salt stress at the level of 1.5 ds.m$^{-1}$resulted in an increase in the number of main branches compared to the control and salt stress of 6 ds.m$^{-1}$(Table 2). Total sugars of fruit decreased by the highest salt stress (6 ds.m$^{-1}$) compared to the lowest salt stress level of 3 ds.m$^{-1}$ (Table 2). Phenol content showed a significant increase in the salt stress level of 3 ds.m$^{-1}$ compared to the control (Table 2).The treatment containing salt stress level of 6 ds.m$^{-1}$had the highest

**Table (1): The main effect of different salt stress on some characteristics of tomato.**

| RZT (°C) | Leaf area cm | Stem diameter cm | Number of main branches | Total sugars | Phenol content of 100 /fruit(mg g(FW) | EC | pH | Anthocyanin (n/mg (FW)) | Flavonoid content of fruit g(FW)/(nm |
|---|---|---|---|---|---|---|---|---|---|
| 20 | 28.07 b | 11.16 a | 15.88 b | 0.311 a | 0.024 a | 172.38 ab | 4.04 a | 2.14 a | 8.351 a |
| 25 | 33.10 a | 12.20 a | 16.16 b | 0.346 a | 0.034 a | 166.60 b | 3.97 a | 2.16 a | 7.088 a |
| 30 | 30.64 ab | 11.43 a | 18.53 a | 0.309 b | 0.030 a | 178.16 a | 4.20 a | 2.54 a | 7.988 a |

**Within a column means followed by the same letter are not significantly different at P < 5% according to the least significant difference test. Low temperature (20°C), optimum temperature (25°C) and high-temperature stress (30°C).**

**Table (2): The main effect of different salt stress on some characteristics of tomato.**

| Salt stress $ds.m^{-1}$ | Leaf area cm | Stem diameter cm | Number of main branches | Total sugars | Phenol content of 100 /fruit(mg g(FW) | EC | pH | Anthocyanin (n/mg (FW)) | Flavonoid content of fruit g(FW)/(nm |
|---|---|---|---|---|---|---|---|---|---|
| Control | 27.19 b | 11.84 a | 16.04 c | 0.331 b | 0.044 b | 151.26 b | 3.80 b | 1.78 b | 7.113 b |
| 1.5 | 29.05 ab | 11.69 a | 18.54 a | 0.318 b | 0.050 b | 177.14 a | 4.19 a | 2.37 ab | 7.230 b |
| 3 | 33.21 a | 12.04 a | 17.33 b | 0.391 a | 0.074 a | 181.58 a | 4.15 a | 2.96 a | 7.625 ab |
| 6 | 32.97 a | 10.84 b | 15.50 c | 0.249 c | 0.064 a | 179.54 a | 4.14 a | 2.01 b | 9.268 a |

**Within a column means followed by the same letter are not significantly different at P < 5% according to the least significant different test.**

level of flavonoid (Table 2) showed pH and E.C. increased in salt stress levels of 1.5 and 3 ds.m$^{-1}$ compared to the control. The results in Table (2) showed that anthocyanin decreased in control and salt stress level of 6 ds.m$^{-1}$ compared to thesalt stress level of 3 ds.m$^{-1}$.

**The interaction effect of salt stress and root zone temperature**

The results in fig.(1) showed that the interaction between salt stress and root zone heating caused an increase in leaf area. The greatest leaf area was observed in $T_7$ (salt stress level of 3 ds.m$^{-1}$and root zone heating of 20$^{o}$C), while the lowest amount was seen in $T_1$ (a salt level of 0 ds.m$^{-1}$and root zone temperature of 20$^{o}$C). The results also showed that combination of the salt level of 3 ds.m$^{-1}$and root zone heating of 25$^{o}$C ($T_8$)increased stem diameter, while the combination of the salt level of 1.5 ds.m$^{-1}$and root zone temperature of 30$^{o}$C($T_6$) had the lowest stem diameter (Fig. 2). As seen in Fig. (3), the highest number of main branches was observed in $T_6$ (a salt level of 1.5 ds.m$^{-1}$and root zone heating of 30$^{o}$C), whereas the lowest branch number was due to $T_1$ (control (0 ds.m$^{-1}$) and root zone temperature of 20$^{o}$C).

