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The Influence of Salinity on Seedling Growth of Some Pumpkin Varieties Used as Rootstock

Rana KURUM[1*], Kamile ULUKAPI[2], Köksal AYDINŞAKİR[1], Ahmet Naci ONUS[3]

[1]*Bati Akdeniz Agricultural Research Institute, 25 Paşakavaklari, Antalya, Turkey; ranakurum@yahoo.com (*corresponding author)*

[2]*Akdeniz University, Vocational High School of Technical Sciences, Organic Agriculture Program, Antalya, Turkey*

[3]*Akdeniz University, Faculty of Agriculture, Department of Horticulture, Antalya, Turkey*

Abstract

In this study, the effects of different salinity levels (0, "control", 2, 4, 8, and 12 dS m^{-1}) on seedling growth of Obez, RS 841 and Ferro F_1 pumpkin varieties, widely used around the world as rootstock, were investigated. Seedlings grown under saline conditions were investigated for plant main stem length, plant length, root length, shoot length, root fresh weight, root dry weight, shoot fresh weight, shoot dry weights and ion concentrations (Ca^{++}, K^+, Na^+) in the leaves of pumpkin varieties. The results revealed that root length, shoot length, root fresh weight, root dry weight, shoot fresh weight and shoot dry weights tend to decrease when the electrical conductivity of the solution is increased. Results indicated that these varieties responded different to some investigated parameters under saline conditions.

Keywords: NaCl, pumpkin, rootstock, salinity, seedling

Introduction

Salinity stress is one of the most important abiotic stress factors that limits crop production in arid and semi-arid regions. Salinity negatively affects plant growth when salts accumulate in the root zone. High levels of salinity affect seed germination and plant growth by water deficit (osmotic stress), ion toxity and ion imbalance (ionic stress) or a combination of these factors (Läuchli and Grattan, 2007; McNeil *et al.,* 1999; Reinhardt and Rost, 1995). The osmotic effect initially reduces the ability of the plant to absorb water. Several minutes after the initial decrease in leaf growth, a gradual growth recovery takes place until a new steady state is reached, depending on the salt concentration outside the root (Munns, 2002).

The ions effect is the result of salt accumulation in leaves, leading to salt toxicity in the plant, primarily in the older leaves (i.e. salt-specific effect). Salt toxicity may result with the death of leaves and therefore reduce the total photosynthetic leaf area. As a result, a reduction occure in the supply of photosynthate to the plant, affecting the overall carbon balance necessary to sustain the growth. Salt toxicity primarily occurs in the older leaves where Na and Cl build up in the transpiring leaves over a long period of time. Leaf injury and death are probably due to the high salt load in the leaf that exceeds the capacity of salt compartmentation in the vacuoles, causing salt to build up in the cytoplasm to toxic levels (Munns *et al.*, 2006; Munns and Termaat, 1986; Munns, 2002; Yeo and Flowers, 1983).

Salinity is an important constraint to crop production in the world. Because of salinity problems, researchers are trying to get the salt resistant vegetables to meet the need of mankind. One of the most effective way to overcome salinity problems is the introduction of salt tolerance to crops. Most of the literature indicates that vegetable crops are particularly susceptible to salinity during the seedling and early vegetative growth stages as compared to germination. Examples are reported in melon (Botia *et al.,* 1998; Nerson and Paris, 1984), cowpea (Maas and Poss, 1989), lettuce (Coons *et al.,* 1990), beans (Goertz and Coons, 1991), zucchini squash (Graifenberg *et al.,* 1996), pepper (Chartzoulakis and Klapaki, 2000), spinach (Wilson *et al.,* 2000), tomato (Del Amor *et al.,* 2001), cabbage (Jamil and Rha, 2004), and watermelon (Yetişir and Uygur, 2009). The present study was therefore initiated to investigate the effects of different salt concentrations on plant growth of pumpkin rootstocks varieties.

Materials and methods

The experiments were carried out in a glasshouse, ventilated naturally with side and ridge openings, at Bati Akdeniz Agricultural Research Institute (BATEM), in Antalya, Turkey to evaluate the effects of different salinity levels in irrigation water on growth characterisctics of three pumpkin rootstocks, Ferro, Obez, and RS 841.

Seeds were sown in a mixture of peat and perlite in a 1:1 ratio and germinated in greenhouse conditions. Seedlings at the 2-true-leaf stage were transplanted to 3 L pots filled with a mixture of peat and perlite in a 1:1 ratio and amended with 0.4 g N L^{-1}, 0.175 g P L^{-1}, 0.332 g K L^{-1}, and 0.4 g Ca L^{-1}.

