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Effect of Salt Stress on Growth and Na, K Contents of Pepper (*Capsicum annuum* L.) In Germination and Seedling Stages

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Abstract: The effects of NaCl concentration (0, 50, 100 and 150 mM NaCl) on the growth and development of pepper varieties, Demre, Çetinel 150 and Ilica 256, were investigated for germination and seedling growth. The parameters measured were germination (speed and germination rate), radicle length, hypocotyl length, radicle fresh weight, hypocotyl fresh weight and the ratio of hypocotyls to radicle fresh weight in the germination stage. High NaCl had a negative effect on plant growth and development. In the seedling stage, the negative effects were relative growth rate for root and shoot length; Relative growth rate for fresh and dry weights of roots, shoots and whole plant; fresh and dry weight of shoot to root indices, relative growth rate for leaf number and area. Leaf content of K^+ and Na^+ and K^+/Na^+ were negatively affected by increased NaCl concentrations. It was found that the Na^+ concentration increased whereas the K^+ and K^+/Na^+ values decreased in leaves. Ilica 256 showed better NaCl tolerance in the germination stage and Demre was the most tolerant at the seedling stage than other varieties. This research demonstrated different NaCl tolerances for the varieties tested based on growth and development ability at germination and seedling development.

Key words: Relative growth rate, NaCl, salt stress, pepper (*Capsicum annuum* L.), K^+, Na^+, growth, development

INTRODUCTION

One of the most effective ways to overcome salinity problems is the use of salt tolerance species and varieties. The response of plants to increasing salt concentration may vary considerably among plant species as a function of their inherent salt tolerance[1]. Salt tolerance may alter according to environmental conditions of crops and plant growth stages. NaCl salinity affects water and ion transport process in plants and these may change the nutritional status and ion balance as well as many physiological processes[2]. The burden of high salt concentrations for plants is due to the osmotic retention of water and to specific ionic effects on the protoplasm. Water is osmotically held in salt solutions, so that as the concentration of salt increases water becomes less and less accessible to the plants. An excess of Na^+ and, to an even greater extent, excess Cl^- in the protoplasm leads to disturbances in the ionic balance (K^+ and Ca^{2+} to Na^+) as well as ion-specific effects on enzyme proteins and membranes[3].

When the salt concentration of the soil solution increases and the water potential decreases, the pressure potential of plant cell declines and cells ultimately cease to divide and elongate. Under these situations of water stress conditions, stomata close usually. This results in the reduction of photosynthesis. Protein breakdown is changed and plants ultimately show poor or negative growth and may lose biomass[4]. Salinity damage is common in vegetables and vegetables are often ranked according to tolerance and damaging effects for example S*olanaceae*. Pepper is considered moderately sensitive to salinity[5].

The effect of salt varies at different plant stages. Thus, choice of plants to be grown on a saline soil depends on plant behavior in germination. Plants may be sensitive at the germination stage, but they can be more tolerant in later growing periods[6,7].

The purpose of this study was to investigate of pepper varieties for; (I) characteristics of germination and seedling growth (ii) comparison of salt tolerance in germination and seedling stages (iii) determination of leaf

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K^+, Na^+ and K^+/Na^+ ratios (iv) identify relationship between elements and salt tolerance at the seedling stage.

MATERIALS AND METHODS

In this investigation, three particular varieties (*Capsicum annuum* L. cv. Demre, Cetinel 150 and Ilica 256) of pepper extensively cultivated in Turkey were used as plant material. The experiments were carried out in germination and seedling stages.

Germination experiments: For each cv. 25 seeds of healthy and almost uniform size were washed with deionised water. These seeds were transferred to sterile petri dishes (10 cm diameter) containing two layers of the filter papers moistened with 10 ml of tap water (control) and the tap water added with 50, 100 and 150 mM NaCl for treatments. In order to avoid water losses, 5 ml of these solutions added petri dishes, every day. Seeds were allowed to germinate at about 25±1°C in the dark. In the 14th days, seed growth in terms germination rate and period; young plant growth in terms length and fresh weight for radicle and hypocotyl were recorded.

Seedling experiments: Twenty plants of each variety were seeded in a seedling pots (of 10 cm diameter) filled with a standard soil:farm manure (2:1); and they were grown in a greenhouse with natural light, air temperature of average 30/25°C (day/night), relative air humidity of 85±10. The seedlings were watered tap water to the three-four leaf stage (30 days) and then were irrigated every two days with 0 (control), 50, 100 and 150 mM NaCl contained tap water (50 ml). Seedlings were harvested and washed with deionised water in the 60 days (150 mM NaCl showed negative effects on the plants). Washed seedlings were separated into root, shoot and leave and used for the determination of RGR values for elongation, fresh weight, dry weight, number and area of roots, shoots and leaves. Dry weight was determined after oven drying the samples at 65°C to for 3 d.