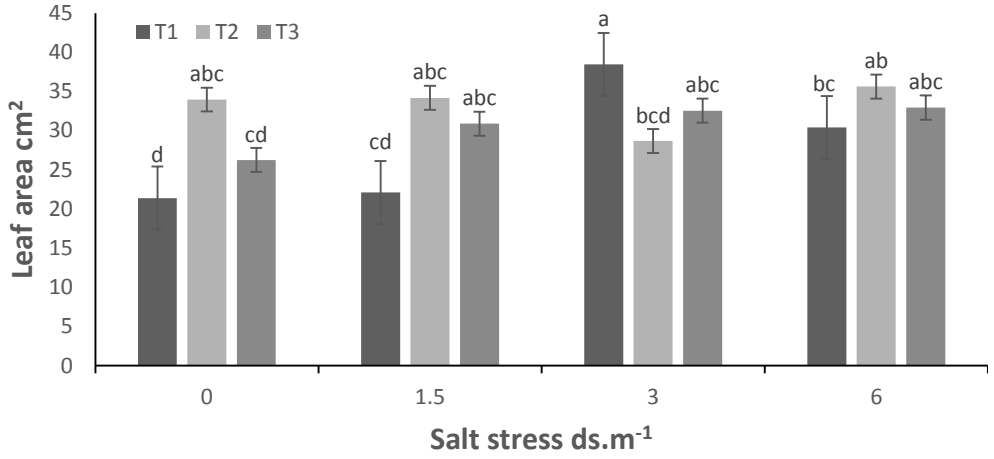


**Fig. (1): The interaction effect of root zone temperature and different levels of salt stress on the leaf area cm$^2$. Low temperature (T1 = 20°C), optimum temperature (T2 = 25°C), and high temperature stress (T3 = 30°C).**

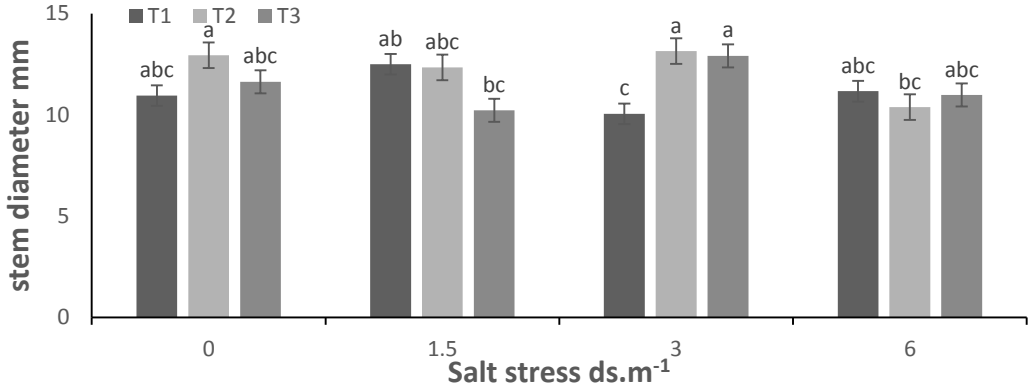


**Fig. (2):The interaction effect of root zone temperature and different levels of salt stress on the stem diameter mm. Low temperature (T1 = 20°C), optimum temperature (T2 = 25°C), and high temperature stress (T3 = 30°C).**

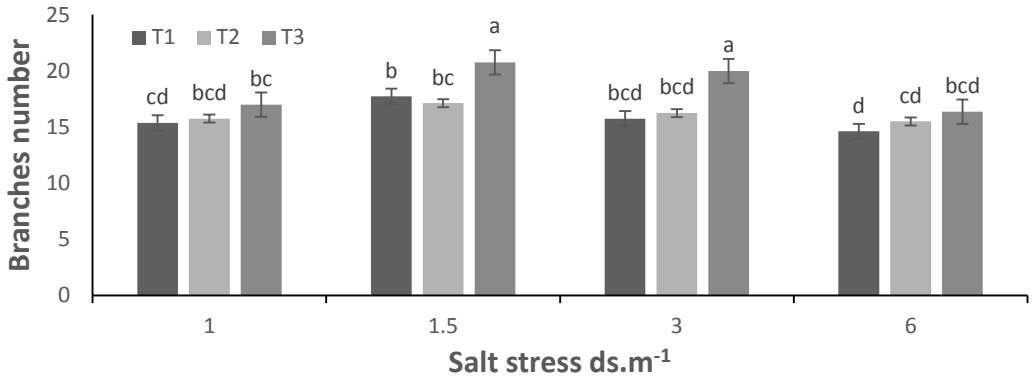


**Fig. (3): The interaction effect of root zone temperature and different levels of salt stress on the branches number. Low temperature (T1 = 20°C), optimum temperature (T2 = 25°C), and high temperature stress (T3 = 30°C).**