Seedlings were irrigated with tap water (EC: 0.54 dS m^{-1} and pH:6.5) for one week and then plants were subjected to salt treatments. Plants were irrigated every 2 days with 5 different saline treatments which were consisted of tap water and NaCl. Salinity levels with different concentrations of 4, 8, 12, and 16 dS m^{-1}, measured by electrical conductivity, were prepared and used as salt source of NaCl while tap water was used as control treatment.

The experiment was formed according to split plot design with two factors (three different rootstock seeds, five different concentration levels) and ten replications. The main plots were rootstock varieties and the subplots were salinity levels. Plants were harvested and evaluated for their response to salinity at the end of four weeks which was the beginning of flowering stage in the control treatment. Roots and shoots of the plants were separated from the growth medium and plant main stem length was measured. After plant roots were cleaned- off growth medium under running water, root and shoot fresh weight were measured. In order to determine the shoot and root dry weight, plant materials were dried for 48 h at 70°C. Na^+, K^+, and Ca^{2+} concentrations in the leaves were determined by ICP (Varian 720 ES) after nitric acid digestion (Zarcinas *et al.,* 1987). Ca^{++}/Na^+ and K^+/Na^+ ratios, and percent reduction in dry weight, as compared to the control treatment, were also calculated. Variance analysis was applied for the obtained data using MSTAT-C program and the differences between the means were compared using Duncan's multiple range test ($p<0.05$) (Gomez and Gomez, 1984).

Results and discussion

Shoot length: Shoot length of the three pumpkin rootstocks ranged from 14.4 to 107.1 cm and the variety, concentration and interactions of variety-concentration was found to be significant at 0.1% confidence level (Tab. 1). While the lowest shoot lengths was recorded in Ferro x 16 dS m^{-1} interaction (14.4 cm), the highest shoot length was obtained from Obez x control interactions (107.1 cm). For the three varieties studied, the shoot length was higher in the control group than in the other treatments.

Root length: The impact of different salt consentrations on root length of pumpkin rootstocks seedlings were investigated and found that the effect of variety on root length was not statistically significant. On the other hand concentration and interactions of concentration and variety were found to be statistically significant at 0.1% and 5% confidence levels, respectively (Tab. 2). While the longest root was obtained in control group (54.3 cm), the shortest root was recorded in 16 dS m^{-1} (30.1 cm). In other words, the root length decreased in accordance with increasement on salt concentration.

Root fresh weight: The effects of different salt concentrations on fresh root weight of pumpkin rootstocks were investigated and found that salt concentration and interactions of variety-concentration were significant at 0.1% and 5% confidence levels, respectively. In the study, root fresh weights varied between 3.8 g (Ferro x 16 dS m^{-1} interaction) and 16.0 g (RS841 x control) (Tab. 3).

Tab. 1. The effects of different salt concentrations on shoot length of varieties (cm)

Varieties	Salt Concentrations (dS m^{-1})					Mean of varieties
	Control	4	8	12	16	
Ferro	97.1 b	56.4 d	31.7 f	24.4 fg	14.4 g	44.8 AB[z]
Obez	107.1 a	68.4 c	42.1 e	30.0 f	19.1 g	53.3 A
RS 841	70.7 c	42.1 e	31.4 f	23.5 fg	16.7 g	36.9 B
Mean of Concentrations	91.6 A	55.6 B	35.1 C	26.0 CD	16.7 D	

Significance: Variety (V): ***[y]; Concentrations (C): ***; V × C: ***

[z]: Means different according to Duncan test at 5% confidence level are shown using different letters;

[y]: ***, significant at 0.1% confidence level

Tab. 2. The effects of different salt concentrations on root length of varieties (cm)

Varieties	Salt Concentrations (dS m^{-1})					Mean of varieties
	Control	4	8	12	16	
Ferro	54.3 a	45.6 ac	45.0 ac	43.6 bc	30.1 e	43.7
Obez	50.5 ab	48.4 ac	41.2 bd	41.3 bd	33.0 de	42.9
RS 841	49.4 ac	41.1 bd	40.1 bd	39.5 ce	32.1 de	40.4
Mean of Concentrations	51.4 A[z]	45.3 AB	42.1 AB	41.5 B	31.7 C	

Significance: Variety (V): N.S. [y] Concentrations (C): ***; V × C: *

[z]: Means different according to Duncan test at 5% confidence level are shown using different letters;

[y]: N.S., ***, and *, non significant, significant at 0.1%, and 5%, confidence level

Root dry weight: It was found that as the salt concentration increased, the root dry weights differed depending on varieties (Tab. 4). The highest root dry weights were obtained in control group for all the varieties studied. A decreasing trend was observed in accordance with salt concentrations as the highest root dry weight was obtained from RS 841 x control interaction (0.73 g) and the lowest root dry weight was obtained Ferro x 16 dS m^{-1} interaction (0.12 g).

