Turk J Agric For
30 (2006) 365-373
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Effect of Salinity Stress on Dry Matter Production and Ion Accumulation in Hybrid Maize Varieties

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Received: 04.08.2006

Abstract: The salt stress tolerance of 19 hybrid maize (*Zea mays* L.) varieties was tested in nutrient solution during the early growth stage under controlled environmental conditions. For the salt stress treatment, sodium chloride (NaCl) was applied to nutrient solution at a concentration of 250 mM for 6 days before the harvest. Plants were harvested after 17 days of growth and analyzed for shoot and root dry matter production, severity of leaf damage (necrotic patches on older leaves), and the concentrations of potassium (K), sodium (Na) and calcium (Ca) in the roots and shoots. The varieties differed greatly in their response to the NaCl treatment. The development time and severity of leaf symptoms caused by 250 mM NaCl were varied markedly among the varieties. Based on the severity of leaf symptoms, the varieties Maverik and C.7993 were classified as the most tolerant and sensitive varieties, respectively. The decreases in the shoot dry matter production as a consequence of the NaCl treatment were higher than the decreases in root growth. There was also a marked genotypic variation in concentrations of K, Ca and Na in roots and particularly in shoots. The higher salt tolerance in maize varieties based on the severity of leaf symptoms was associated with significantly lower Na concentrations in shoots. The K/Na and Ca/Na ratios were significantly greater in most of the tolerant varieties. The most sensitive variety, C.7993, contained a 4-fold greater Na concentration in shoots than the most tolerant variety, Maverik. The varieties RX.9292 and MF.714 also contained very high Na in shoots and showed severe toxicity symptoms on leaves. Besides Maverik, P.3394 and P.3223, with their low shoot Na concentrations, could also be considered tolerant varieties. Under salt treatment significant correlations were found between K/Na ratios and shoot dry matter production ($r = 0.541^{***}$), K/Na ratios and leaf damage ($r = -0.411^{***}$), and Ca/Na ratios and shoot dry matter production ($r = 0.444^{***}$). The results indicate the existence of a large genotypic variation in tolerance to NaCl toxicity in maize that should be exploited in breeding programs aiming to develop maize varieties with high NaCl tolerance during the early growth stages. Among the ions measured, shoot Na concentration was a reliable screening parameter in ranking varieties for their tolerance to salt stress.

Key Words: Genotypic variation, ion accumulation, maize, salt stress, sodium chloride

Tuz Stresinin Hibrit Mısır Çeşitlerinde Kuru Madde Üretimi ve İyon Birikimi Üzerine Etkisi

Özet: Toplam 19 hibrit mısır çeşidinin (*Zea mays* L.) büyümenin erken döneminde tuz stresine toleransı besin çözeltisinde ve kontrollü koşullarda yürütülen bir deneme ile araştırılmıştır. Tuz stresinin oluşturulması için besin çözeltisine 250 mM sodyum klorür (NaCl) uygulandı ve uygulama hasattan 6 gün önce yapıldı. Bitkiler 17 gün yetiştirildikten sonra yeşil aksam ve kök kuru madde verimi, yaprak zararlanma oranı (yaşlı yapraklarda nekrotik lekeler), yeşil aksam ve kökte potasyum (K), sodyum (Na) ve kalsiyum (Ca) konsantrasyonları belirlenmiştir. Çeşitler NaCl uygulamasından oldukça farklı şekilde etkilenmiştir. Besin ortamına 250 mM NaCl uygulaması sonucu yapraklarda oluşan toksisite semptomlarının gelişme zamanı ve şiddeti bakımından çeşitler arasında önemli farklılıklar belirlenmiştir. Yaprak semptomlarının şiddetine göre Maverik ve C.7993, sırasıyla en tolerant ve en duyarlı çeşitler olarak belirlenmiştir. Buna benzer şekilde NaCl uygulaması sonucu yeşil aksam büyümesindeki azalma çeşitler arasında önemli varyasyon göstermiş, kök büyümesinde ise belirgin bir etki oluşmamıştır. Tuzluluk stresinden dolayı yeşil aksam büyümesinde ortaya çıkan azalmalar, kök büyümesinde ortaya çıkan azalmalardan daha fazla olmuştur. Özellikle yeşil aksamda ve kısmen kökte K, Ca ve Na konsantrasyonu bakımından önemli düzeyde genotipsel varyasyon bulunmuştur. Mısır çeşitlerinde semptom şiddetine göre yüksek toleransın yeşil aksamda düşük Na konsantrasyonu ile ilişkili olduğu anlaşılmıştır. Bununla uyumlu olarak K/Na ve Ca/Na oranları

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tolerant çeşitlerin çoğunda önemli düzeyde yüksek bulunmuştur. Toleransı en düşük olan C.7993 çeşidi, en dayanıklı olan Maverik çeşidine göre yeşil aksamında 4 kat daha fazla Na konsantrasyonuna sahip olmuştur. RX.9292 and MF.714 çeşitleri de yeşil aksamda yüksek düzeyde Na konsantrasyonuna sahip olmuş ve şiddetli semptomlar göstertmiştir. Maverik çeşidinin yanı sıra P.3394 and P.3223 çeşitleri de sahip oldukları çok düşük Na konsantrasyonlarıyla dayanıklı çeşitler olarak dikkate alınabilir. Tuz stresi altında yeşil aksam K/Na oranı ile yeşil aksam kuru madde üretimi (r = 0.541***), K/Na oranı ile yaprak zararlanma oranı (r = –0.411***), Ca/Na oranı ile yeşil aksam kuru madde üretimi (r = 0.444***) arasında çok önemli korelasyonlar belirlenmiştir. Sonuçlar mısır bitkisinde NaCl toksisitesine karşı oldukça geniş bir genotipik varyasyon olduğunu ve erken dönemde tuz toksisitesine tolerans için yürütülecek ıslah çalışmalarında bu varyasyondan yararlanılabileceğini göstermektedir. Ölçülen iyonlar arasında yeşil aksam Na konsantrasyonunun çeşitlerin tuz toleransına göre sınıflandırılmasında güvenilir parametre olduğu bulunmuştur.