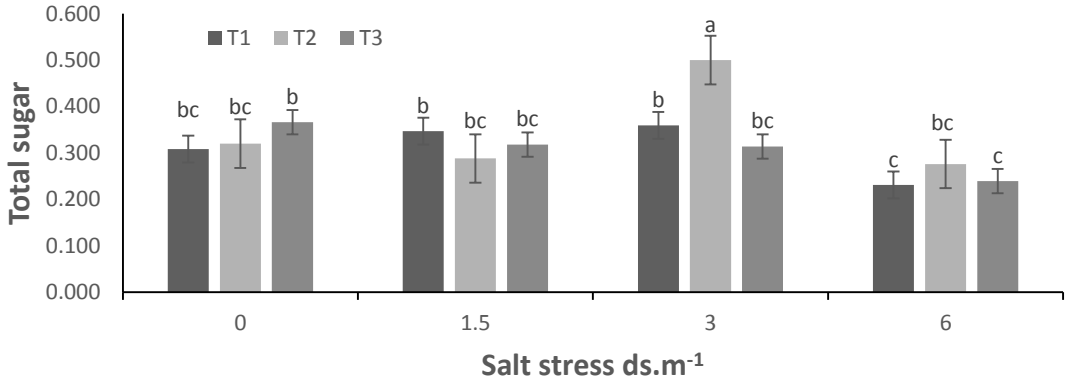


**Fig. (4): The interaction effect of root zone temperature and different levels of salt stress on the total sugar content of fruit. Low temperature (T1 = 20°C), optimum temperature (T2 = 25°C), and high temperature stress (T3 = 30°C).**

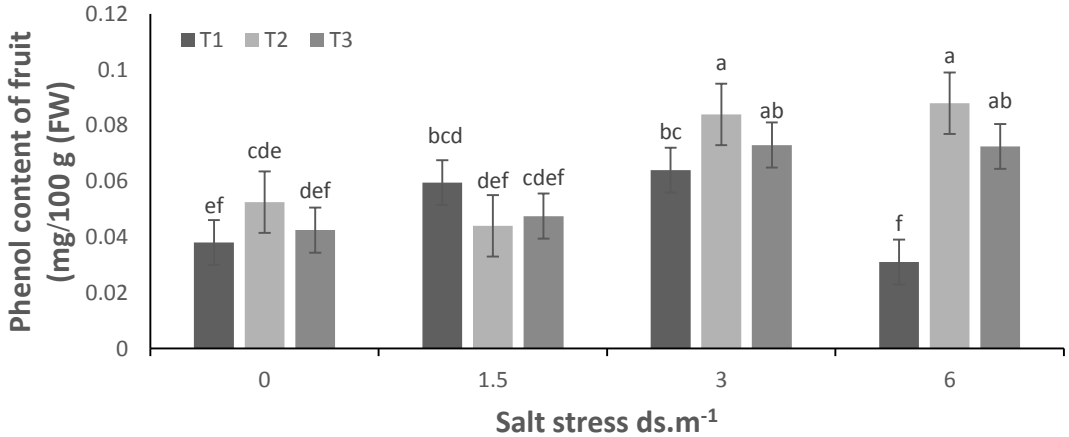


**Fig. (5): The interaction effect of root zone temperature and different levels of salt stress on the Phenol content of fruit (mg/100 g (FW) of fruit. Low temperature (T1 = 20°C), optimum temperature (T2 = 25°C), and high temperature stress (T3 = 30°C).**

Total sugars were significantly increased in $T_8$ (3 ds.m$^{-1}$and root zone heating of 25$^o$C) compared to $T_{10}$ (6 ds.m$^{-1}$and root zone heating of 20$^o$C) (Fig. 4).

