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The Effect of Irrigation Water Salinity on the Morphological and Physiological Traits of Swiss Chard (*Beta vulgaris* L. var. *cicla* Moq.)

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ABSTRACT

Swiss chard (*Beta vulgaris* L. var. *cicla* Moq.), which is grown as a vegetable in Turkey and well adapted to the Marmara region, was used in our experiments. Provided by a producer, chard seedlings were grown in 6 L plastic bags in a non-heated plastic greenhouse. Starting from the 4-5 true-leaf stage to harvest, sodium chloride (NaCl)-added tap water at 5 different electricity conductivity (EC) values [(0. 4 (tap water, control), 8, 16, 24 and 32 dS/m)] was used as irrigation water. The results showed that the EC of the irrigation water affected some of the morphological and physiological properties of chard. An increase in the EC value of irrigation water led to a decrease in the number of leaves, leaf weight, leaf area, plant length, root length, chlorophyll content and increase in the injury level in the leaves and leaf thickness of Swiss chard. The changes observed upon the application of irrigation water with an EC of 16 dS/m were 50% greater than those observed in the control plants, whereas irrigation water with an EC of 32 dS/m results in severe discoloration and yellowing, but the plant was still alive. Therefore, chard growing can be suggested in agricultural areas with salinity problems.

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Introduction

Swiss chard (*Beta vulgaris* L. var. *cicla* Moq.) is an important vegetable in terms of its vitamin and antioxidant content. Although it is regarded as relatively poor in minerals, Swiss chard is very rich in ascorbic acid, i.e., vitamin C (Pokluda and Kuben, 2002; Alibaş and Okursoy, 2012). Chard leaves contain a high amount of vitamin A and sodium. They also contain minerals, such as calcium, phosphorus, magnesium, iron and potassium; fatty acids, such as palmitic acid, oleic acid (omega-9) and linoleic acid; citric acid, folic acid, pectin, ascorbic acid, phospholipid, glycolipid and polysaccharides (Bolkent et al., 2000).

Drought and salinity are the two of the major abiotic stress factors that limit agricultural production over the world. 45% of the world's total agricultural area is frequently exposed to drought and 6% is exposed to salinity (Ashraf and Foolad, 2007).

Abiotic stress conditions cause significant losses to agricultural production and intensive efforts have been devoted to investigating the effect of salinity on the survival mechanism of plants and find solutions to maintain maximum yield and quality under these conditions. In this way, a maximum efficiency can be achieved for the available agricultural area and water resources. In Turkey, salinity and alkalinity problems exist in approximately 1.5 million hectares. This accounts for 32.5% of the area suitable for irrigation. Factors such as irrigation, drainage, soil properties and climate have significant effects on the salinization and alkalinization of soil (Ekmekçi et al., 2005).

In plants directly exposed to NaCl, primary root growth is blocked because cell expansion and the cell cycle are suppressed (Wang et al., 2009). Leaf growth is more sensitive to salinity than root growth, even though the root system is directly exposed to salt. Therefore, the root/shoot ratio increases in the presence of salt. Although the mechanism behind this increase has not been determined to date, it has been attributed to the changes taking place in the cell walls of the roots and leaves due to salinity (Munns and Tester, 2008).

The aim of this study was to investigate the effects of the irrigation water salinity on growth of Swiss chard. For this purpose, the growth and development of the plants were determined using various parameters and the changes that occur at the different levels of salinity revealed. Field experiments were performed in the Cekmeköy district in a non-heated plastic greenhouse between October 2012 and April 2013.

Materials and methods

Seeds of a Swiss chard cv. Sarma (*Beta vulgaris* L. var. *cicla* Moq.), a well-adapted cultivar to the Marmara region, was obtained from the Argeto Seed Company and used as the vegetable material for study.

Field experiments were performed in a plastic, non-heated greenhouse, located in the Çekmeköy district, Istanbul (41°.02'05 N, 29°.08'26 E) to allow control over the irrigation and salt application process. Temperatures and relative humidity during the experiment varied and are presented in the Table 1. Chemical analyses were carried out at the Horticultural Laboratory of Namik Kemal University and Tekirdag Commodity Exchange Laboratory.

The chemical properties of the soil samples taken from the greenhouse are presented in Table 2.

Swiss chard seedlings were planted in 6-liter plastic bags filled with greenhouse soil. All necessary practices were carried out according to Şalk et al. (2008). Starting from the 4-5 true-leaf stage to harvest, the plants were irrigated with salt water prepared at different salt concentrations at each irrigation period. Each plant was given an equal amount of irrigation water (625 mL).