Shoot fresh weight: Experiment results revealed that salt concentrations and interactions of variety-concentration were significant at 0.1% and 5% confidence levels, respectively (Tab. 5). While the highest shoot fresh weight (95.8 g) was obtained from control treatment in 'Obez', the lowest shoot fresh weight (9.0 g) was obtained from 16 dS m^{-1} in 'Ferro'. As can be seen in Tab. 5 shoot fresh weight decreased as salt concentration increased in all the varieties.

Shoot dry weight: The shoot dry weight values changed by increasing salt concentration (Tab. 6). The highest shoot dry weight was obtained from control treatment. On the other hand a decrease was observed on shoot dry weight values in accordance with increasement on salt concentration. The highest and shoot dry weight (8.30 g) was obtained from control treatment in 'Ferro' and the lowest shoot dry weight (1.45 g) was obtained from 16 dS m^{-1} in 'Ferro'.

All results obtained indicated that different growing characteristics were significantly affected by salinity stress. The first plant part interacts with salt is the roots and it is almost inevitable that the crops are affected by salt concentration. Therefore the results obtained in present study are in agrement with previous studies reporting increase on salt concentration negatively affects root and shoot development (Ashraf and Tufail, 1995; Dash and Panda, 2001; Delgado and Sanchez-Raya, 2007; Munns, 2002; Reinhardt and Rost, 1995). The reason that the root and shoot length are affected negatively by salt stress is due to toxic effect of salts as well as inhibition of cytokinesis and cell expansion. Additionally, the decrease in hormones

Tab. 3. The effects of different salt concentrations on root fresh weight of varieties (g)

Varieties	Salt Concentrations (dS m^{-1})					Mean of varieties
	Control	4	8	12	16	
Ferro	12.5 b	6.1 df	6.0 df	5.6 df	3.8 f	6.8
Obez	12.0 b	10.6 bc	7.9 de	5.7 df	5.2 ef	8.3
RS 841	16.0 a	8.4 cd	8.0 ce	7.6 de	4.0 f	8.8
Mean of Concentrations	13.5 A[z]	8.4 AB	7.3 B	6.3 BC	4.4 C	

Significance: Variety (V): N.S.[y]; Concentrations (C): ***; VxC: *

[z]: Means different according to Duncan test at 5% confidence level are shown using different letters;

[y]: N.S., ***, and *, non significant, significant at 0.1%, and 5%, confidence level

Tab. 4. The effects of different salt concentrations on root dry weight of varieties (g)

Varieties	Salt Concentrations (dS m^{-1})					Mean of varieties
	Control	4	8	12	16	
Ferro	0.62 b	0.34 eg	0.28 gı	0.21 hj	0.12 j	0.31
Obez	0.53 bc	0.49 cd	0.34 fg	0.31 fh	0.21 hj	0.37
RS 841	0.73 a	0.45 ce	0.40 df	0.29 fı	0.17 ıj	0.41
Mean of Concentrations	0.62 A[z]	0.43 B	0.34 BC	0.27 CD	0.17 D	

Significance: Variety (V): N.S.[y]; Concentrations (C): ***; V × C: *

[z]: Means different according to Duncan test at 5% confidence level are shown using different letters;

[y]: N.S., ***, and *, non significant, significant at 0.1%, and 5%, confidence level

Tab. 5. The effects of different salt concentrations on shoot fresh weight of varieties (g)

Varieties	Salt Concentrations (dS m^{-1})					Mean of varieties
	Control	4	8	12	16	
Ferro	89.0 a	42.6 d	28.7 ef	29.0 ef	9.0 h	39.7
Obez	95.8 a	56.5 c	37.0 df	26.0 fg	16.4 gh	46.4
RS 841	74.2 b	37.8 de	35.5 df	26.0 fg	12.3 h	37.2
Mean of Concentrations	86.3 A[z]	45.6 B	33.7 C	27.0 C	12.6 D	

