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Genotypic variation in the response of pepper to salinity

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Abstract

Using 102 pepper (*Capsicum annuum*) genotypes, a greenhouse experiment has been conducted to study genotypic variation in tolerance to 100 mM sodium chloride (NaCl) in nutrient solution. Based on the severity of leaf symptoms caused by the NaCl treatment there was a substantial genotypic variation in salt tolerance. From this screening experiment, six sensitive and six tolerant genotypes were chosen to study dry matter production and root and shoot concentrations of sodium (Na), potassium (K) and calcium (Ca) in a growth chamber experiment in a nutrient solution with and without 150 mM NaCl. The genotypes selected as sensitive were highly damaged and developed severe chlorosis and necrosis under NaCl treatment, while the genotypes selected as tolerant were slightly affected. On average, decreases in shoot dry matter production caused by NaCl were greater in the sensitive than the tolerant genotypes. Application of salt increased shoot Na concentration at greater amount in the sensitive than the tolerant genotypes. Of the tolerant genotypes, the genotype Cac (*Capsicum annuum* var. *cerasiforme*) and 1245 F1 had around 2.45% Na in shoot while the sensitive genotypes Kandil and Pazarcik contained, on average, 5.4% Na. All sensitive and tolerant genotypes exhibited more or less similar shoot concentrations of K and Ca. There was very significant and positive correlation between severity of leaf symptoms and shoot Na concentration, but no correlation could be found in the case of K or Ca concentrations with the severity of leaf symptoms. The results indicate existence of substantial genotypic variation in tolerance to NaCl stress in pepper. It seems very likely that exclusion of Na from roots into growth medium plays a critical role in expression of high Na tolerance in pepper.

Keywords: *Capsicum annuum*; Genotypic variation; Pepper; Salt tolerance; Sodium chloride

1. Introduction

Accumulation of salts at excessive amounts in cultivated soils is a common problem, especially under irrigated conditions, threatening food production globally (Bohnert and Jensen, 1996; Zeng et al., 2003). A commonly adopted method to reduce the salt level in soil is the leaching of salt accumulated in soil profile. This strategy is, however, not sustainable and cost-effective because it is a time consuming and expensive approach, and the supply of good quality water for leaching and irrigation is generally limited (Sohan et al., 1999). Salinity stress depresses plant growth and development at different physiological levels. The reduction in plant growth by salinity stress might be related to adverse effects of excess salt on ion homeostasis, water balance, mineral nutrition and photosynthetic carbon metabolism (Zhu, 2001; Munns, 2002). The mechanisms by which salt stress damage plants are still a discussing matter due to very complex nature of the salt stress in plants.

In many crop production areas, use of low quality water for irrigation and application of excess amounts of mineral fertilizer are the major reasons for increased salinity problem in cultivated soils. Due to very rapid accumulation of salts in soil under greenhouse conditions, salinity problem is also a critical constraint to vegetable production (Shannon and Grieve, 1999). Among the vegetables, pepper (*Capsicum annuum* L.) is very susceptible to salt stress, and salt-affected pepper shows severe decreases in growth and disturbances in membrane permeability, water channel activity, stomatal conductance, photosynthesis and ion balance (Sonneveld, 1988; Bethke and Drew, 1992; Carvajal et al., 1999; Shannon and Grieve, 1999; Navarro et al., 2003; Cabanero et al., 2004).

Among the factors studied for characterization of salt tolerance in crop plants, an increasing attention has been paid to the nutritional status of plants with potassium (K) and calcium (Ca). Increasing supply of NaCl impairs root uptake of K and Ca and interferes with their physiological functions (Rengel,

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1992; Marschner, 1995; Zhu, 2001; Yoshida, 2002). Therefore, ability of plant genotypes to maintain higher levels of K and Ca and low levels of Na within tissue is one of the key mechanisms contributing to expression of high salt tolerance. In most cases, salt-tolerant genotypes are capable of maintaining higher K/Na ratios in tissues (Mansour, 2003; Zeng et al., 2003). By reducing Na uptake and promoting K uptake, Ca greatly contributes to maintenance of higher K/Na ratios in plant as shown in pepper plants (Rubio et al., 2003). Ability of genotypes to extrude Na from roots in to growth medium is an

Table 1
Scale score (1–5) of 26 days old 102 pepper genotypes grown in nutrient solution with 100 mM NaCl and genotypes origin and types

