J. Agr. Sci. Tech. (2021) Vol. 23(3): 661-671

Responses of Growth, Physiological and Anatomical Characteristics of Resistant and Sensitive Cultivars of *Cucumis inodorous* L. to Salt Stress

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ABSTRACT

In order to study the effect of irrigation with saline water on physiology, biochemical, and anatomy characteristics, growth, and yield of different melon cultivars, an experiment was performed in split-plot with randomized complete block design with three replications. Treatments were two melon cultivars resistant to salinity (Sooski and Diamond) and two sensitive cultivars (Daregzi and Zard Ivanaki) with two salinity levels [0 (control) and 8 dS m^{-1} of NaCl] of irrigation water. The results showed that fresh and dry weight of shoot, fruit and seed weight, fruit acidity, fruit firmness, fruit length/diameter, fruit number and yield, photosynthesis rate, transpiration, and stomatal conductance decreased by irrigation with saline water. Under salinity condition, resistant cultivars showed the highest amount of the abovementioned parameters and had higher proline, phenol, and antioxidant activity. Generally, the results showed that by applying salt stress, growth parameters, photosynthetic parameters, and quantitative characteristics of the fruit decreased. This decrease was lower in Sooski resistant cultivar. It seems that melon cultivars achieved their resistant; firstly, improving yield instead of vegetative growth; secondly, increasing antioxidant and phenol content to reduce deleterious salinity effect, and finally, increasing TSS to have more osmotic adjustment for promoting photosynthesis at the acceptable level for producing enough assimilate for commercial yield under salinity conditions.

Keywords: DPPH, Endemic melons, Melon, Phenol content.

INTRODUCTION

The combined effect of irrigation and agricultural practices have increased soil salinity. The salinity of water from wells in the central part of Iran has increased because of the excess water withdrawal and lowering of the groundwater level. Farmers use this saline water, so, it is necessary to introduce the local cultivars resistant to salinity and recommend them for cultivation in this region. Salt tolerance is the relative ability of a plant to endure the effects of excess salts in the soil rooting medium and to produce a satisfactory stand or yield. The mode of tolerance can vary. Most plants avoid salinity, some evade or resist salinity, and a few actually tolerate salinity. Plants respond to salinity differently, but generally, plants grown under salinity have lower growth rates, with a dwarf structure, and their leaves are mostly small, with a dark green color (Mer *et al*., 2000). Salinity reduces the development of leaves, which leads to a decrease in the total leaf area (El-Hendawy, 2004).

Resistant genotypes have reasonable growth in saline stress, while the sensitive genotypes show high reductions in their shoot and root dry weights, plant height and leaf area (Kusvuran *et al*., 2012)

Physiological changes in plants growing under saline conditions have been developed

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[DOR: 20.1001.1.16807073.2021.23.3.3.0]

as effective indices for resistant screening in plant breeding programs (Ashraf and Foolad, 2007; Cha-um and Kirdmanee, 2009). The first physiological response of plants to salt stress conditions is the reduction of photosynthesis. This reduction is partly due to a reduced stomatal conductance and the consequent restriction of the availability of CO_2 for carboxylation (Razzaghi *et al*., 2011). Leaf water potential decreases with salinity (Munns, 2002).

Morpho-anatomical alterations of halophytes in salinity condition include increase in cell volume, especially of epidermal cells, spongy and scalar parenchyma, increase in leaf thickness, and decrease in the number of stomata (Polic *et al*., 2009). Longstreth and Nobel (1979) reported that leaf epidermal thickness and mesophyll thickness increased with increasing NaCl concentration, both in salt-tolerant and salt-sensitive plants. Reduced yield by salinity could be attributed to increased activity of Na^+ and Cl^- ions in the root zone (Greenway and Munns, 1980). Salinity often reduces the yield of vegetable crops, but in many cases, it improves the fruit quality in both soil and soilless culture (Francois and Mmass, 1986). Salinity improved fruit quality by increasing fruit dry matter, Total soluble solids (TSS) contents, and by decreasing pH (Navarro *et al*., 2002). Application of saline water reduced fruit yield in sensitive cultivars and increased fruit quality (TSS) in both resistant and sensitive cultivars in melon (Botia *et al*., 2005).