The relative growth rates were designed and calculated with RGR= (ln IV-ln FV).$(t-t_0)^{-1}$ according to Grunberg and Taleisnik[8], Krug[9] and Windt and Van Hasselt[10]. RGR = Relative Growth (elongation, fresh weight, dry weight, number and area for roots, shoots and leaves) Rates; IV= Initial value of any parameter (elongation, fresh weight, dry weight, number and area for roots, shoots and leaves); FV= Final value of any parameter (elongation, fresh weight, dry weight, number and area for roots, shoots and leaves); t= final time of measure for any parameter value (elongation, fresh weight, dry weight, number and area for roots, shoots and leaves); t_0= initial time of measure for any parameter value (elongation, fresh weight, dry weight, number and area for roots, shoots and leaves).

Na^+ and K^+ concentrations were determined from dry, powdered plant tissue before extraction in the dry ashing procedure methods[11], using an atomic absorption spectrophotometer (Perkin Elmer 3110, USA).

Statistical analysis: In both stages (germination and seedling) of the experiment was used completely randomized blocks experimental design with three replications (of 25 seeds and 20 plants in for germination and seedling stages, respectively). Results were expressed as means of three replications (n=3) by F test. Differences among the means were compared by Duncan's multiple range test.

RESULTS

Generally, on increasing the concentration of NaCl; the percent and period of germination, lengths and weights (fresh and dry) of radicles and hypocotyls decreased, in comparison to control. The percentage and period of germination, lengths and weights of radicles and hypocotyls decreases changed according to the varieties (Table 1). Generally, Ilica 256 had better mean values than Çetinel 150 and Demre for all seed germination and growth parameters in 50 mM NaCl but not hypocotyls length and hypocotyl fresh weight. At the 100 mM NaCl, Çetinel 150 was better than other two varieties for radicle length, hypocotyl length, radicle fresh weight and hypocotyl/radicle fresh weight index. And Ilica 256 was more superiority other varieties for germination rate, germination period and hypocotyl fresh weight. At high salinity concentration (150 mM NaCl), Demre was successful variety for germination rate, hypocotyl length and hypocotyl fresh weight criterions than the other two varieties. Also, Ilica 256 was better than others for radicle length and radicle fresh weight. On the other hand, Çetinel 150 had the greatest value for germination period hypocotyl/radicle fresh weight index.

Increasing NaCl concentration caused reduction in relative root, shoot and whole plant growth of pepper seedling (Table 2 and Table 3).

Variety response was different to increasing salinity. At low salinity level (50 mM NaCl), Ilica 256 and Çetinel 150 varieties behaved similar for radicle length and hypocotyl length parameters. While Ilica 256 had the

Table 1: The effect of NaCl and varieties on germination and radicle-hypocotyl growth

NaCl (mM)	Variety	GR %	GP day	RL mm	HL mm	RFW mg $plant^{-1}$	HFW mg $plant^{-1}$	HRFWI mg $plant^{-1}$
0	Ç. 150	78.3c	5.2g	28.7a	29.9a	161.3c	482.0b	3.0ab
0	Demre	85.0ab	6.5e	30.0a	25.1c	186.7a	557.7b	2.1cd
0	Ilica 256	80.0bc	5.3g	30.3a	28.6ab	170.3b	672.0a	3.5a
50	Ç. 150	76.7cd	6.6e	23.3c	27.2bc	135.0e	369.0c	2.1cd
50	Demre	71.7d	6.0f	22.5c	27.3bc	142.7d	528.7b	2.0cde
50	Ilica 256	88.3a	4.5h	25.9b	24.8c	146.0d	495.0b	3.6a
100	Ç. 150	53.3e	7.7d	19.7d	19.4d	115.7f	290.7d	2.5bc
100	Demre	50.0e	9.6b	14.3e	18.3d	87.0h	275.0d	1.6def
100	Ilica 256	76.7cd	6.3ef	17.6d	14.7e	106.7g	301.0cd	1.7de
150	Ç. 150	35.0f	8.2c	7.4f	11.6f	79.7h	194.7e	1.5def
150	Demre	51.7e	11.8a	3.9g	11.9f	65.3i	280.3d	1.0f
150	Ilica 256	35.0f	8.3c	13.1e	7.3g	80.0h	228.3de	1.4ef

(GR: Germination Rate, GP: Germination Period, RL: Radicle Length, HL: Hypocotyl Length, RFW: Radicle Fresh Weight, HFW: Hypocotyl Fresh Weight, HRFWI: Hypocotyl/Radicle Fresh Weight Index). All parameters are significant at P = 0.01 in variance analyses. Comparisons between means were made with Duncan's multiple range tests within each column. Values followed by a common letter are not significantly different, P = 0.05