Phenol content of fruit increased in $T_8$, $T_9$, $T_{11}$ and $T_{12}$(salt level of 3 ds.m$^{-1}$and root zone heating of 25$^o$C, salt stress 6 ds.m$^{-1}$ and root zone heating 20$^o$C,salt stress 6 ds.m$^{-1}$ and root zone heating 25$^o$C and salt stress 6 ds.m$^{-1}$ and root zone heating 30$^o$C) respectively, among which $T_{11}$ had the highest phenol content (Fig. 5). The results also showed that flavonoid content decreased in treatment containing a salt level of 1.5 ds.m$^{-1}$and root zone heating of 25$^o$C ($T_5$) compared to $T_{10}$ (salt stress 6 ds.m$^{-1}$ and root zone heating 20$^o$C)(Fig. 6). Furthermore, regarding pH, a significant effect was found by the interaction between salt stress and root zone heating, so that the highest amount of pH was observed in $T_6$ (salt level of 1.5 ds.m$^{-1}$and root zone heating of 30$^o$C), and the lowest rate was seen in $T_2$ (control (0 ds.m$^{-1}$) and root zone heating of 25$^o$C) and $T_1$ (control (0 ds.m$^{-1}$) and root zone heating of 20$^o$C).

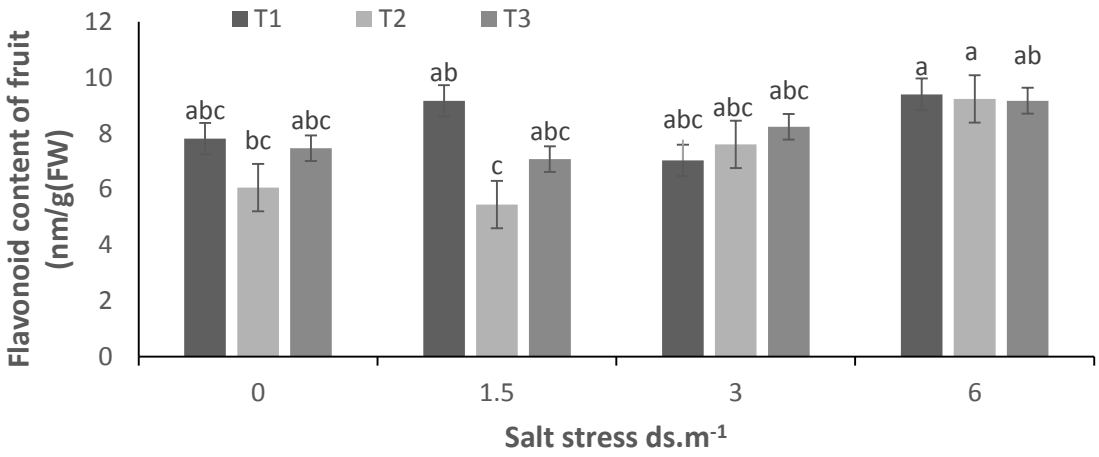


**Fig. (6): The interaction effect of root zone temperature and different levels of salt stress on the flavonoid content of fruit. Low temperature (T1 = 20°C), optimum temperature (T2 = 25°C), and high temperature stress (T3 = 30°C).**

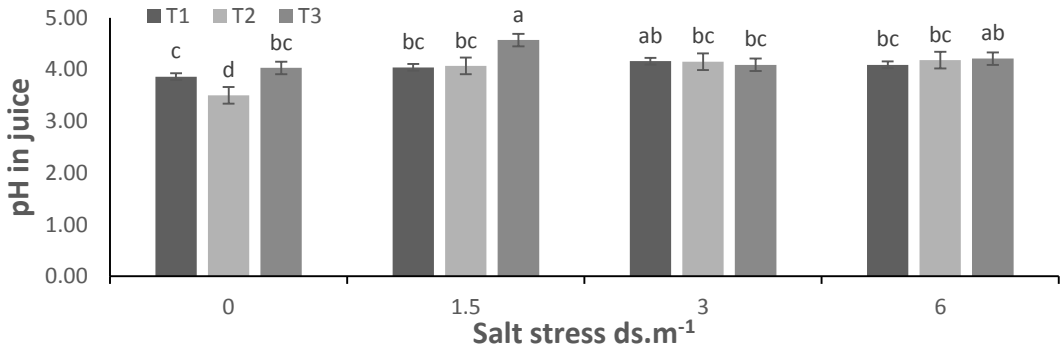


**Fig. (7): The interaction effect of root zone temperature and different levels of salt stress on the pH in juice of fruit. Low temperature (T1 = 20°C), optimum temperature (T2 = 25°C), and high temperature stress (T3 = 30°C).**