Melon is a common crop in many arid and semi-arid regions of the world and Iran (Botia *et al*., 2005). Several researchers reported that although melon has moderate resistance to salinity (Mangal *et al*., 1988; Meiri *et al*., 1982), the fruit weight and, to greater extent, the number of fruits and size decreased by salinity (Nukaya *et al*., 1980; Meiri *et al*., 1981). Shannon and Francois (1978) showed that total soluble solid content of melon increased by salinity. Field experiments have demonstrated that melons are a potential crop for irrigation with saline water (Goldberg *et al*., 1971; Pasternak *et al*., 1987, 1980), but there were no reports on comparing the changes of local melon of Iran in salinity. Therefore, this experiment aimed to compare common native/local melons cultivated in the central part of Iran under salinity conditions in terms of growth and quality of fruits and to introduce the best endemic melon for cultivation in the central part of Iran, as well as best varieties for future breeding purposes.

MATERIALS AND METHODS

The experiment was carried out during spring-summer 2015–2016 in Isfahan Research Center, Isfahan, Iran. In the pre-tested experiment, 16 native melon cultivars were used to evaluate the salinity resistance. According to the results of pre-tested investigation, two sensitive and resistant cultivars were chosen for this experiment. In the pre-test experiment, to study the response of Iranian melon cultivars to salinity stress, an experiment was designed and conducted with four salinity treatments (0, 6.6, 8, and 12 dS m^{-1} NaCl) and 16 melons in the greenhouse of Isfahan Research Station. Resistance and sensitive cultivars were chosen according to parameters such as fresh and dry weight of shoot and root, chlorophyll fluorescence, electrolyte leakage, RWC, Na^+ and K^+ concentration of leaf and K^+/N^+ ratio.

Two resistant cultivars (Sooski and Diamond) and two sensitive cultivars (Daregzi and Zard Ivanaki) were chosen. Seeds were sown in April 2015. The experiment was conducted in the field in Isfahan Research Center, Isfahan, Iran. This semi-arid region has a mean temperature of 15°C, with a latitude of 1545 m asl and the mean rainfall of 112 mm. Soil texture is clay loam with pH= 7-8 and high EC more than 8 dS m^{-1}. The amount of OC% and soil nitrogen is low. In May 2015, when all plants had two-three leaves, they were transplanted to the field in a row with spacing of 50 cm. Irrigation was carried out

[DOR: 20.1001.1.16807073.2021.23.3.3.0]

using a drip irrigation system with tape, using two salinity levels of NaCl [0 (control) and 8 dS m^{-1}]. The salinity treatment (8 dS m^{-1}) was chosen according to the salinity of wells of this region. Melons grew for 90 days to harvest. The split-plot experiment was based on RBCD designed with 3 replicates. Irrigation was applied when it was needed.

Photosynthetic rate was determined in the youngest fully expanded leaves at 30 days after sowing for 3 replications per treatment and leaf area was measured using a portable leaf area meter (Li-Cor Li-3000, USA) from 10:00 to 11:00 am on a clear day (without clouds).

At the end of the growth period (90 days after sowing), fresh and dry weight of shoot was measured in convection oven at 70°C overnight. Flesh, skin and seed of fruits were separated and dried at 70°C. The number of fruits were counted during the growing season. Yield was measured by weighing fruits of each individual bush. Fruit length and fruit diameter were measured with ruler and caliper (Mitutoyo Corp, Japan), respectively. Plant height was measured by the meter. Fruit shape was presented by the ratio of length/ diameter of fruit.

Fruit flesh firmness was measured with Penetrometer (DA 600, Japan) and TSS with a portable refractometer (PAL-1 Brix, Japan) (Raeisi *et al*., 2014). The acidity of fruit was determined using titration of 10 mL of fruit extract with 0.1% N NaOH and calculated as citric acid (Mazumdar, 2003).