Table 2: The effect of NaCl and varieties on the root, shoot and whole plant growth of sweet pepper seedlings

		Relative Length Rate (μm $μm^{-1}$ day^{-1})		Relative Fresh Weight Rate (μm $μm^{-1}$ day^{-1})			Relative Dry Weight Growth Rate (μm $μm^{-1}$ day^{-1})		
NaCl(mM)	Variety	Root	Shoot	Root	Shoot	Whole	Root	Shoot	Whole
0	Ç. 150	219.4a	282.1a	31.8a	76.5b	69.3b	43.9a	69.5b	64.4b
0	Ilica 256	195.6b	227.3c	29.5ab	74.4b	67.5b	42.4a	70.2b	64.8b
0	Demre	199.9b	269.0b	28.2b	82.8a	74.7a	27.8e	76.6a	69.0a
50	Ilica 256	164.0c	150.6e	20.2c	50.8f	44.8f	37.7b	54.4d	50.6d
50	Ç. 150	156.4c	159.4e	16.5d	56.1e	49.7e	33.2c	61.7c	56.4c
50	Demre	133.4de	198.8d	9.5fg	69.8c	61.4c	28.6de	73.6a	66.3ab
100	Ilica 256	137.0d	92.6g	12.3ef	42.0g	36.6g	9.7h	34.9f	29.6f
100	Ç. 150	133.1de	119.4f	13.4e	51.5f	44.9f	24.0f	48.0e	43.3e
100	Demre	81.2h	116.4f	9.3fg	65.6d	57.4d	31.3cd	62.5c	56.7c
150	Ilica 256	96.6g	45.2i	7.8gh	30.5h	26.1h	4.1i	12.3g	10.3g
150	Ç. 150	124.2ef	77.5h	5.9h	44.0g	37.4g	15.5g	36.2f	32.0f
150	Demre	116.3f	38.2i	9.0g	54.8ef	47.3ef	22.6f	48.3e	43.2e

All parameters are significant at P = 0.01 in variance analyses. Comparisons between means were made with Duncan's multiple range tests within each column. Values followed by a common letter are not significantly different, P = 0.01

Table 3: The effect of NaCl and varieties on shoot/root weight indices and leaf number and leaf area of sweet pepper varieties

		Shoot/Root Weight Indices		Relative Growth Rate: Leaf Number (no no^{-1} $day.10^{-3}$)	Relative Growth Rate: Leaf Area (cm^2 cm^{-2} $day.10^{-3}$)
NaCl(mM)	Variety	Fresh	Dry		
0	Ç. 150	10.7cd	6.6cd	9.9bc	50.9NS
0	Demre	14.2b	12.0a	19.8a	49.0NS
0	Ilica 256	11.4c	5.9cd	17.8a	39.2NS
50	Ç. 150	8.1e	6.8cd	8.6bcd	47.9NS
50	Demre	16.7a	10.7b	11.0b	41.5NS
50	Ilica 256	8.3e	6.4cd	10.4bc	36.4NS
100	Ç. 150	8.7de	6.0cd	7.6cde	34.7NS
100	Demre	14.9ab	7.2c	5.7de	17.3NS
100	Ilica 256	7.2ef	5.7d	7.2cde	17.0NS
150	Ç. 150	8.7de	5.4d	5.3e	11.2NS
150	Demre	10.8cd	6.0cd	5.2e	5.9NS
150	Ilica 256	5.8f	3.5e	4.9e	3.5NS

All parameters are significant at P= 0.01 in variance analyses, except Relative Leaf Area Rate. Comparisons between means were made with Duncan's multiple range tests within each column. Values followed by a common letter are not significantly different, P= 0.01

Table 4: The effect of NaCl and varieties on the Na^+ and K^+ contents and K^+/Na^+ values of whole leaves in the pepper seedlings

NaCl (mM)	Varieties	Na^+ (g kg^{-1})	K^+ (g kg^{-1})	K^+/Na^+
0	Cetinel 150	2.5g	34.2d	14.0b
0	Demre	1.9g	38.7a	20.5a
0	Ilica 256	2.5g	36.4b	14.6b
50	Cetinel 150	24.7d	31.9ce	1.3cd
50	Demre	24.2de	34.1c	1.4cd
50	Ilica 256	17.0f	36.2b	2.1c
100	Cetinel 150	26.6d	33.4cd	1.3cd
100	Demre	24.0de	30.6ef	1.3cd
100	Ilica 256	21.8e	28.8fg	1.3cd
150	Cetinel 150	56.4b	29.0fg	0.5d
150	Demre	44.4c	29.9fg	0.7d
150	Ilica 256	62.4a	28.3g	0.5d

