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IMPACTS OF IRRIGATION WATER SALINITY ON LEAF CARBON ISOTOPE DISCRIMINATION, STOMATAL CONDUCTANCE AND YIELDS OF SWEET CORN (*Zea mays saccharata)*

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Abstract

This study was carried out in a greenhouse in the pot experiments in order to determine the effects of the irrigation water salinity on leaf carbon isotope ratio of maize (Zea mays saccharata). Salinity treatments were imposed by irrigation water containing NaCl, $CaCO_3$, $MgSO_4$ salts and having electrical conductivity of 0, 1.5, 3, 5 and 10 dS/m. The experiment was conducted in a completely randomized plot design with 3 replications. The fully expanded leaves were collected for carbon isotope analysis. Then plant samples were harvested for dry matter analysis. Carbon isotope ratio of leaves and crop yields were strongly affected by increasing irrigation water salinity. According to the results of the research, leaf carbon isotope ratio (ΔL; ^{13}C ‰) has increased in parallel with the increase of the irrigation water salinity. While the average carbon isotope ratio of leaves in T_1 is at the lowest (4.00), the average carbon isotope ratio of leaves in T_5 is found as the highest (4.65). Crop yields decreased with increasing irrigation water salinity. The highest yield (33.14 g) and the lowest yield (8.22 g) was found. A negative correlation was observed between crop yields and carbon isotope ratio of leaves.

***Key words**: Carbon isotope discrimination, corn, irrigation water salinity.*

INTRODUCTION

Salinity is one of the most important environmental factors limiting crop production of marginal agricultural soils in many parts of the world. Salinity effects on plants include ion toxicity, osmotic stress, mineral deficiencies, physiological and biochemical perturbations, and combinations of these stresses (Hasegawa et al., 2000; Munns and Tester, 2008; Neumann, 1997; Yeo, 1998). Salt stress affects many aspects of plant metabolism and, as a result, growth and yields are reduced and excessive concentrations kill growing plants (Garg and Gupta, 1997; Mer et al., 2000; Donahue et al., 1983).

Determination of stable carbon isotopic composition (δ13 C) of plants has resulted in significant progress toward understanding the influence of environmental stresses (water, light, salinity and air pollution) on CO_2 fixation and transpiration (O'Leary, 1981, Farquhar et al., 1989, Ehleringer et al., 1993). Stable isotope techniques are useful in studying carbon metabolism involved with photosynthesis and salinity (Farquhar et al., 1989). Relatively little information is available concerning the effects of salinity on carbon isotope composition in C_4 plants (Meinzer et al., 1994). Farquhar et al. (1989) reported that $\delta^{13}C$ increased with rising salinization degree whether the plant is halophyte or not, which was also proven by Yang et al. (2006). The change of $\delta^{13}C$ in soil carbonates or plants may reflect the soil salinization degree (Yang et al., 2006). There is a negative correlation between $\delta^{13}C$ value of plant and CO_2 molefraction in the intercellular air space for plants growing under different saline environments (Wei et al., 2008). A positive relationship observed between carbon isotope ratio with increasing soil salinity in C_4 species (Maricle and Lee, 2006).

The present study was undertaken to examine effects of irrigation water salinty concentrations on Δ and crop yield of corn.

MATERIALS AND METHODS

Experiment side characteristics

Experimental site was located in Isparta (37°45'N and 30°33'E) of Mediterranean Region of Turkey. The pot experiment was conducted under greenhouse conditions (humidity around 65-75 %, air temperature 23-30°C) in Suleyman

Demirel University's Agricultural Research and Training Center.

The plastic pots were filled with 2.5 kg of soil. The experimental soil was taken from Aridisol great soil group. It was calcareous (26.44 % $CaCO_3$), clay loamy in texture (clay 41.20 %, silt 28.71 % and sand 30.09 %), slightly alkaline (pH 7.74, EC 0.30 dS/m; both in 1:2.5 water extract). Field capacity and wilting point on the volume basis were 34.50% and 21.20 % respectively. According to soil fertility analysis results for basal fertilizer, 10 ml NPK (sodium phosphorus potassium, 3.5 % NH_4, 5.5 % NO_3, 10 % urea 19N-19P-19K+micro elements) was applied to the pots.