The method proposed by Bates *et al*. (1973) was applied to analyze the proline in leaf tissues. A 520-nm UV–VIS spectrophotometer (UV-600A, England) was used to measure the absorbance of the chromophore. In order to determine the total phenol content, leaves sample was mixed with 5 mL Folin-Ciocalteu and 4 mL aqueous Na_2CO_3 separately. The phenols were determined by spectrophotometer at 765 nm as Gallic Acid Equivalents per gram Dry Weight (mg GAE g^{-1} DW) (Kahkonen *et al*., 1999). The antioxidant activity of cucumber leaves was estimated, according to Yu *et al*. (2002). Three mg of the sample was dissolved in 5 mL methanol stock and 1.4 mL of this solution was blended with 0.6 mL of antioxidants solution. After 30 minutes, the absorbance of the solution was recorded at 515 nm with a spectrophotometer (V-530, JASCO, Japan) against methanol as a blank. For the measurement of leaf water potential, a leaf was detached from the shoot and placed in the pressure chamber (3115 model) with the cut end protruding from the chamber and exposed to atmospheric pressure (Turner, 1988). To investigate the anatomical structure of the leaves, the samples were fixed in 70% alcohol, cutting by blade manually and after removing the color with bleach, coloring with methylene blue and carmen-zagi, and, after permanent fixation on the lam, was examined under an optical microscope (Leica Galen III) (Polic *et al*., 2009).

All data were subjected to two-way ANOVA by using Statistix 8 software (Tallahassee FL, USA) and the means were compared for significance by the Least Significant Difference (LSD) test at $P< 0.05$.

RESULTS

Growth and Fruit Characteristics of Resistant and Sensitive Cultivars

When salinity was applied, fresh and dry weight of shoot decreased in all cultivars, except Zard Ivanaki, which did not change significantly. Plant height was not affected by salinity, in all cultivars. Leaf area decreased in Daregzi, Zard Ivanaki, and Diamond by salinity (Table 1).

Salinity did not affect fruit weight and yield in Sooski and Diamond, but the number of fruit decreased in Zard Ivanaki and Diamond (Table 2). Fruit length decreased in Zard Ivanaki significantly, while fruit width decreased in Zard Ivanaki and Diamond. Salinity decreased fresh weights of flesh, skin, and seed of fruits in Zard Ivanaki, but it did not affect other

[DOR: 20.1001.1.16807073.2021.23.3.3.0]

Table 1. The interactive effect of salinity and resistant and sensitive varieties on growth indices of melon plants.[a]

	Length plant (cm)	Leaf area (cm^2)	Fresh weight shoot (g)	Dry weight shoot (g)	Fruit weight (Kg)	Number fruit per plant	Yield (ton h^{-1})
Control							
Daregzi	111.67 ab	477.50 d	404.87 bc	42.91 b	1.08 d	13.86 bc	17.64 c
Zard Ivanaki	91.33 a-c	556.83 cd	328.83 cd	36.14 bc	1.49 b	16.00 b	23.88 b
Sooski	113.00 ab	705.80 b	782.03 a	89.28 a	2.28 a	14.93 bc	27.28 a
Diamond	96.33 ab	919.10 a	431.07 bc	46.30 b	1.26 c	27.73 a	24.88 ab
8 dS m^{-1}							
Daregzi	70.67 bc	329.97 e	174.33 e	19.18 d	0.91 e	14.93 bc	15.15 d
Zard Ivanaki	51.00 c	309.33 e	225.97 de	23.73 d	1.01 de	8.53c	18.01 c
Sooski	115.67 a	614.53 bc	459.93 b	47.04 b	2.20 a	13.86 bc	26.74 a
Diamond	109.67 ab	582.63 b-d	261.40 de	26.83 cd	1.12 cd	11.73 bc	23.22 b

[a] Means with different letters in each column are significantly different at $P < 0.05$ according to the LSD test.

Table 2. The interactive effect of salinity and resistant and sensitive varieties on fruit characteristics of melon plants. [a]