Significance: Variety (V): N.S.[y]; Concentrations (C): ***; V × C: *

[z]: Means different according to Duncan test at 5% confidence level are shown using different letters;

[y]: N.S., ***, and *, non significant, significant at 0.1%, and 5%, confidence level

Tab. 6. The effects of different salt concentrations on shoot dry weight of varieties (g)

Varieties	Salt Concentrations (dS m^{-1})					Mean of varieties
	Control	4	8	12	16	
Ferro	8.30 a	3.73 de	2.62 eh	2.61 eh	1.45 ı	3.74
Obez	7.92 a	5.11 c	3.38 df	2.56 fh	1.94 gi	4.18
RS 841	6.82 b	3.93 d	3.59 df	2.97 dg	1.50 hı	3.76
Mean of Concentrations	7.68 A	4.26 B	3.20 C	2.71 C	1.63 D	

Significance: Variety (V): N.S.[y]; Concentrations (C): ***; VxC: *

[z]: Means different according to Duncan test at 5% confidence level are shown using different letters;

[y]: N.S., ***, and *, non significant, significant at 0.1%, and 5%, confidence level

that stimulate the growth and increase in hormones that hinder growth can cause shorter root and shoot lengths (Ashraf and O'leary, 1997; Foolad, 1996; Prakash and Prathapasenan, 1990; Taiz and Zeiger, 1998). The increase in osmotic pressure around the roots as a result of saline environment can also prevent water uptake by root and results with short root and shoot length (Al-Karaki, 2001; Bohnert *et al.,* 1995; Werner and Finkelstein, 1995).

Salinity stress had remarkable effects on other plant growth parameters such as plant fresh and dry weight.

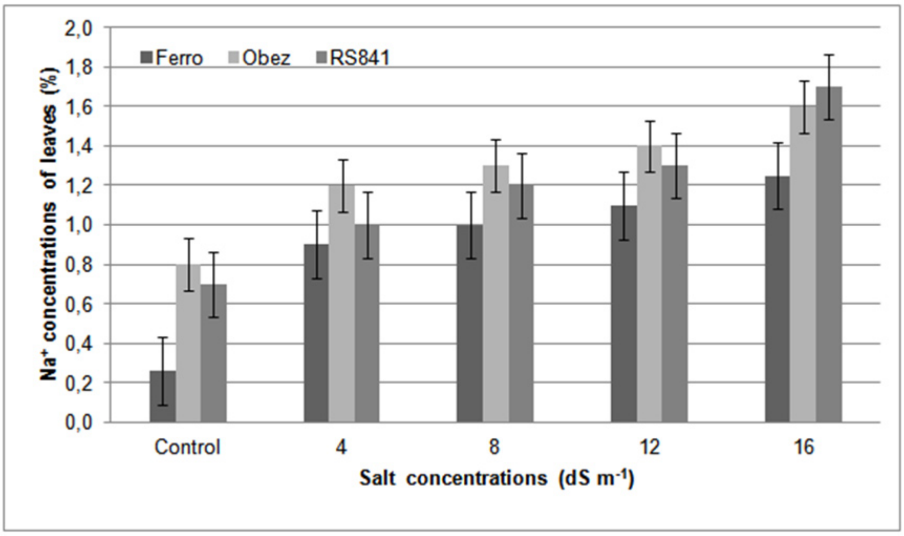

Fig. 1. The effect of NaCl salinity on Na^+ concentration in the leaves of pumpkin varieties (significant at 0.01 level)