No.	Genotypes	Origin	Type	Symptom score	No.	Genotypes	Origin	Type	Symptom score
1	*Cac* *	INRA	*W*	1.3^{f}	52	845_{F1}	WMARI	*C*	$3.0^{a–e}$
2	Kraska	OM	*C*	$1.6^{e–f}$	53	5020_{F1}	WMARI	*C*	$3.0^{a–e}$
3	Olenka	OM	*C*	$1.6^{e–f}$	54	$Ethem_{F1}$	Seminis	*C*	$3.0^{a–e}$
4	CS-279	INRA	*L*	$1.6^{e–f}$	55	AT-61	Golden Seed	*C*	$3.0^{a–e}$
5	Firat 558 F1	WMARI	*C*	$1.6^{e–f}$	56	AT-47	Golden Seed	*C*	$3.0^{a–e}$
6	AT-44	Golden Seed	*L*	$2.0^{d–f}$	57	AT-51	Golden Seed	*C*	$3.0^{a–e}$
7	HAD 174	INRA	*L*	$2.0^{d–f}$	58	Urfa 247	CUAF	*L*	$3.0^{a–e}$
8	AT-63	Golden Seed	*L*	$2.0^{d–f}$	59	E 2853 F1	Seto Seed	*C*	$3.0^{a–e}$
9	Ilıca-256	Sount Seed	*C*	$2.0^{d–f}$	60	N53	GG	*C*	$3.0^{a–e}$
10	Charlee F1	Seminis Seed	*C*	$2.0^{d–f}$	61	N55	GG	*C*	$3.0^{a–e}$
11	Elazıg Dolma	CUAF	*C*	$2.0^{d–f}$	62	Urfa Yerli	CUAF	*L*	$3.0^{a–e}$
12	Perennial	INRA	*W*	$2.0^{d–f}$	63	AT-64	Golden Seed	*C*	$3.0^{a–e}$
13	Julia F1	Rito Seed	*C*	$2.3^{c–f}$	64	AT-42	Golden Seed	*C*	$3.0^{a–e}$
14	AT-56	Golden Seed	*L*	$2.3^{c–f}$	65	AT-53	Golden Seed	*C*	$3.0^{a–e}$
15	AT-43	Golden Seed	*L*	$2.3^{c–f}$	66	HAD 160	INRA	*L*	$3.0^{a–e}$
16	37-01 F1	Rito Seed	*C*	$2.3^{c–f}$	67	Podstollina	OM	*C*	$3.0^{a–e}$
17	PM-217	INRA	*W*	$2.3^{c–f}$	68	AT-57	Golden Seed	*C*	$3.0^{a–e}$
18	Demre F1	WMARI	*C*	$2.3^{c–f}$	69	AT-59	Golden Seed	*C*	$3.0^{a–e}$
19	AT-45	Golden Seed	*L*	$2.3^{c–f}$	70	AT-49	Golden Seed	*C*	$3.0^{a–e}$
20	AT-46	Golden Seed	*L*	$2.3^{c–f}$	71	AT-54	Golden Seed	*C*	$3.0^{a–e}$
21	AT-48	Golden Seed	*L*	$2.3^{c–f}$	72	Melis F1	Rito Seed	*C*	$3.0^{a–e}$
22	Islahiye-3	CUAF	*L*	$2.3^{c–f}$	73	AT-69	Golden Seed	*C*	$3.0^{a–e}$
23	AT-41	Golden Seed	*C*	$2.3^{c–f}$	74	Alata-38	EMARI	*L*	$3.0^{a–e}$
24	AT-65	Golden Seed	*C*	$2.3^{c–f}$	75	Yolo Wonder	INRA	*C*	$3.0^{a–e}$
25	AT-62	Golden Seed	*C*	$2.3^{c–f}$	76	AT-67	Golden Seed	*C*	$3.3^{a–d}$
26	Alata-42	EMARI	*C*	$2.3^{c–f}$	77	Kale F1	Seto Seed	*C*	$3.3^{a–d}$
27	40157 F1	WMARI	*C*	$2.6^{b–f}$	78	Balo F1	Rito Seed	*C*	$3.3^{a–d}$
28	AT-73	Golden Seed	*C*	$2.6^{b–f}$	79	*C. baccatum*	CUAF	*L*	$3.3^{a–d}$
29	AT-74	Golden Seed	*C*	$2.6^{b–f}$	80	AT-72	Golden Seed	*C*	$3.3^{a–d}$
30	AT-70	Golden Seed	*C*	$2.6^{b–f}$	81	Beldi-3	INRA	*C*	$3.3^{a–d}$
31	Donna F1	Seto Seed	*C*	$2.6^{b–f}$	82	AT-52	Golden Seed	*C*	$3.3^{a–d}$
32	Alata-43	EMARI	*C*	$2.6^{b–f}$	83	AT-71	Golden Seed	*C*	$3.3^{a–d}$
33	Alata-29	EMARI	*C*	$2.6^{b–f}$	84	Sirena F1	Rito Seed	*C*	$3.3^{a–d}$
34	1245 F1	WMARI	*C*	$2.6^{b–f}$	85	AT-68	Golden Seed	*C*	$3.3^{a–d}$
35	948 F1	WMSRI	*C*	$2.6^{b–f}$	86	Yanka F1	Rito Seed	*C*	$3.3^{a–d}$
36	Urfa 238-7	CUAF	*C*	$2.6^{b–f}$	87	Punto F1	Rito Seed	*C*	$3.3^{a–d}$
37	Yağız 375 $_{F1}$	WMARI	*C*	$2.6^{b–f}$	88	AT-50	Golden Seed	*C*	$3.6^{a–c}$
38	15914 F1	WMARI	*C*	$2.6^{b–f}$	89	Kekova F1	Antalya Tarım	*C*	$3.6^{a–c}$
39	Alata-41	EMARI	*C*	$2.6^{b–f}$	90	AT-58	Golden Seed	*C*	$3.6^{a–c}$
40	SC 81	INRA	*L*	$2.6^{b–f}$	91	AT-60	Golden Seed	*C*	$3.6^{a–c}$
41	N 52	GG	*C*	$2.6^{b–f}$	92	Hülya F1	WMARI	*C*	$3.6^{a–c}$
42	Ozarowska	OM	*C*	$2.6^{b–f}$	93	Telimena	OM	*C*	$3.6^{a–c}$
43	Alata-4	EMARI	*L*	$2.6^{b–f}$	94	AT-55	Golden Seed	*C*	$3.6^{a–c}$
44	Pegasus F1	Seto Seed	*C*	$2.6^{b–f}$	95	AT-40	Golden Seed	*C*	$3.6^{a–c}$
45	AT-66	Golden Seed	*C*	$2.6^{b–f}$	96	*C. pubescens*	CUAF	*W*	$3.6^{a–c}$
46	PM-702	INRA	*C*	$3.0^{a–e}$	97	*C. frutescens*	CUAF	*W*	$4.0^{a–b}$
47	Alata-17	EMARI	*L*	$3.0^{a–e}$	98	KM2-3	CUAF	*L*	$4.0^{a–b}$
48	Alata-20	EMARI	*L*	$3.0^{a–e}$	99	Cango F1	Seto Seed	*C*	$4.0^{a–b}$
49	1590 F1	WMSRI	*C*	$3.0^{a–e}$	100	Madison	GG	*C*	$4.0^{a–b}$
50	Alata-7	EMARI	*L*	$3.0^{a–e}$	101	Kandil	GAP Seed	*C*	$4.0^{a–b}$
51	Alata-10	EMARI	*L*	$3.0^{a–e}$	102	Pazarcik	CUAF	*L*	4.3^{a}