	Fruit length (cm)	Fruit width (cm)	Fresh flesh weight (g)	Fresh skin weight (g)	Fresh seed weight (g)	Dry weight 100g flesh (g)	Dry weight 100 g skin (g)	Dry weight seed (g)	Flesh diameter (mm)	Skin diameter (mm)	Cavity diameter of fruit (mm)
Control											
Daregzi	20.89 b-d	12.83 a-c	801.9 c	212.16 de	84.16 bc	4.53 de	8.37 bc	25.21 bc	20.49 c	1.96 bc	83.40 ab
Zard Ivanaki	28.95 a	14.16 ab	1191.1 b	285.60 cd	149.38 a	6.42 c	8.31 bc	25.36 b	20.13 c	2.63 bc	96.14 a
Sooski	29.89 a	14.50 a	1701.3 a	436.64 a	151.24 a	11.63 a	12.58 a	34.80 a	29.89 a	4.60 a	87.98 a
Diamond	21.27 b-d	14.16 ab	837.9 c	339.95 bc	96.48 bc	9.70 b	9.53 b	22.21 bc	26.53 ab	3.36 b	81.87 ab
8dS/m											
Daregzi	16.1 d	11.83 bc	647.1 c	188.67 de	56.56 c	3.28 e	6.32 cd	10.95 d	12.94 d	1.53 c	88.12 a
Zard Ivanaki	21.46 bc	10.5 c	777.2 c	174.51 e	74.1 bc	3.34 e	5.01 d	23.33 bc	23.76 bc	2.76 b	51.13 c
Sooski	26.06 ab	13.66 ab	1684.8 a	409.81 ab	111.66 ab	9.66 b	10.66 ab	24.96 bc	23.53 bc	3.50 b	82.60 ab
Diamond	17.44 cd	11.33 c	777.1 c	258.03 cd	99.90 b	4.85 d	9.23 b	17.90 cd	23.78 bc	2.46 bc	60.82 bc

[a] Means with different letters in each column are significantly different at P< 0.05 according to the LSD test.

cultivars. Dry weight of 100 g flesh decreased in all cultivars, except Daregzi. Dry weight of 100 g skin decreased significantly in Zard Ivanaki. Dry weight of seed decreased in Daregzi and Sooski (Table 2). In salinity treatment, flesh diameter decreased in Daregzi and Sooski and skin diameter showed the same trend in Sooski significantly. Cavity diameter decreased in Zard Ivanaki significantly (Table 2), and length/diameter did not change significantly in salinity condition (data not shown).

Salinity decreased fruit firmness and acidity in Daregzi and Zard Ivanaki. Total soluble solid increased in Daregzi and Sooski with salinity (Figures 1-a, -b and -c).

Photosynthesis activity improved in all cultivars under salinity; and transpiration decreased in Daregzi and Zard Ivanaki (Figures 2-a and -b). Leaf water potential was lower in Zard Ivanaki with salinity (Figure 2-c).

Salinity increased phenol content in Daregzi, Sooski and Diamond and proline in Daregzi and Zard Ivanki significantly (Figures 3-a and -b). The total antioxidant activity was enhanced by salinity in all

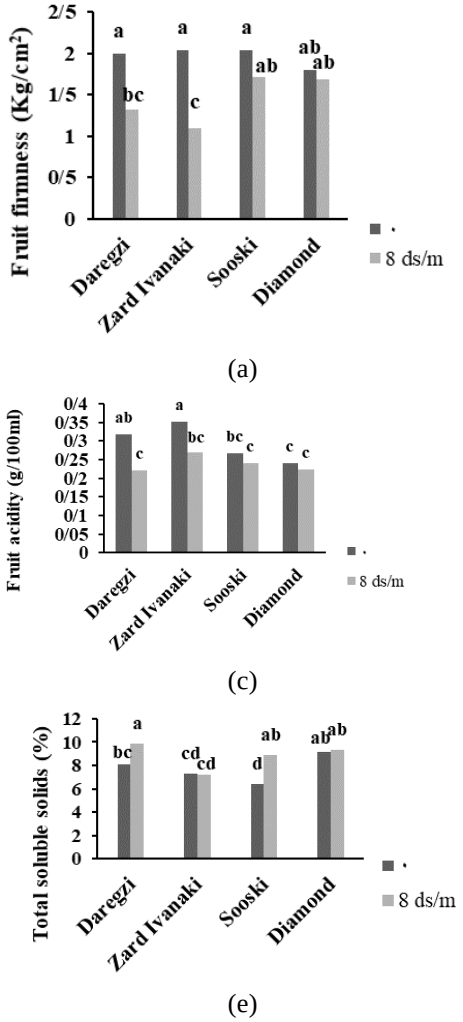

Figure 1. The interactive effect of salinity and resistant and sensitive cultivars on fruit firmness (a), fruit acidity, (b) and total soluble solids (c).