The experimental design was completely randomized block design. The experiment consists of 5 different irrigation water salinity given below with 3 replications. Salinity treatments were; $\mathbf{T_1}$; 0 dS/m (control), $\mathbf{T_2}$; 1.5 dS/m, $\mathbf{T_3}$; 3 dS/m, $\mathbf{T_4}$; 5 dS/m, $\mathbf{T_5}$; 10 dS/m.

Salinity levels were supplied from a mixture of NaCl, $CaCO_3$ and $MgSO_4$ salts in the ratio of Ca:Mg 1:1 and Sodium Adsorpsion Ratio (SAR) around 5. Pots were weighed regularly and irrigation treatments were made when soil moisture content decreased below the 50 % field capacity.

Sweet corn (*Zea mays saccharata*) of Merit F_1 variety was used in this study and this genotype was moderately sensitive to the salt stress. Five seeds were planted in each pot and watered to field capacity to facilitate germination, salinity stress was imposed when the seedlings were 15 days old. After 2 weeks, the seedlings were thinned to 3 per pot.

Plants were maintained for 80 days in their respective salinity treatments. Before harvest, 2 complete middle-aged leaves were sampled from similar-sized shoots from all plants for subsequent isotope analysis. The leaves were dried at 65°C for 48 h, then ground to a fine powder in order to carbon isotope analysis.

Carbon isotopes were analyzed on the leaf samples. Carbon isotope ratio ($^{13}C/^{12}C$) of the samples ($^{13}C/^{12}C_{sample}$) and the standard ($^{13}C/^{12}C_{standard}$) was determined by means of a mass spectrophotometer (Turkey Atomic Energy Agency/Ankara). $^{13}C/^{12}C$ value was transformed into $\delta^{13}C$ (‰; per mil) with the help of the equation [1].

$$\delta\ ^{13}\mathrm{C}\ (‰) = \frac{(^{13}\mathrm{C}/^{12}\mathrm{C})_{\mathrm{sample}} - (^{13}\mathrm{C}/^{12}\mathrm{C})_{\mathrm{standard}}}{(^{13}\mathrm{C}/^{12}\mathrm{C})_{\mathrm{standard}}} x1000 [1]$$

The standard used to evaluate the carbon is known as PDB (Pee Dee Beliminate). PDB standard is the CO_2 isotope ratio obtained from the Belemnite limestone present in the Peedee formation in South Carolina (Akhter et al., 2008). $\delta^{13}C$ values is transformed into the carbon isotope ratio/difference (Δ) using the equation [2] developed by Farquhar et al. (1982):

$$\Delta = \frac{\delta_a - \delta_p}{1 + \delta_p} \qquad [2]$$

where δa and δp are the carbon isotope composition of source air and plant material, respectively. Carbon isotope composition of air was taken as 8.00% while transforming the $\delta^{13}C$ value into Δ (Keeling et al., 1979; Farquhar et al., 1989; Bolger and Turner, 1998).

Plants were cut at ground level, dried in the oven at 70°C until constand weight and after which they were weighed for dry matter.

Variance analysis of data for all variables was computed using MINITAB computer package (Minitab Release 10.51) program (Version 3.00) was used to compare treatment means by Tukey test.

RESULTS AND DISCUSSIONS

Treatment effects on crop yields

The effects of salinity on the yield (dry matter production) were summarized Table 1.

The highest yield (33.14 g) was found at 0 dS/m, which is 75.2% more than the lowest yield (8.22 g) produced at 10 dS/m.

Table 1. Yield of sweet corn (g/plant) for different salinity treatments

Treatments	Average	Range (%)
T_1	33.14[a]	100.00
T_2	30.04[ab]	90.65
T_3	29.26[bc]	88.29
T_4	25.39[c]	76.61
T_5	8.22[d]	24.80

The relationship between irrigation water salinity and crop yield

The present study showed that irrigation water salinity inhibited plant growth and caused a decrease in yield of sweet corn plant. For each treatment relationship between salinity and crop yield and correlation coefficient are provided in the Figure 1.