L: line, W: wild, C: cultivar. (INRA) Insitut National de la Recherche Agronomique, Avignon, France; (OM) Ozarow Mazowiecki Seed Company, Krakow, Poland; (WMARI) Western Mediterranean Agriculture Research Institute, Antalya, Turkey; (EMARI) Eastern Mediterranean Agriculture Research Institute, Mersin, Turkey; (GG) Gauntier Graines Seed Company, Holland; (CUAF) Cukurova University, Faculty of Agriculture, Adana, Turkey. Values indicated with different superscripts are significantly different from each other according to the Duncan multiple range test ($P < 0.05$).

* *Capsicum annuum* var. *cerasiforme*.

important plant trait contributing to higher K/Na ratios and expression of high salt tolerance (Yoshida, 2002; Zhu, 2002). According to Munns and James (2003), Na exclusion mechanisms correlated very well with salinity tolerance in tetraploid wheat genotypes. In Arabidopsis (Elphick et al., 2001) and yeast (Almagro et al., 2001) systems, higher sensitivity to NaCl was found to be associated with poor ability of genotypes to activate Na efflux system. In screening plant genotypes for high tolerance to salt stress the K/Na and Na/Ca ratios and tissue Na concentration are, therefore, widely used parameters for different crop species (Munns and James, 2003).

In the case of pepper, very rare information is available in literature regarding to genetic variability for salt tolerance and the role of K/Na ratio in identification of genotypes with high tolerance to salt stress. Another concern is that in these studies only a few pepper genotypes have been considered to study genetic variability for tolerance to NaCl (Cornillon and Palloix, 1997; Chartzoulakis and Klapaki, 2000). For a reasonable and useful genetic variation to use in breeding programs, large number of genotypes should be considered in screening for tolerance to NaCl. In the present study, 102 pepper genotypes have been screened for their tolerance to NaCl based on the severity of leaf symptoms caused by 100 mM NaCl treatments. By considering the results (severity of leaf symptoms) from this screening study, six tolerant and six sensitive genotypes were selected for analysis of shoot and root growth and the concentrations of K, Na and Ca in root and shoot tissue.