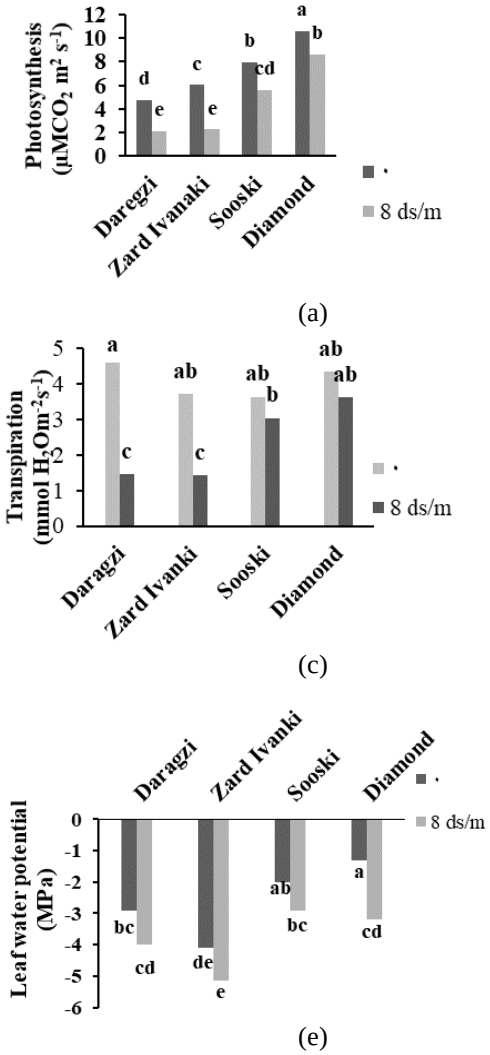

Figure 2. The interactive effect of salinity and resistant and sensitive cultivars on photosynthesis (a), transpiration (b) and leaf water potential (c).

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cultivars (Figure 3-c).

Leaf parenchyma scalar length increased in Daragzi and Zard Ivanki and parenchyma sponge enlarged in Daragzi with salinity (Figures 4-a and –b, 5-a and -b). Epidermal upper thickness increased in Zard Ivanki and Sooski (Figures 4-c, 6-a and -b), epidermal under thickness did not change with salinity in different cultivar (data not shown).

DISCUSSION

Decrease in vegetative growth under salinity in terms of growth rates, with a dwarf structure and small leaves with a dark green color have been reported previously (Greenway and Munns, 1980; Franco *et al*. 1993). Generally, plant length, fruit weight, and yield did not decrease in both resistant cultivars in salinity. Also, leaf area and the number of fruits did not change in the more tolerant cultivar Sooski. It seems that increase in the growth of cultivar was related to reproductive growth rather than vegetative growth. In other words, growth potential results in increasing yield rather than vegetative growth. As shown by the resistant cultivar, Sooski, all fruit flesh, skin and size did not change in salinity stress and the greater decrease in vegetative growth

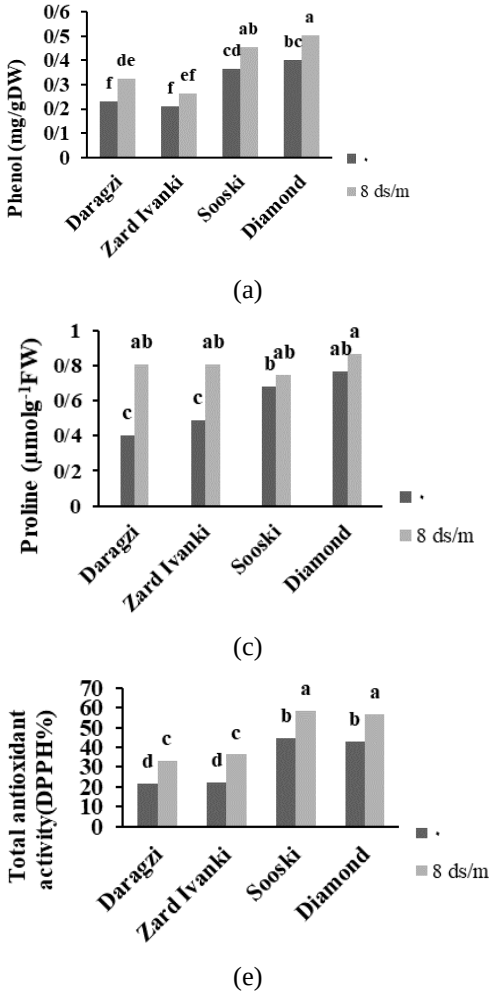

Figure 3. The interactive effect of salinity and resistant and sensitive cultivars on phenol (a), proline (b) and total antioxidant activity (c).