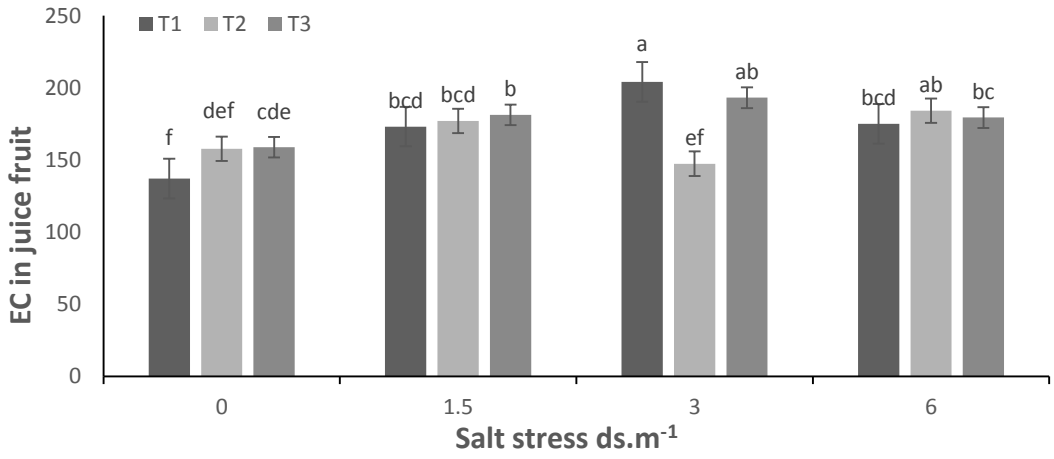


**Fig. (8): The interaction effect of root zone temperature and different levels of salt stress on the EC in juice of fruit. Low temperature (T1 = 20°C), optimum temperature (T2 = 25°C), and high temperature stress (T3 = 30°C).**

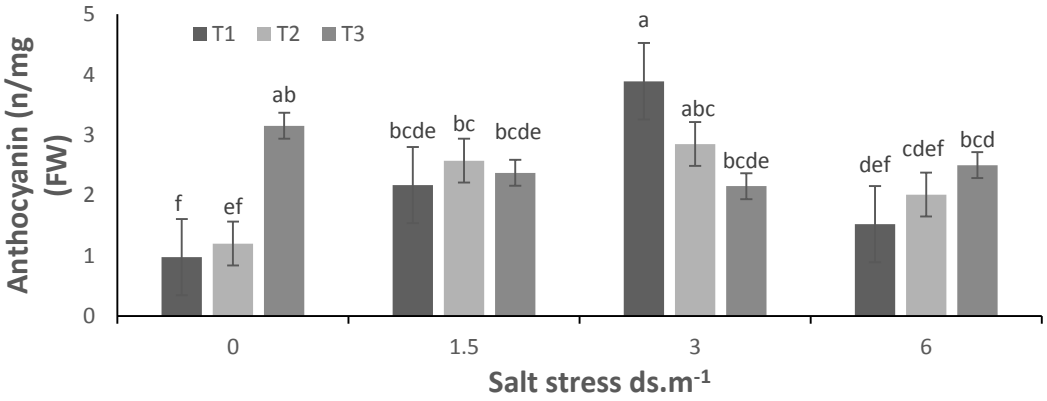


**Fig. (9): The interaction effect of root zone temperature and different levels of salt stress on the anthocyanin (n/mg (FW) fruit. Low temperature (T1 = 20°C), optimum temperature (T2 = 25°C), and high temperature stress (T3 = 30°C).**

$T_7$ (a salt level of 3 ds.m$^{-1}$and root zone heating of 20ºC) increased the amount of EC in fruit, but, by contrast, $T_1$ reduced the rate of EC (Figs. 7 & 8). Based on the results observed in Fig.(9), the content of anthocyanin increased in $T_7$anddeceased in $T_1$.

## Discussion

In the present study, total sugar decreased by salt stress level of 6 ds.m$^{-1}$ and root zone temperature of 20 and 30ºC (Tables 1 & 2). In cherry tomato *var*. *cerasiforme* (Al Hassan *et al*., 2015) reported that total sugar was decreased by salt stress. The same results were also reported in red leaf lettuce cv. red wave by Sakamoto & Suzuki (2015b) who showed that total sugar decreased under low root zone temperature. Al Hassan *et al.* (2015) also stated that phenolic and flavonoid content in cherry tomato affected by salt stress, which is agreement with our results and (Ali & Ismail, 2014) reported salt stress increased flavonoid content in tomato fruits. Phenol content in leaf lettuce cv. red wave was reported to decrease in root zone

temperature of 20, 25 and 30$^{o}$C compared to root zone temperature of 10$^{o}$C (Sakamoto & Suzuki, 2015a). Salt stress in the current study led to a significant increase in phenolic content.