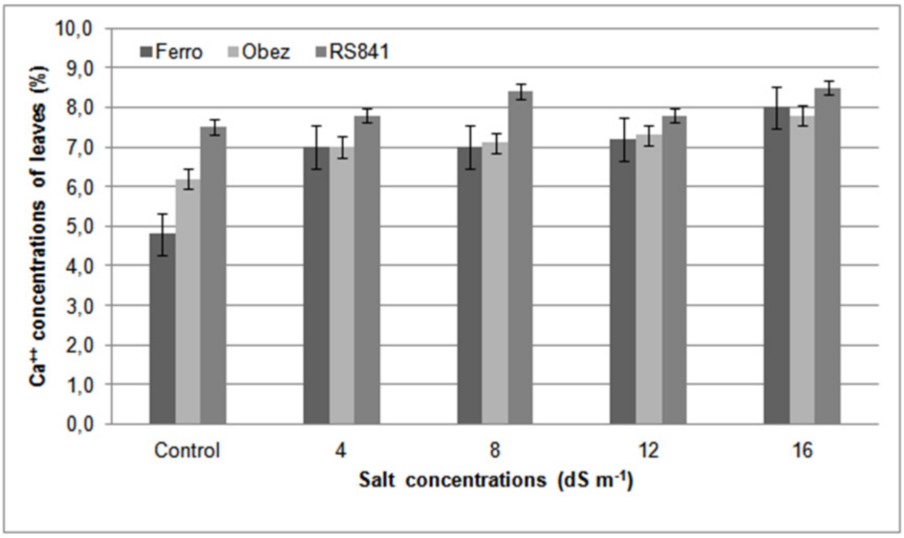

Fig. 2. The effect of NaCl salinity on Ca^{++} concentration in the leaves of pumpkin varieties (significant at 0.01 level)

Tab. 7. The effects of different salt concentrations on Ca^{++}/Na^{+} and K^{+}/Na^{+} ratios in the leaves of varieties

Varieties	Ca^{++}/Na^{+} Salt Concentrations (dS m^{-1})					Mean of varieties
	Control	4	8	12	16	
Ferro	18.5 a	7.8 c	7.0 cd	6.5 ce	6.4 ce	9.2 A[z]
Obez	7.8 c	5.8 de	6.5 ce	5.2 de	4.9 e	6.0 B
RS 841	10.7 b	7.8 c	7.0 cd	7.0 cd	5.0 e	7.5 B
Mean of Concentrations	12.3 A[z]	7.1 B	6.8 B	6.2 B	5.4 B	
Varieties	**K^{+}/Na^{+} Salt Concentrations (dS m^{-1})**					**Mean of varieties**
	Control	4	8	12	16	
Ferro	5.8 a	2.1 b	2.0 b	1.9 b	1.9 b	2.7
Obez	2.3 b	1.6 b	1.6 b	1.6 b	1.4 b	1.7
RS 841	2.6 b	2.0 b	1.8 b	1.6 b	1.4 b	1.7
Mean of Concentrations	3.2 A[z]	1.9 AB	1.8 AB	1.7 AB	1.6 B	

Significance: Variety (V): ***; Concentrations (C): ***; VxC: ***
Significance: Variety (V): N.S.[y]; Concentrations (C): ***; VxC: ***
[z]: Means different according to Duncan test at 5% confidence level are shown using different letters;
[y]: N.S. and ***, non significant and significant at 0.1%, confidence level

High foliar concentration of Na^{+} is capable of reducing CO_2 assimilation because of ionic toxicity (Cachorro *et al.,* 1993). Reduction in shoot fresh and dry weights in response to salt stress has been reported for other crops, such as cucumber (Van de Sanden and Veen, 1992), strawberry (Awang *et al.,* 1993), eggplant (Chartzoulaki and Loupassaki, 1997), broadbean (De Pascale and Barbieri, 1997), pepper (Chartzoulakis and Klapaki 2000), melon (Sivritepe *et al.,* 2005), tomato (Yurtseven *et al.,* 2005), watermelon (Yu-feng, 2006), and okra (Ünlükara *et al.,* 2008).

The results of the present study showed that NaCl treatments caused an increase in Na^{+} concentration. Increased Na^{+} concentration in leaves is one of the primary plant response to salinity as reported earlier (Shachtman and Munns, 1992). Sodium accumulation was significantly affected by the varieties and salinity level (Fig. 1). The lowest Na^{+} concentration was observed in the leaves of 'Ferro' variety, while the highest Na^{+} concentration was in the leaves of 'Obez' control treatment plants. The highest increase in leaf Na^{+} concentration was observed in 'RS841', whereas there was a little increase in 'Ferro' variety. It is assumed that sodium concentration increased in response to salt treatment and these results are in aggrement with previous studies in eggplant (Chartzoulakis and Loupassaki, 1997), pepper (Chartzoulakis and Klapaki, 2000), tomato (Alian *et al*., 2000), and watermelon (Yetişir and Uygur, 2009).