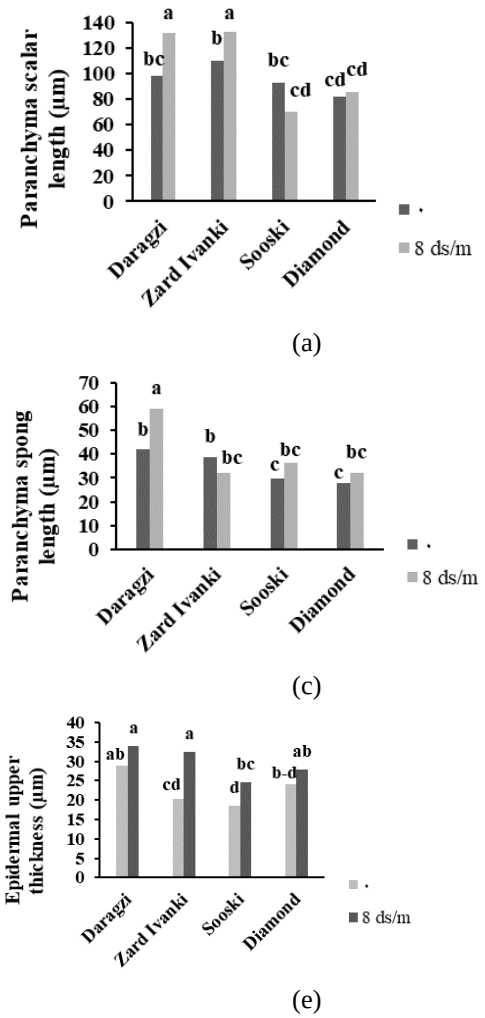

Figure 4. The interactive effect of salinity and resistant and sensitive cultivars on parenchyma sponge length (a), parenchyma scalar length (b), epidermal upper thickness (c).

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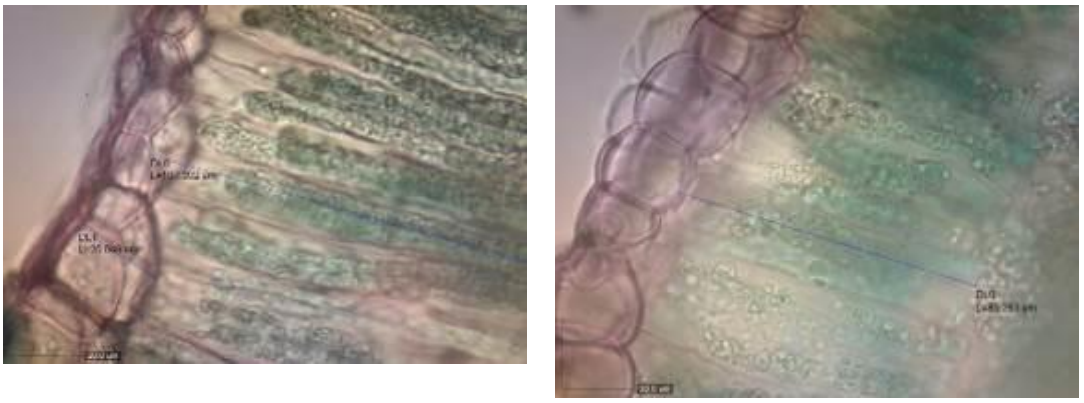
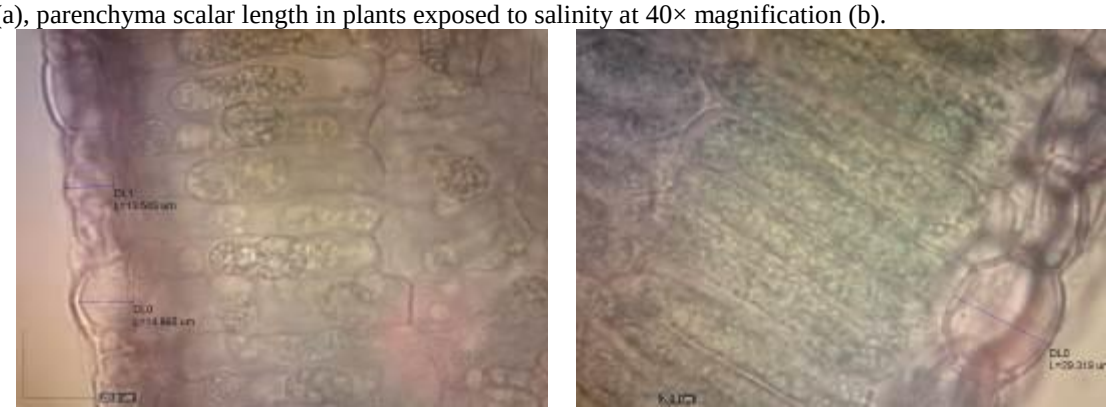