Our results also showed that flavonoid increased in the lowest root zone heating (20$^{o}$C)and salt stress level of 6 ds.m$^{-1}$ (Tables 1 & 2). A significant increase was found in phenolic and flavonoid contents in the plants submitted to the salt treatments. There are manystudies reporting the increase in the levels of phenolic compounds and flavonoids in tomato fruit under the conditions of abiotic stress (Ali & Ismail, 2014; Krauss*et al.*, 2006). In fact, due to having high phenolic compounds and flavonoids, thetomato has been recommended to be consumed for reducing the risk of cancer (Holiman*et al*., 1996). Dixon & Paiva(1995) reported that environmental stresses such as salt stress lead to the accumulation of polyphenol constituents. Also suggest that salt stress induce the biosynthesis of these acids as salt-stress-enhance components that could play an important role in diminishing the oxidative processes. These results support the theory that polyphenols as secondary metabolites protect plant tissues against oxidative stress generated by salt stress and contribute to salinity tolerance. Salinity treatment affected the content of total anthocyanin's(Table 2).

It is well known that anthocyanin's are members of the flavonoid class of plant secondary metabolites that are not usually synthesized in tomato fruits(Mes *et al.*, 2008). In the present experiment, the content of anthocyanin's in fruits grown under salinity stress decreased by (0 & 6) ds.m$^{-1}$ compared to that of the levels (3 & 6) ds.m$^{-1}$. In the present investigation, root zone temperature had almost no effect on anthocyanin content. The increase found in the content of anthocyanin's in medium levels of salt stress is inconsistent with the results reported by Borghesi *et al.* (2011) who showed that salinity stress caused an enhancement (2-fold) in the accumulation of total anthocyanins in fruits of Sun Black, while it reduced it in fruits of anthocyanin (10-fold decrease). Our results showed that root zone heating at all the three levels had no desirable effect on stem diameter, but the effect of salt stress at the level of 3 ds.m$^{-1}$on stem diameter was significant. Similar results were reported by Suwa *et al.* (2008) and Ekinci*et al.*(2012).

They are shown the reduction in stem diameter under salinity stress. The results of the present study are consistent with findings that the stem diameter shrinks during the day because of the loss of water in transpiration and swells at night owing to the uptake and storage of water(Suwa*et al*., 2006).It showed that water potential recorded during the night time. The water potential was saturated during these conditions. however, the difference in the change in stem diameter at night time was treated as an irreversible component, called sink growth (Abdel-Mawgoud*et al.*, 2005) reported that leaf area in Sweet Pepper was affected by high root zone heating. In the present experiment, leaf area was significantly reduced under root zone heating of 25 and 30$^{o}$C. The increase observed in leaf area under salt stress level of 3 ds.m$^{-1}$ is in line with the findings of Al-Maskri*et al.* (2010), in which the same increase of lettuce leaf under different salinity levels was shown. In the present study, the number of main branches was reduced in the treatment of low root zone heating (Table 1).

It seems that low- temperature stress caused higher damage than high- temperature stress. Also in our experiment, high-

temperature treatment of the root zone heating increased the number of branches. Treatment with high root-zone heating in hydroponically grown tomato plants may increase growth while preserving the accumulation of secondary metabolites. Similar results were observed by(Sakamoto & Suzuki, 2015b) who found carrot plant growth affected by root zone stress. Our results also showed that high root zone heating triggered a greater increase in pH and EC of fruit compared to low root zone heating; in addition, a higher amount of pH and E.C. was observed in salt stress levels of 1.5 and 3 $ds.m^{-1}$ compared to the control.

## Conclusions

Results showed that Root zone temperature (25 °C) had improved the total sugar and phenol content of tomato fruits, whereas, High zone temperature have enhanced the pH, EC and anthocyanin levels. Study also found that the Root zone temperature did not effect on Flavonoid and anthocyanin levels. Furthermore, salt stress at the level of 3 $ds.m^{-1}$ increased stem diameter, total sugar, pH and EC of fruit, but flavonoid and anthocyanin levels were decreased.

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