2. Materials and methods

As plant material, 102 pepper genotypes were used. The name and origins of all these genotypes were presented in Table 1. Two different experiments have been conducted with different number of pepper genotypes. The experimental design was completely randomized with five independent replications. For each replication one plant has been used.

In the first experiment, seeds of 102 pepper genotypes (Table 1) were germinated for 12 days at 30 °C in perlite under greenhouse conditions, and the seedlings emerged were then transferred to 50 L plastic pots containing aerated nutrient solution consisted of 2 mM $Ca(NO_3)_2$, 0.88 mM K_2SO_4, 1.0 mM $MgSO_4$, 0.25 mM KH_2PO_4, 10 μmol H_3BO_3, 0.5 μmol $MnSO_4$, 1 μmol $ZnSO_4$, 100 μmol Fe EDTA; 0.2 μmol $CuSO_4$ and 0.02 μmol $(NH)_6Mo_7O_{24}$. Nutrient solutions were renewed every 3 days. The pepper plants were grown in the nutrient solution without salt treatment up to 6–7 true leaf stage (14 days after transfer to nutrient solution). Then, NaCl was supplied as 25 mM increments every 12 h until a final concentration of 100 mM (10.9 dS/m at 25 °C). The plants were grown for 10 days under 100 mM NaCl. On day 26 in nutrient solution, genotypes were scored and classified according to the severity of leaf symptoms caused by 100 mM NaCl by using a 1–5 symptom scale (Fig. 1). As shown in Fig. 1, severity of leaf symptoms (leaf chlorosis and necrosis) was ranked as following: (1) no or very slight, (2) slight, (3) mild, (4) severe and (5) very severe. Based on the severity of leaf symptoms, six sensitive and six tolerant genotypes have been selected and used in the second experiment.

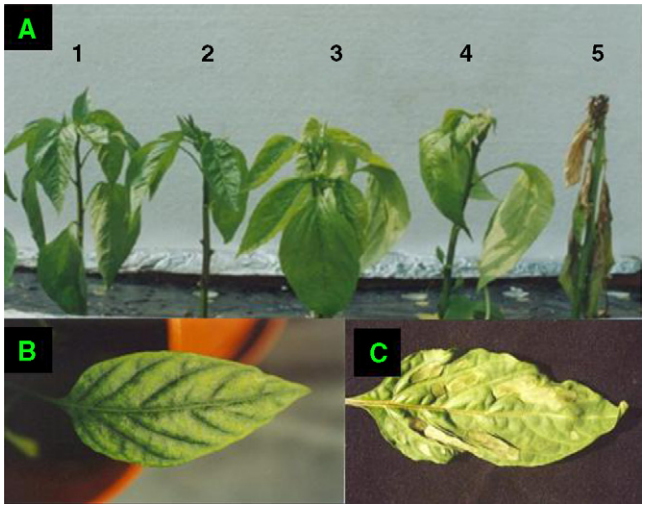

Fig. 1. Severity of leaf symptoms on 26 days old pepper plants grown in nutrient solution containing 100 mM NaCl. All genotypes were scored by using 1–5 scores. (A) Leaf chlorosis and necrosis: 1, no or very slight; 2, slight; 3, mild; 4, severe and 5, very severe). (B) Chlorosis and (C) necrotic damage on the leaf.

Like in the first experiment, 12 selected genotypes were germinated under greenhouse conditions for 12 days in perlite, and the seedlings were then transferred to 2.5 L plastic pots containing same nutrient solution as described above. Plants were grown in a plant growth chamber under controlled climatic conditions (light/dark regimes of 16/8 h, temperature 26/22 °C, relative humidity 60–70% and light intensity 400 μmol m^{-2} s^{-1} at plant height).

When the plants were at 6–7 true leaf stage (e.g., following 14 days of growth without NaCl treatment in nutrient solution), 25 mM NaCl was supplied into nutrient solution every 12 h until the final concentration reached to 150 mM NaCl in the nutrient solution (EC: 15.35 dS/m). The plants were grown in nutrient solution containing 150 mM NaCl for 10 days. At day 27 following the transfer of plants to nutrient solution, plants were harvested and separated to shoots and roots. All plant parts were dried at 70 °C for 48 h, and weighed for the determination of dry matter production, and then ground and digested for mineral element analysis. Plant samples were ashed at 550 °C and dissolved in 1% (v/v) HCl, and analysed for Na, K and Ca by using an atomic absorption spectrometer (Varian Spectra AA 220 FS). Measurements of Na, K and Ca have been checked by using reference leaf samples from the National Institute of Standards and Technology (Gaithersburg, MD). All measurements have been realized by five independent replications. For statistical analysis, see legends of tables and figures.