(a) (b)

Figure 5. Microscopic image of parenchyma scalar length in control plants at 40× magnification (a), parenchyma scalar length in plants exposed to salinity at 40× magnification (b).



(a) (b)

Figure 6. Microscopic image of parenchyma scalar length in control plants at 40× magnification (a), parenchyma scalar length in plants exposed to salinity at 40× magnification (b).

belonged to leaf area. The highest leaf area decrease was observed in Zard Ivanki, with 44% compared to the control. Researchers have reported that reducing leaf area under saline conditions may be associated with a decrease in cellular swelling or a change in the sending of hormonal messages from the root to the leaves (El-Hendawy, 2004).

The decrease in yield and fruit weight of the sensitive cultivars was related to increase in fruit cavity and decrease in flesh diameter of fruit. In line with this result, it was observed the growth improvement in Zard Ivanki too. Study of the anatomy of leaves showed that the parenchyma sponge and scalar length increased in the sensitive cultivar Zard Ivanki and Daragzi with 17 and 25%, respectively. With increasing salinity, thickness of the upper epidermis increased in Daragzi, Zard Ivanki, and Sooski cultivar. The results of Polic *et al*. (2009) on the plant (*Suaeda maritime* L.) are similar to the results of this study. Investigations showed that in Suaeda species, salt accumulation in the medium epidermis cell size of the epidermis cells was increased, and their number decreased in two types of halophytes, namely, *Nitraria retusa* and *Atriplex halimus*. An increase in the thickness of the epidermis has been reported with increasing sodium chloride concentration (Boughalleb *et al*. 2009). With increasing epidermis cell, the stomata become smaller and keep cell water more effectively.

The soluble solid content of melons was found to increase with increasing salinity, but fruit size decreased (Shannon and

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Francois, 1978). The same results were seen in this experiment. Total soluble solid increased in Daragzi, Sooski and Diamond with salinity. The highest soluble solid observed in Diamond cultivar was 9.9%. The presence of higher TSS in the tolerant cultivar in salinity may help to maintain osmotic regulation in fruits, which results in maintenance of photosynthesis in stress conditions, prevent decreasing the production of assimilate in leaves and preventing reduction in fruit weight. The data of this experiment confirm them. Salinity decreased fruit quality in terms of firmness and acidity in Daragzi and Zard Ivanki and these parameters did not change in resistant cultivars. Increasing fruit quality like increasing TSS and decreasing pH was reported in melon irrigated with saline water (Botia *et al*., 2005).

Photosynthesis is the most important physiological process of the plant, which is the main determinant of plant growth and yield (Mobin and Khan, 2007). The reduction of plant growth is because of the limited photosynthesis. The decline of photosynthesis can be attributed to the lack of stomatal conductance that decreases under stress (Ashraf and Harris, 2004). Photosynthesis decreased in all of the cultivars under salinity stress, and this decrease was highest in sensitive cultivars Daragzi and Zard Ivanki variety with 58% and 62%, respectively. Leaf water potential was lower in cultivar Diamond with salinity. It conclusion more water absorption with plants.