The experiment was set up as randomized block design with four repetitions (Açıkgöz, 1984). Plants were placed in accordance to the experiment pattern, which consists of 20 parcels of the five levels of water salinity (EC_{iw}) comprised of one tap water (0.4 dS/m, control) and salt water at four different salt concentrations (EC = 8, 16, 24 and 32 dS/m) with 16 plants in each parcel and a total of 320 plants. The irrigation water at different EC values was obtained upon adding an appropriate amount of NaCl to tap water.

A total of 10 criteria were used to examine the morphological and physiological changes in the plants that were harvested. Leaves with a length of >2 cm were used for the number of leaves, leaf weight and leaf thickness measurements. Similarly, the leaf area was determined using leaves whose lengths are >2 cm. After being processed through a scanner, the leaf area was calculated using a computer program (Kraft, 1995; Deveci et al., 2006).

Leaf damage was evaluated during harvest for each individual plant using a visual score based on a reference scale from 0 to 5: 0 = Normal growth, no leaf symptoms, 1 = growth retardation (related to the control plants), 2 = beginning of discoloration in the lower leaves, 3 = discoloration and rolling (closure) of the upper leaves, 4 = severe discoloration and necrosis in the leaves and the beginning of drying at the leaf edges, and 5 = discoloration in the plants and drying in the lower leaves (Kuşvuran et al., 2008).

Table 1 Some temperature and humidity data in the greenhouse in the period of 2012-2013

Months	Max.Temp. (ºC)	Min.Temp. (ºC)	Relative Humidity (%)	Soil Temp (20 cm) (ºC)
December 2012	26	5.5	78.3	11.8
January 2013	22	0.5	81.9	11.0
February 2013	22	-2.5	80.6	9.5
March 2013	27	2.0	78.5	13.2
April 2013	32	7.5	76.0	17.8

Table 2 The chemical characteristics of the greenhouse soil

Parameter	Unit	Result	Method
pH		6.59	Saturation
Salt	(%)	0.29	Saturation
Lime	(%)	0/04	Calcimetry
Saturation		57.00	Saturation
Organic matter	(%)	4.95	Walkey-Black
Total Nitrogen	(%)	0.25	Kjeldahl
Phosphorus	(ppm)	189.45	Olsen-ICP
Potassium	(ppm)	374.67	A. Asetat-ICP
Calcium	(ppm)	3028.80	A. Asetat-ICP
Magnesium	(ppm)	354.22	A. Asetat-ICP
Iron	(ppm)	51.63	DTPA-ICP
Copper	(ppm)	2.55	DTPA-ICP
Zinc	(ppm)	16.13	DTPA-ICP
Manganese	(ppm)	29.80	DTPA-ICP

The membrane damage index (MDI) was calculated by measuring the electrolyte released from the cell (Dlugokecka and Kacperska-Palacz, 1978; Fan and Blake, 1994). During the harvest period, disks with a diameter of 17 mm were taken which leaves, from the outside a few leaves of the stressed and control plants and kept in ionized water for 5 h, and the electricity conductivity (EC) measured. The same disks were kept in an autoclave at 100°C for 10 min and the EC value of the solution measured again. Using the obtained EC values, the MDI in the leaf cells (%) was calculated using the following equation.

$$\mathrm{MDI}=\frac{100\ (\mathrm{Lt}-\mathrm{Lc})}{1-\mathrm{Lc}}$$

Lt: EC before autoclaving/EC after autoclaving of the leaf under drought stress.

Lc: EC before autoclaving/EC after autoclaving of the control leaf.

The total chlorophyll content was determined during the harvesting period, using three plants from each parcel by averaging the readings obtained from two different regions of the leaf close to the main vein. The total chlorophyll content was measured using a Konica Minolta SPAD-502 portable chlorophyll meter (Geravandi et al., 2011).

Statistical analysis of the data obtained from the experiment was carried out using the MSTAT version 3.00/EM package program (Akdemir et al., 1994) and the least significant differences (LSD) between means were calculated for P=0.05.

Results and Discussion

The effect of irrigation water with different EC values on some of the morphological and physiological properties of Swiss chard was found to be significant at 1% (Table 3).

Leaf damage increased as the irrigation water salt concentration increased. The highest damage (Score = 4.25) was observed in the plants irrigated with water having 32dS/m EC and no damage was observed in the leaves of control group plants (EC: 0.4 dS/m) (Table 3). with irrigation water salinity the 32 dS/m where the leaf damage was highest, severe discoloration and yellowing in the leaves were observed and they began to dry from their edges. This result is an important indicator of stress in the plants irrigated with water containing a salt concentration of 32 dS/m. On the other hand that the control group plants which were irrigated with water containing a salt concentration of 0.4 dS/m were not under stress and completed their normal development.

The MDI of plants irrigated with tap water (control, EC=0.4 dS/m) was observed to be the lowest (4.43%). The highest MDI (69.33%) was found in the 32 dS/m EC_{iw} (Table 3).