3. Results

The genotypes tested showed a large variation in tolerance to 100 mM NaCl treatment based on severity of leaf symptoms. Among the 102 genotypes screened, *Capsicum annuum* var.

Table 2
Shoot and root dry matter production of 27 days old 12 pepper genotypes grown in nutrient solution with (150 mM) and without NaCl treatment

	Symptom score*	Shoot dry weight (mg plant^{-1})		Root dry weight (mg plant^{-1})		Shoot/root (mg plant^{-1})	
		−NaCl	+NaCl	−NaCl	+NaCl	−NaCl	+NaCl
Tolerant genotypes							
Cac	1.3	978	667	146	120	6.70	5.56
1245 F1	2.3	1491	898	156	130	9.56	6.91
Demre F1	1.5	1692	1182	230	160	7.36	7.39
Donna F1	1.8	1440	1049	240	160	6.00	6.56
Kraska	1.5	1400	652	190	110	7.37	5.93
40157 F1	1.8	1215	1080	210	180	5.79	6.00
Mean	1.7	1357	921	195	143	7.02	6.44
Sensitive genotypes							
5020 F1	2.5	1732	984	160	100	10.83	9.84
1590 F1	3.3	1691	913	320	170	5.28	5.37
Cango F1	3.5	1004	853	190	120	5.28	7.11
PM-702	3.3	885	703	175	130	5.06	5.41
Kandil	4.0	1440	607	210	110	6.86	5.52
Pazarcik	4.3	1410	813	260	210	5.42	3.87
Mean	3.5	1360	812	224	140	6.07	5.80
$LSD_{0.05}$		378	171	65	44		

Data represent means of five independent replications.
* 1–5 scores (leaf chlorosis and necrosis: 1, no or very slight; 2, slight; 3, mild; 4, severe and 5, very severe).

cerasiforme (Cac) with a score 1.3 was found to be the most salt tolerant genotype followed by Olenka, Kraska, CS-279 and Firat 558 F1 with 1.6 score values (Table 1). All these genotypes were less affected from salt treatment and showed only slight cholorosis. The genotype Pazarcik was the most sensitive genotype to salinity with 4.3 score, followed by Kandil, KM2-3, Cango F1, Madison and *C. frutescens* with the score of 4.0 (Table 1). The remaining genotypes were placed in different scores between 2.0 and 4.0. Nearly the half of the genotypes tested in the present study had a score between 3.0 and 3.6 and showed mild tolerance. Based on these observations made on 102 pepper genotypes, 12 genotypes were chosen for the next experiment. Among the selected 12 genotypes, there were the 6 tolerant genotypes (Cac, Kraska,

Table 3
Shoot Na, K and Ca concentration (%) and K/Na, Ca/Na rations of 27 days old 12 pepper genotypes grown in nutrient solution with (150 mM) and without NaCl treatment

	Shoot (concentration, %)									
	−NaCl					+NaCl				
	Na	K	Ca	K/Na	Ca/Na	Na	K	Ca	K/Na	Ca/Na
Tolerant genotypes										
Cac	0.45	7.07	1.25	15.7	2.78	2.35	4.26	0.66	1.81	0.28
1245 F1	0.26	5.99	0.86	23.3	3.31	2.53	4.32	0.57	1.71	0.23
Demre F1	0.32	5.28	0.76	16.5	2.38	2.49	4.46	0.50	1.79	0.20
Donna F1	0.23	4.09	0.64	23.1	2.78	2.76	4.28	0.58	1.55	0.21
Kraska	0.23	6.09	1.42	26.5	6.17	2.98	4.05	0.79	1.36	0.27
40157 F1	0.39	6.31	1.08	16.2	2.77	2.71	4.69	0.61	1.73	0.23
Mean	0.31	5.18	1.00	19.3	3.36	2.64	4.34	0.62	1.66	0.23
Sensitive genotypes										
5020 F1	0.29	5.08	0.83	17.5	2.86	3.15	3.88	0.49	1.23	0.16
1590 F1	0.30	5.64	1.25	18.8	4.17	3.58	4.44	0.65	1.24	0.18
Cango F1	0.30	7.13	0.98	23.7	3.27	3.86	3.98	0.62	1.03	0.16
PM-702	0.40	7.24	1.28	18.1	3.20	4.28	4.32	0.92	1.01	0.21
Kandil	0.23	6.20	1.08	26.9	4.70	4.97	3.69	0.66	0.74	0.13
Pazarcik	0.31	5.50	1.16	17.7	3.74	5.86	4.19	0.65	0.72	0.11
Mean	0.32	6.13	1.10	20.1	3.66	4.31	4.08	0.67	1.01	0.16
$LSD_{0.05}$	0.06	0.67	0.21			0.58	0.34	0.07		

Data represent means of five independent replications.