The highest amounts of phenol and antioxidant activity were in resistant cultivars Sooski and Diamond. It seems that the other resistant mechanism that helps melon to keep growth in salinity condition is increasing phenol and antioxidant content. Rezazadeh *et al*. (2012) investigated the effect of salinity stress on phenolic compounds and antioxidant activity of artichoke leaves and showed that increasing salinity increased phenolic compounds. Some researchers have found that antioxidant activity is associated with phenolic compounds, but others reported a weak correlation or lack of correlation between antioxidant activity and phenolic compounds of the leaf (Keutgen and Pawelzik, 2007). Proline accumulated in leaves of all cultivars. During stress, proline is accumulated in all parts of the plant. However, its accumulation in leaves is faster and more than other organs. Repeated reports have shown proline synthesis under stress conditions and preventing its oxidation, resulting in proline accumulation in tissues (Misra and Gupta, 2005). Increasing proline concentration under salinity conditions may be due to biosynthesis or reduction of proline oxidation to glutamate or conversion of protein to proline (Flowers *et al*., 1977).

CONCLUSIONS

Generally, the results showed that, by applying salt stress, growth parameters, photosynthetic parameters, and qualitative and quantitative characteristics of the fruit decreased more in the sensitive cultivars. Biochemical indices such as phenol and antioxidant activity increased under salinity stress and their accumulation in Sooski and Diamond was higher. On the other hand, due to the tolerance mechanisms studied in the physiological parameters, in this study, melon seems to be able to resist through changes in the biochemical parameters such as antioxidants and phenols, and through the plant's water relations such as transpiration and water potential, and TSS through getting a better osmotic adjustment. Moreover, the vegetative traits of the aerial parts, such as plant length and weights, were affected by stress more than yield. Additionally, the qualitative characteristics of the fruit were improved even in some traits such as TSS.

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پاسخ‌های مختلف ارقام خربزه (*Cucumis inodorous* L.) از گروه inodorous مقاوم و حساس به تنش شوری از نظر رشد، خصوصیات فیزیولوژیکی و آناتومیکی

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چکیده

به‌منظور بررسی تأثیر شوری آب آبیاری بر صفات فیزیولوژیک، بیوشیمیایی، آناتومی، رشد و عملکرد ارقام مختلف خربزه، آزمایشی در قالب اسپلیت پلات بر پایه طرح بلوک‌های کامل تصادفی با سه تکرار به اجرا درآمد. تیمارها شامل دو رقم متحمل به شوری (سوسکی و دیاموند) و دو رقم حساس به شوری (درگزی و زرد ایوانکی) با دو سطح شوری کلرید سدیم (صفر و ۸ دسی زیمنس بر متر) بودند. نتایج نشان داد تحت شوری آب آبیاری وزن‌تر و خشک اندام هوایی، وزن میوه و بذر، اسیدیته

[DOR: 20.1001.1.16807073.2021.23.3.3.0]

میوه، سفتی بافت میوه، نسبت طول به قطر میوه، تعداد میوه و عملکرد، فتوسنتز، تعرق، هدایت روزنه ای کاهش یافت.ارقام متحمل (سوسکی و دیاموند) تحت شوری بیشترین میزان پارامترهای ذکر شده در بالارا نشان دادند. ارقام متحمل بیشترین میزان پرولین، فنول، فعالیت آنتی اکسیدانی کل در شرایط شوری داشتند. به طور کلی نتایج نشان داد تحت تنش شوری پارامترهای رشد، پارامترهای فتوسنتزی و خصوصیات کیفی میوه کاهش یافت. این کاهش در رقم متحمل سوسکی کمتر بود. به نظر می رسد ارقام خربزه مقاومت خود را به دست آورند؛ اولا، منجر به رشد به عملکرد به جای رشد رویشی شد؛ ثانیا، افزایش میزان آنتی اکسیدان و فنل باعث کاهش اثرات مضر شوری گردید و در نهایت افزایش قند (TSS) با تنظیم اسمزی بیشتر برای پیشرفت فتوسنتز در سطح قابل قبول برای تولید آسیملایت کافی برای عملکرد تجاری در شوری است. در نتیجه، ارقام سوسکی و دیاموند را می توان به کشاورزان منطقه مرکزی ایران و شرایط مشابه سایر بخش های جهان و آبیاری با آب شور تا ۸ دسی زیمنس بر متر را توصیه کرد.

[DOR: 20.1001.1.16807073.2021.23.3.3.0]