Similarly, the total chlorophyll content (37.30) was highest in the control group. The control group was followed by the 8, 16 and 24 dS/m groups, respectively and the lowest total chlorophyll content was found in the leaves irrigated with a water containing a salt concentration of 32 dS/m (16.88) (Table 3).

The total leaf area decreased by the increasing salt concentration. The leaf area was highest (959.13 cm^2) in the control group, followed by the he plants irrigated with water having 8 dS/m EC (685.53 cm^2). When the EC value of the irrigation water reached 32 dS/m, the leaf area decreased to 327.10 cm^2, which is a 65.9% reduction when compared to the control plants (Table 3).

The leaf thickness increased with the increasing salt concentration. The leaf thickness was 0.26 mm in the control group and 0.45 mm in the 32 dS/m EC_{iw} (Table 3).

The results show that the leaf damage, membrane damage, total chlorophyll content and leaf area decreased with the increasing salt concentration. Although there is no indisputable correlation between these criteria and salinity tolerance (Shannon and Grieve, 1999), these physiological parameters are important indices in the measurement of salt tolerance. The unsustainability of net photosynthesis and stoma conductivity due to the salt induced low osmotic potential, excessive salt in photosynthetic tissues, ion imbalance, etc. affect the salt tolerance (Ashraf, 2004).

Table 3 The effect of irrigation water salinity on the morphological and physiological properties of Swiss chard

Properties	$LSD_{0.01}$	Control, 0.4 dS/m	8 dS/m	16 dS/m	24 dS/m	32 dS/m
Leaf damage index	0.644	0.00^{e}	0.85^{d}	1.50^{c}	2.75^{b}	4.25^{a}
Number of leaves	2.344	23.43^{a}	18.25^{b}	16.40^{b}	13.93^{c}	10.78^{d}
Leaf fresh weight (g)	11.793	34.42^{a}	24.50^{ab}	23.67^{a}b	21.17^{b}	14.42^{b}
Leaf thickness (mm)	0.068	0.26^{c}	0.27^{c}	0.33^{bc}	0.35^{b}	0.45^{a}
Leaf area (cm^2)	45.236	959.13^{a}	685.53^{b}	557.38^{c}	456.47^{d}	327.10^{e}
Plant length (cm)	11.630	78.80^{a}	52.95^{b}	29.75^{c}	21.30^{cd}	12.10^{d}
Root length (cm)	4.221	33.70^{a}	28.90^{b}	23.25^{c}	17.88^{d}	14.15^{d}
Root weight (g)	3.069	27.83^{a}	20.25^{b}	17.75^{b}	11.58^{c}	9.75^{c}
Membrane damage index (%)	2.293	4.43^{e}	6.77^{d}	21.26^{c}	45.11^{b}	69.33^{a}
Total chlorophyll content (SPAD)	10.086	37.30^{a}	31.90^{ab}	26.35^{bc}	22.55^{bc}	16.88^{c}

*There is no difference in the level of 0.001 among averages that have the same letter.

Table 3 shows the average number of leaves varied between 10.78 and 23.43. The highest number of leaves was determined in the control group (23.43). In the 8 and 16 dS/m salinity levels, no significant difference was observed related to leaf number (18.25 and 16.40, respectively) and the lowest number of leaves was obtained in the 32 dS/m water salinity (10.78). The number of leaves decreased by 22–54% when compared to the control group with the increasing salt concentration in the irrigation water.

The highest leaf fresh weight was observed in the control group (34.42 g), followed by the 8 dS/m EC_{iw} (24.50 g) while the lowest leaf fresh weight observed in the 32 dS/m EC_{iw} (14.42 g). The leaf fresh weight decreased upon increasing the salt concentration. Similarly, Shannon et al. (2000) reported that the yield decreases significantly with the increasing salinity in the range of 3–23 dS/m and a 50% reduction in the yield was observed at 14.5 dS/m. The highest yield was recorded at the lowest salt level when saline water applied during later growth stages.

The salt threshold for yield reduction was reported as 7 dS/m and slope (based on top-fresh-weight) was 5.7% (Grieve et al., 2012). The salt threshold and slope may vary because significant interactions between the salinity and important environmental factors such as temperature, humidity, light, wind and air pollution (Shannon and

Grieve, 1999). According to the results of our study, when the irrigation water EC value was increased to 8 dS/m, the leaf fresh weight decreased by 29%.

Table 3 reveals that the highest plant height (78.80 cm) was observed in the parcels irrigated with tap water, followed by the 8 dS/m EC_{iw} (52.95 cm) and the lowest plant height (12.10 cm) was observed in the 32 dS/m EC_{iw}.