Demre F1, Donna F1, 1245 F1 and 40157 F1) and the 6 susceptible genotypes (PM 702, 1590 F1, 5020 F1, Kandil, Cango F1 and Pazarcik) by considering severity of leaf symptoms caused by 100 mM NaCl treatment (Table 1).

In a separate experiment, the selected 12 genotypes were exposed to 150 mM NaCl. There was large variation in shoot and root dry matter production between 12 genotypes under 150 mM NaCl treatment. On average, shoot dry matter production of the sensitive genotypes was much more affected by NaCl treatment than the tolerant genotypes. For example, in sensitive genotypes Kandil, 1590 F1 and Pazarcik shoot dry matter production was reduced by 54, 46 and 42%, respectively, while in tolerant genotypes the reductions in shoot dry weights were 11% in 40157 F1, 32% in Cac and 30% in Demre F1 and 27% in Donna F1 (Table 2). Interestingly, the tolerant genotype Kraska (based on the severity of leaf symptoms) showed large decrease in shoot dry matter production, while in the sensitive genotype PM-702 reduction in shoot growth by NaCl was less (Table 2). Decreases in shoot dry matter production caused by salt stress were similar to the decreases in root dry weight, but in a lesser extend (Table 2). According to the shoot and root ratio values, salinity stress affects shoot growth much more than the root growth (Table 2).

When NaCl was not supplied, the sensitive and tolerant genotypes had very similar Na and Ca concentrations in shoot (Table 3). Potassium concentration of plants under without NaCl treatment was higher in the sensitive than the tolerant genotypes. On average, the K/Na and Ca/Na ratios were very similar between sensitive and tolerant genotypes when NaCl was not supplied (Table 3). With the NaCl supply, there was a marked increase in Na concentration of plants, particularly in the sensitive genotypes with severe leaf symptoms. The highest increase in Na concentration of shoots by NaCl supply was found in the most sensitive genotype Pazarcik (Table 3). Consequently, shoot Na concentration at NaCl treatment very significantly correlated with the severity of leaf symptoms caused by NaCl toxicity (Fig. 2). Such a very significant correlation could not be found between the K or Ca concentrations and leaf symptoms (Fig. 2). NaCl treatment exerted a reducing effect on K concentration of plants, and this reduction tended to be clearer in the sensitive genotypes. NaCl treatment also reduced Ca concentration of plants.

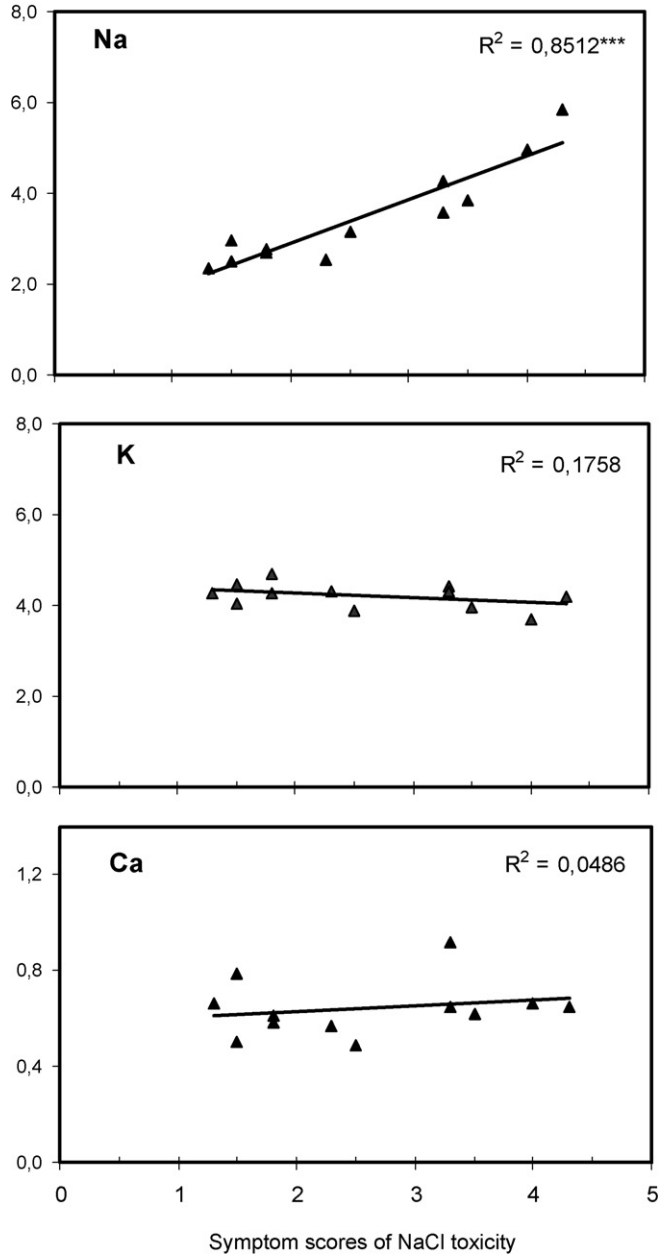

Fig. 2. Relationships between shoot Na, Ka and Ca concentration (%) of 27 days old 12 pepper genotypes grown in nutrient solution with 150 mM NaCl treatment, *n*: 12.