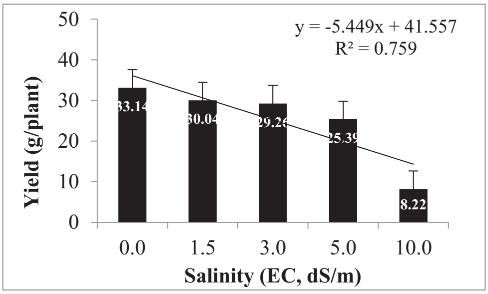

Figure 1. Relationship between salinity and yield

A negative relationship was found between irrigation water salinity and the yield. Results indicated that the yield of sweet corn plants were significantly decreased in with increase of the irrigation water salinity. The reduction in yield shows specific ions accumulated in high concentrations to have toxic effect on plants, as well as that the osmotic pressure in soil to be high and thus the plant has not benefited enough from availability of water. Similar results were found by Ashraf and McNeilly, (1990); Ungar, (1982); Khan et al. (1984); Çiçek et al. (2002); Maricle and Lee, (2006); Turan et al. (2009); Kachout et al. (2009).

According to variance analysis results, a statistical difference of 0.05 was observed between irrigation water salinity and yield. Variance analysis results were provided in the Table 2.

Table 2. Crop yields variance analysis values

Variation Source	D.F	S.S	M.S	F	Table F 0.05
Blocks	2	17.4	8.7	1.76	4.46
Treatments	4	1173.6	293.4	238.0*	4.46
Error	8	39.4	4.9		
General	14	1230.5			

* Statistically significant at $P<0.05$

Carbon Isotope Ratio of Leaves

The average leaf carbon isotope ratio (ΔL; ^{13}C ‰) values were calculated using the average carbon isotope composition ($\delta^{13}C$) values. The values obtained from the leaf carbon isotope (ΔL; ^{13}C ‰) ratios are provided in the Table 3. The value of the average carbon isotope in T_1 is registered 4.00, which is the lowest whereas in T_5 it was the highest value with 4.65. As a result, carbon isotope ratio has increased in parallel with the increase of the irrigation water salinity. The concentration of salinity signify-cantly increased carbon isotope ratio of leaves at T_5 level by 13.98 from T_1 salinity level.

Table 3. Carbon isotope ratio (ΔL, ^{13}C ‰) of leaf samples for different salinity treatments

Treatments	Average	Range (%)
T_1	4.00[c]	86.02
T_2	4.18[bc]	89.89
T_3	4.37[abc]	93.98
T_4	4.56[ab]	98.06
T_5	4.65[a]	100

The Relationship between Irrigation Water Salinity and Δ of Leaves

The sweet corn plant in this study showed increases in leaf $\Delta^{13}C$ with increasing irrigation water salinity. For each treatment relationship between salinity and leaf $\Delta^{13}C$ and correlation coefficient are provided in the Figure 2.