According to the root length and root weight measurements during the harvest period, the highest root length (33.70 cm) was measured in the control group, followed by the 8 dS/m EC_{iw} (28.90 cm) and the lowest root length (14.15 cm) was observed at the highest salt concentration. Similarly, the highest root weight (27.83 g) was measured in the control group, followed by the 8 dS/m application (20.25 g) and the lowest root weight (9.75 g) was observed in the 32 dS/m EC_{iw}.

When the EC value of the irrigation water was 24 dS/m, the leaf area and root biomass exhibit a >50% decrease compared to the control group. On the other hand, a >50% decrease in the number of leaves and root length was observed when the EC value of the irrigation water was 32 dS/m (Figure 1). Although this assessment was made with respect to the EC value of irrigation water, it can be speculated that Swiss chard is resistant to high EC values. Since a 20–25% leaching fraction was employed in the study, the electrical conductivity of the soil extract (ECe) can be predicted to be $1.5EC_{iw}$ (Allen et al., 1998). Under these conditions the ECe values can be estimated as 0.6, 12, 24, 36 and 48 dS/m when the EC_{iw} is 0.4, 8, 16, 24 and 32, respectively.

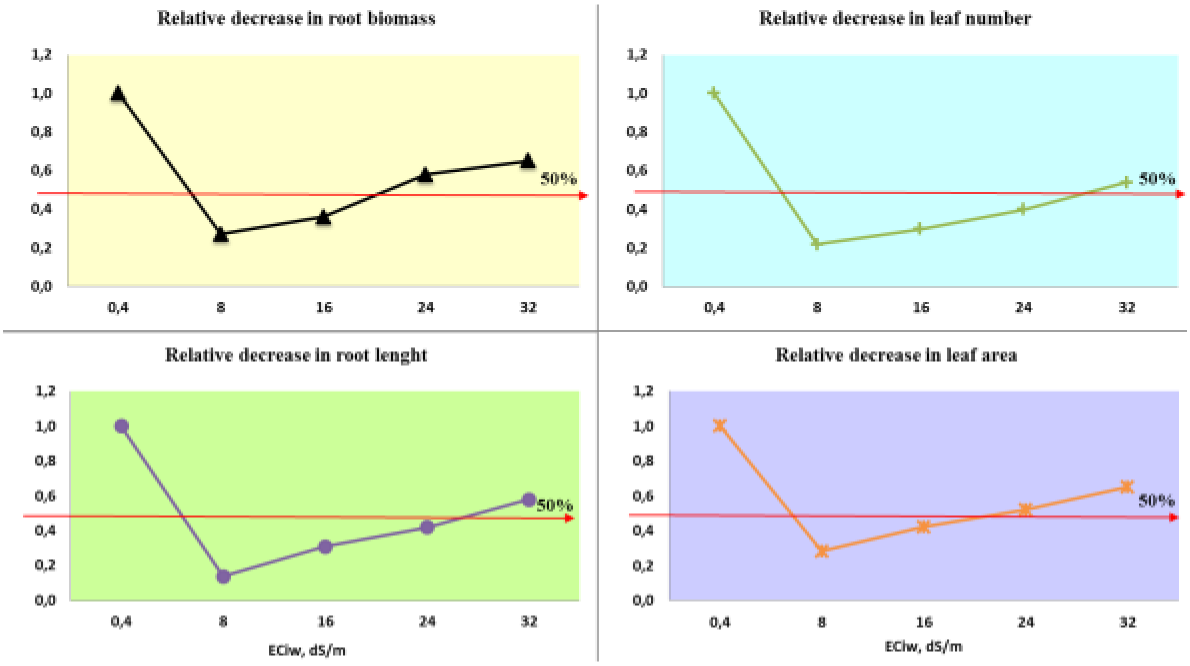

Figure 1 The changes observed in some of the growth related indices in response to the irrigation water salinity

Conclusions

When the results obtained from our experiment were evaluated; the number of leaves, leaf weight, leaf area, plant height, root length, root weight and chlorophyll content decreased by increasing levels of irrigation water salinity. On the other hand, the degree of damage in the leaf cells and leaf thickness increased with the increasing salinity in the irrigation water.

The results showed that Swiss chard can grow at salinity levels that can be detrimental for many vegetable species. Even at the highest salinity of 32 dS/m, the plants still survived even though severe discoloration and yellowing on the leaves and drying of the leaf edges were observed.

When the electrical conductivity of the irrigation water increased to 24 dS/m, the decrease in the leaf area and root weight was higher than 50% when compared to the control plants and when ECiw higher than 24 dS/m, more than 50% reduction in the number of leaves, leaf weight, root length and chlorophyll content were observed.

Due to the decrease in water resources and water quality in our country as well as in the world, new alternatives have been investigated in recent years. It is a known fact that the problem of soil salinity is inevitable with decreasing water resources. Based on our results, Swiss chard has commercial importance, especially in highly saline regions where many plant species can not grow due to the presence of high amounts of sodium chloride.

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