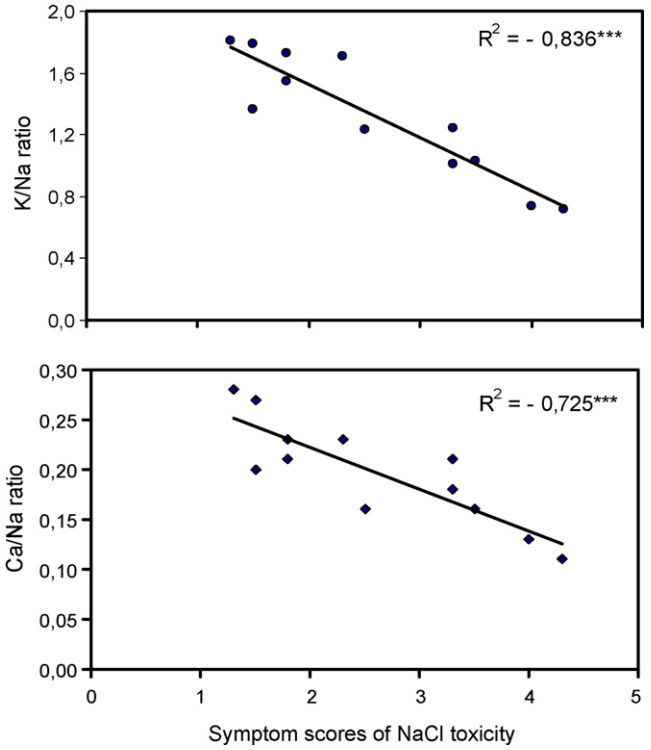

Fig. 3. Relationships between shoot K/Na and Ca/Na ratios and the severity of leaf symptoms caused by salt stress in 27 days old 12 pepper genotypes grown in nutrient solution containing 150 mM NaCl.

Table 4
Root Na, K and Ca concentration (%) and K/Na, Ca/Na rations of 27 days old 12 pepper genotypes grown in nutrient solution with (150 mM) and without NaCl treatment

	Root (concentration, %)									
	−NaCl					+NaCl				
	Na	K	Ca	K/Na	Ca/Na	Na	K	Ca	K/Na	Ca/Na
Tolerant genotypes										
Cac	0.54	6.55	0.25	12	0.46	4.75	2.52	0.16	0.53	0.03
1245 F1	0.25	4.51	0.18	18	0.72	5.99	2.71	0.18	0.45	0.03
Demre F1	0.15	3.78	0.16	25	1.07	6.94	2.74	0.19	0.39	0.03
Donna F1	0.14	5.14	0.18	37	1.29	5.54	2.43	0.19	0.44	0.03
Kraska	0.20	6.61	0.23	33	1.15	6.12	3.30	0.21	0.54	0.03
40157 F1	0.20	3.93	0.17	20	0.85	3.32	1.39	0.09	0.42	0.03
Mean	0.25	5.09	0.20	21	0.79	5.44	2.52	0.17	0.46	0.03
Sensitive genotypes										
5020 F1	0.19	3.76	0.16	20	0.84	6.75	4.02	0.28	0.60	0.04
1590 F1	0.16	5.49	0.18	34	1.13	4.00	2.10	0.14	0.53	0.04
Cango F1	0.15	3.55	0.14	24	0.93	6.04	3.38	0.21	0.56	0.03
PM-702	0.24	5.92	0.24	25	1.00	5.63	2.66	0.26	0.47	0.05
Kandil	0.16	5.26	0.17	33	1.06	5.41	2.29	0.17	0.42	0.03
Pazarcik	0.13	4.5	0.19	35	1.46	5.72	2.49	0.20	0.44	0.03
Mean	0.17	4.75	0.18	28	1.05	5.59	2.82	0.21	0.50	0.04
$LSD_{0.05}$	0.06	0.96	0.04			1.42	0.80	0.06		

Data represent means of five independent replications.