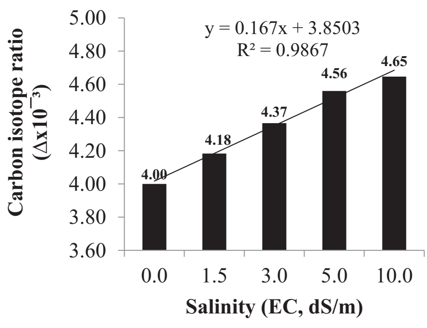

Figure 2. Relationship between salinity and leaf $\Delta^{13}C$

Analysis of variance indicated that there was a highly significant effect of salinity on Δ. A positive correlation was found between irrigation water salinity and the leaf $\Delta^{13}C$. Results indicated that the leaf $\Delta^{13}C$ of sweet corn plants were significantly decreased in with increase of the irrigation water salinity. While a positive relationship observed between carbon isotope ratio with increasing soil salinity in C_4 (corn, sugarcane, sorghum etc) species (Maricle and Lee, 2006), a negative relationship was observed in C_3 (wheat, barley, sugar beet etc) plants (Guy et al., 1986). The difference in $^{13}C/^{12}C$ ratio between C_3 and C_4 plants is related to difference in the isotopic fractionation existing between the ribulose 1.5 biphosphate carboxylase oxygenase (Rubisco) activity in C_3 plants and the phosphoenol pyruvate carboxylase (PEPC) activity in C_4 plants. Rubisco discriminates more against ^{13}C (around 29 ‰) (Estep et al., 1978) than PEPC (around 2.2. ‰) (Delens et al., 1983). Similar results were found by Meinzer et al. (1994); Sandquist and Ehleringer (1995); Bowman et al. (1989); Henderson et al. (1992); Maricle and Lee (2006); Kafi et al. (2007); Dubey and Chandra (2008).

Analysis of variance result, a statistical difference of 0.05 was observed between irrigation water salinity and leaf $\Delta^{13}C$. Variance analysis results were provided in the Table 4.

Table 4. Carbon isotope ratio of the leaf samples (ΔL; ^{13}C ‰) variance analysis

Variation Source	DF	S.F	M.S	F	Table F 0.05
Blocks	2	0.0	0.0	0.21	4.46
Treatments	4	0.8	0.2	33.51*	4.46
Error	8	0.2	0.0		
General	14	1.1			

* Statistically significant at $P<0.05$

The Relationship Between Δ of Leaves and Crop Yields

The $\Delta^{13}C$ values declined while the crop yields values increased with increasing irrigation water salinity in this study. For each treatment, the leaf $\Delta^{13}C$ relationship and crop yields are provided in the Figure 3.

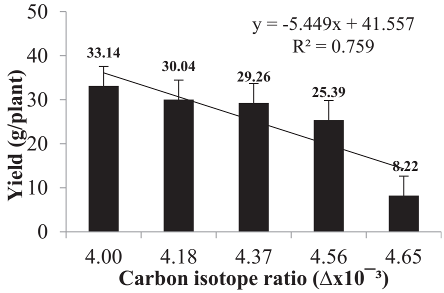

Figure 3. Relationship carbon isotope ratio and yield

A negative and remarkable correlation was found between irrigation water salinity and the crop yields and the leaf carbon isotope ratios. The crop yields were reduced from 33.14 to 8.22 g per plant and carbon isotope ratio of leaves was increased from 4 to 4.65 per plant when EC increased from 0 dS/m to 10 dS/ m. The values obtained from crop yields and the leaf carbon isotope (ΔL; ^{13}C ‰) ratios are provided in the Table 5.

Table 5. Crop yields and leaf carbon isotope ratio values

Treatments	Yield (g)	$\Delta\ ^{13}C$ - Leaf (‰)
T_1	33.14^{a}	4.00^{c}
T_2	30.04^{ab}	4.18^{bc}
T_3	29.26^{bc}	4.37^{abc}
T_4	25.39^{c}	4.56^{ab}
T_5	8.22^{d}	4.65^{a}

Results indicated that there was a negative significant relationship between $\Delta^{13}C$ and crop yields with increasing irrigation water salinity levels. Similar results were found by Ueno et al. (2006); Monneveux et al. (2007).

CONCLUSIONS

At the end of the research study the highest crop yields were determined at the lowest irrigation salinity level. Decline in yield was observed with increasing salinity level. Carbon isotope ratio of leaves values varied between 4.00 and 4.65‰ and were higher at 10 dS/m salinity level. A significant negative correlation was found between carbon isotope ratio of leaves and crop yields. It may be emphasized that pot techniques on carbon isotope studies can provide useful preliminary information about salinity effects on crop yields.

Stable carbon isotope discrimination appears to be a worthful index for assessing whole-plant response to salinity. It can help clarify the effects of salinity on carbon isotope composition in C_4 plants.

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