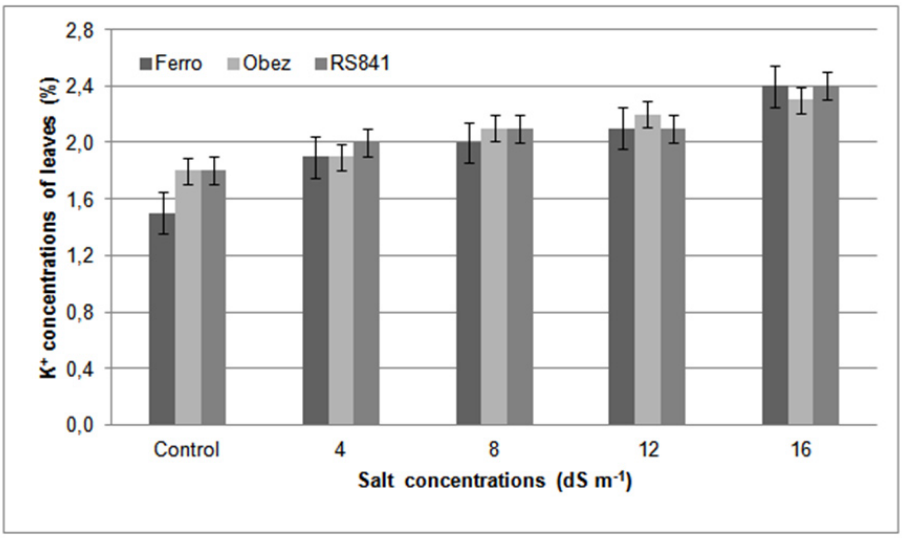

Fig. 3. The effect of NaCl salinity on K^{+} concentration in the leaves of pumpkin varieties (significant at 0.01 level)

Ca^{++} concentration significantly increased in all varieties. The highest Ca^{++} amount was measured in 'RS841' variety, while the lowest Ca^{++} amount in 'Ferro' variety (Fig. 2). It is known that Ca^{++} is important during salt stress, for example on inpreserving membrane integrity (Ashraf and Orooj, 2006; Rengel, 1992) and on signalling in osmoregulation (Mansfield *et al.*, 1990). It was reported that the decrease of calcium attraction under saline conditions was because of the increase in Na^+/Ca^{++} ratio, which also limited the root growth (Garcia-Sanchez *et al,*. 2002; Orcutt *et al.*, 2000). Ca^{++} accumulation in plant tissues suggested that the higher Ca^{++} concentration in the plants might be a factor involved in conferring salt tolerance. In addition, recent studies showed that increased Ca^{++} concentration in melon plants challenged with salinity stress could ameliorate the inhibitory effects of salinity stress on plant growth (Kaya *et al.,* 2003; Navarro *et al.,* 2002).

Similarly K^+ concentration also increased in accordance with salt level (Fig. 3). The effect of salinity on K^+ accumulation in the varieties was statisticly significant. 'Ferro' and 'RS841' had the highest K^+ concentration under 16 dS m^{-1} conditions, while 'Ferro' had the lowest K^+ concentration under control conditions.

Concerning the Ca^{++}/Na^+ and K^+/Na^+ ratios in the leaves of varieties, the highest Ca^{++}/Na^+ ratio was observed in 'Ferro', while the lowest was in 'Obez' variety under control treatment (Tab. 7). The Ca^{++}/Na^+ ratio decreased by increasing the salinity level in all the varieties. Reductions in Ca^{++}/Na^+ ratios in plants due to salinity have also been reported in other studies (Ashraf and Orooj, 2006; Grewal, 2010; Safavi and Khajehpour, 2007; Yetisir and Uygur, 2009). The varieties also differed in terms of the K^+/Na^+ ratio. In control plants, 'Ferro' had the highest K^+/Na^+ ratio, while 'Obez' had the lowest K^+/Na^+ ratio. The K^+/Na^+ ratio decreased in all varieties based on salt concentration as the greatest reduction in K^+/Na^+ ratio was in 'Ferro' and the lowest reduction was in 'RS841' from the control and 4 dS m^{-1} salt treatment.

Conclusions

It is a well known fact that the use of grafted seedlings provides tolerance to abiotic stress. In the present study variations were recorded in terms of salinity tolerance among the rootstocks variety. The most affected pumpkin rootstock variety was 'Ferro' as it had a higher ratio of Ca^{++}/Na^+ and K^+/Na^+ than 'Obez' and 'RS841'. Obez produced the highest plant fresh weight, dry weight and plant length than the others. On the other hand, while 'Ferro' produced the highest root length, in terms of accumulation of Ca^{++} and Na^+ it had the lowest values.

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