All parameters are significant at P = 0.01 in variance analyses. Comparisons between means were made with Duncan's multiple range tests within each column. Values followed by a common letter are not significantly different, P = 0.05

greatest value for relative root dry and fresh rate, Demre was the best variety RGR for fresh and dry of shoot and whole plant. Generally, on increasing the concentration of NaCl, Demre appeared more tolerant variety. In the 100 mM NaCl, Demre was the greatest variety for many parameters as shoot lengths, dry weights of root and fresh and dry weights of shoots and whole plants. On high the concentration of NaCl (150 mM), Demre had better relative seedling growth parameters than Ilica 256 and Çetinel 150, except length of root and shoot. Namely, Demre was seen slightly more tolerant than Çetinel 150 and Ilica 256 in length, fresh and dry weights of root, shoot and whole plant parameters.

Shoot/root fresh and dry weight indices and relative leaf number and area values decreased with increasing NaCl concentrations (Table 3). At all NaCl concentrations, Demre was more tolerant variety than Çetinel 150 and Ilica 256 for shoot/root fresh and dry weight indices. Superiority of Çetinel 150 was observed for relative leaf number and relative leaf area.

Salinity increased the Na^+ content and decreased the K^+ content and K^+/Na^+ value of the pepper seedling leaves (Table 4). The highest Na^+ level was obtained in Centinel 150 and followed by Demre and Ilica 256 at 50 and 100 mM NaCl. At 150 mM NaCl, Ilica 256 accumulated Na^+ more than the other two varieties. It can be see a decrease leaf K content of the varieties with increasing NaCl. K^+ contents of cultivers were very distinctive at all NaCl concentrations. The highest K^+/Na^+ values were obtained from Ilica 256 at 50 and 100 mM the NaCl concentrations. Demre had the best K^+/Na^+ value at the 150 mM NaCl.

DISCUSSION

In this investigation, it was determined that the salinity caused loses of germination characteristics of sweet pepper varieties. Similarly, the seedling stage characteristics of sweet pepper varieties were negatively affected by increasing NaCl. Results were agreed previously investigations[7,12-19]. When salinity increased, loses were observed in germination characteristics and at the seedling stage and these loses varied in a similar manner to varieties tested and cited above.

The differences among the varieties observed in this study are summarised below. Demre was slightly less tolerant to increasing NaCl concentrations than Çetinel 150 and Ilica 256 at the germination stage. In the seedling stage, Demre was found more tolerant to increasing salinity concentrations in the many seedling stage characteristics. The results supported other studies that plant show various adaptation to salinity in different growing stages. As a matter fact, cultivers in less more tolerant to salinity in the early growing stages[7,12,15,20,21,22,23]. The germination and seedling stage characteristics were affected negatively by salinity. In addition, the effects of salinity on the varieties varied with growth stage. This was explained as differences of Na^+ and K^+ uptake among varieties. The K^+ uptake and accumulation of cells tissue were affected negatively by increasing Na^+ as demonstrated elsewhere [14,24-27] In addition, there is accumulation of a controlled amount of salt as an osmoticum, which is different for different cell of plants[28]. There is a significant correlation between salt tolerance and K^+ uptake of plant varieties and species[13,29]. It is reported that salt tolerance of species and varieties can balance osmoticum and adjust Na^+ and K^+ levels[21,30]. This situation can be better explained with K^+/Na^+ ratios in plants. The varieties and species that had high K^+/Na^+ ratios have a higher tolerance to salt[31,32]. In this study, the Na^+ content in leaf increased with increasing salt concentration and the K^+ content of leaf decreased. The increasing salt stress prevented K^+ uptake of plants. According to seedling stage characteristics, Demre showed the higher tolerance to salt than the other varieties. While Na content of this variety was lower and K^+ content and K^+/Na^+ ratios were significantly higher than the other varieties, in especially 150 mM NaCl. The data indicated that salt tolerances of tolerant varieties in peppers related to mechanism of Na^+ uptake under control or ability of regulation of K^+ levels. At the plant cell level, a few strategies can be suggested for the plants survival in the saline conditions. Salt may be taken up by the cells and used as a major osmoticum and ion toxicity could be prevented by compartmentation or by some other mechanism of protecting enzyme and ribosomal activities[30-34]. It has been benefited from genotypes balanced to uptake of K^+ and Na^+ levels to structure at the agricultural as economical. The variation in salt tolerances with growth stage could facilitate successful commercial growing as shown by[4,7,31,33,35]. Determining of varieties to salt tolerance in early growth stages like the seedling stage is very important in determining the time taken to produce commercial crops[14,36].

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