The sensitive and tolerant genotypes were very similar in shoot Ca concentration at NaCl treatment (Table 3). As a consequence of greater increase in Na and decreases in K concentration by NaCl treatment, the sensitive genotypes exhibited much smaller K/Na ratio compared to the tolerant genotypes. The most tolerant and sensitive genotypes Cac and Pazarcik had K/Na ratios 1.81 and 0.72, respectively (Table 3). Also in the case of Ca/Na ratio tolerant genotypes showed greater ratios. Accordingly, Ca/Na and K/Na ratios showed a very significant negative relationship with the severity of leaf symptoms (Fig. 3). The differences in root concentration of Na, K and Ca between the sensitive and tolerant genotypes were very little and statistically not important (Table 4). Only in the case of nil NaCl treatment root Na concentration was higher in the tolerant genotypes. In contrast to the results obtained in shoot, the K/Na and Ca/Na ratios in roots at NaCl treatment did not differ between the sensitive and tolerant genotypes (Table 4).

4. Discussion

Among the pepper genotypes tested there was a large variation in tolerance to salt stress, as judged from the severity of leaf symptoms caused by NaCl treatment (Table 1). To our knowledge, such large variation in tolerance to NaCl by using more than 100 genotypes was not reported in literature. By using, only four pepper genotypes Cornillon and Palloix (1997) reported genotypic variation in tolerance to NaCl. Of the genotypes screened by Cornillon and Palloix (1997) the genotypes SC 81 and Yolo Wonder were classified as tolerant and sensitive genotypes to NaCl, respectively. The same genotypes were also used in the present study and in agreement with the results of Cornillon and Palloix (1997) SC 81 was found to be more tolerant to NaCl than Yolo Wonder (Table 1). However, there were many other genotypes that exhibited much greater tolerance to NaCl than SC 81 and were more sensitive to NaCl than Yolo Wonder (Table 1) was. This observation points out that use of large number of genotypes in a germplasm provides greater potential for a substantial genotypic variation for a given trait.

Under salt stress, severe disturbance in root uptake of mineral nutrients and imbalances between Na, K and Ca at cellular level are commonly observed, and these impairments play a critical role in the extent of salt tolerance of plants (Marschner, 1995; Zhu, 2001; Munns and James, 2003; Lecerda et al., 2005). When absorbed and accumulated at large amounts in plants, Na becomes highly toxic at different physiological levels. Physiological impairments caused by Na toxicity include disruption of K and Ca nutrition, development of water stress and induction of oxidative cell damage. Therefore, maintenance of low Na concentration by preventing Na uptake or regulating Na homeostasis in the cells by higher K/Na ratios or sequestering Na ions in vacuole are the major strategies of plants against Na stress (Rengel, 1992; Bohnert and Jensen, 1996; Zhu, 2001).

Sodium concentration and K/Na ratios of plants are widely accepted and used parameters in screening genotypes for Na tolerance (Gorham et al., 1997; Dasgan et al., 2002; Munns and James, 2003; Shi et al., 2003). In the present study, the genotypes with greater severity of leaf symptoms under salt stress were associated with higher Na concentration (Fig. 2; Table 3). When NaCl was not supplied, the genotypes tested

were more or less similar in Na concentration, but in the case of NaCl treatment, the sensitive genotypes with greater leaf damage had much higher Na concentration in the shoot (Table 3). Regarding to the root concentration of Na, the sensitive and tolerant genotypes were not different (Table 4). Under salt treatment, shoot concentration of K and Ca were also very similar between the sensitive and tolerant genotypes, and thus not related to differential salt tolerance of genotypes (Table 3). Based on these results, it can be suggested that genotypic differences in tolerance to salt stress are primarily related to reduced uptake and thus low accumulation of Na in plants. Possibly, in the salt tolerant pepper genotypes a plasma membrane Na^+/H^+ antiporter protein is activated in root cells upon NaCl exposure to extrude Na from roots into growth medium. Such Na^+/H^+ antiporters with high Na exclusion capacity are well documented in different transgenic plants (Shi et al., 2000, 2002; Waditee et al., 2002).

Interestingly, the genotype Kraska showed a very marked decrease in shoot dry matter production although this genotype showed very slight severity of leaf damage by NaCl (Table 2). Also the genotype PM-702 with severe leaf symptoms showed less reduction in growth (Table 2). These results indicate that in ranking genotypes for their tolerance to salt stress, only scoring symptoms for the severity of leaf symptoms cannot be a reliable screening method at early seedling growth. This screening method should be combined with other approaches such as shoot Na concentration of genotypes (Fig. 2). Low Na concentration in shoot is very closely correlated with salt tolerance as judged from the severity of leaf symptoms caused (Fig. 1). In the case of the germplasms with large number of genotypes, as in this study, scoring symptoms for the severity of leaf symptoms could be helpful in reducing the number of genotypes for further and detailed screening studies.

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