Anahtar Sözcükler: Genotipik varyasyon, iyon birikimi, mısır, tuz stresi, sodyum klorür

Introduction

Salinity is a common abiotic stress factor seriously affecting crop production in different regions, particularly in arid and semi-arid regions. It is estimated that over 800 million hectares of land in the world are affected by both salinity and sodicity (Munns, 2005). In Turkey also, the soil salinity problem is becoming an important constraint on crop production. According to the FAO/UNESCO Soil Map of the World, around 1.5 million hectares of land in Turkey have both salinity and sodicity problems (FAO, 2000).

There are various detrimental effects of salt stress in crop plants, responsible for severe decreases in the growth and yield of plants. Osmotic stress (drought problem), ion imbalances, particularly with Ca and K, and the direct toxic effects of ions on the metabolic process are the most important and widely studied physiological impairments caused by salt stress (Zhu, 2001; Munns, 2002; Munns et al., 2006). Salt stress, like many abiotic stress factors, also induces oxidative damage to plant cells catalyzed by reactive oxygen species (Mittler, 2002; Demidchik et al., 2003; Azevedo-Neto et al., 2006).

Maintaining a better nutrition with K and Ca, while limiting Na uptake, is a highly important trait contributing to high salt stress tolerance in plants. Consequently, higher K/Na or Ca/Na ratios are typical in the tissues of salt-tolerant varieties, and are often used as a screening parameter for identification of salt stress-tolerant varieties (Gorham, 1990; Dasgan et al., 2002; Munns and James, 2003; Poustini and Siosemardeh, 2004; Song et al., 2006). Chen et al. (2005) showed that the genotypic variation in salt tolerance among different barley varieties was closely related to the capacity of varieties to reduce K-efflux from the mature root zones under saline conditions. The varieties with higher salt tolerance had lower K-efflux. Measurement of K-efflux rate is considered a reliable screening parameter for salt tolerance (Chen et al., 2005).

When the plants are not able to control Na uptake over K uptake, an accumulation of Na in plant tissues at excessive levels is expected, causing cell damage. To minimize the toxic effects of salts in plant cells, plants should develop mechanisms to maintain low concentrations of Na in cytosol by compartmentation of Na into vacuoles. Several transporter proteins have been described, which are located on tonoplast membranes and are responsible for an efficient compartmentation of Na into vacuoles (Zhu, 2003; Munns, 2005). In recent years, increasing numbers of salt-tolerant transgenic plants have been generated with overexpression of vacuolar Na^+/H^+ antiporter proteins mediating lower concentrations of Na and higher ratios of K/Na in cytosol (Zhang and Blumwald, 2001; He et al., 2005; Yamaguchi and Blumwald, 2005). These results indicate that both the absolute concentrations of K, Na, and Ca in plants and the magnitude of the imbalance between these ions in the critical cell organelles such as cytosol and vacuoles are important in the differential expression of salt tolerance. Therefore, in many research groups, investigations dealing with the development of salt-tolerant varieties have concentrated on the uptake, transport, and accumulation of K, Na, and Ca in plants (Colmer et al., 2006; Munns et al., 2006). The concentrations of these nutrients and their ratios (e.g., K/Na and Ca/Na) are widely used as screening parameters in ranking varieties for their tolerance to salt toxicity. These parameters are reliable and useful in screening varieties for salt-stress tolerance.

Among the cereal species, maize seems to be sensitive to salt stress (Maas and Hoffman, 1997). Maize is being increasingly cultivated in Turkey. Its cultivation area is expanding to areas having high potential for accumulation of salts in the soil profile, such as the Southeastern Anatolia and Central Anatolia regions. It is, therefore, important to develop new maize varieties with high genetic capacity to tolerate salt stress. The first important step in breeding new varieties with high salt tolerance is to have a useful and substantial genetic variation in tolerance to salinity stress. The present study investigated the response of different maize varieties to a very high NaCl treatment (e.g., 250 mM) at early growth stage in terms of dry matter production, leaf damage (symptoms) caused by NaCl, and the root and shoot concentrations of Na, K, and Ca.

Materials and Methods

Nineteen hybrid maize (*Zea mays* L.) varieties (Premier 626, Pegaso, Konsur, Trebbia, RX.770, DK.6022, Korduna, P.3394, P.3223, Maverik, Tector, Sele, Doge, P.31 G 98, MF.714, Tempra, RX.9292, DK.626, and C.7993) were used in this study. The seeds were provided by the Department of Field Crops, University of Çukurova, and were germinated in perlite moistened with concentrated $CaSO_4$ solution (around 15 mM soluble Ca). Following 5 days' germination at 25 (day)-22 (night) °C, 4 uniformly germinated seedlings were transferred to pots containing 2.7 l of nutrient solution. The plants were grown under controlled growth chamber conditions (light/dark regime of 16/8 h, temperature 25/22 °C, relative humidity 65%-75%, and light intensity 350 µmol $m^{-2} s^{-1}$). The nutrient solutions were changed every 3 days, and deionized water was used in the preparation of the nutrient solutions. The composition of the nutrient solution was modified Hoagland solution as follows: 2.0 mM $Ca(NO_3)_2$, 0.88 mM K_2SO_4, 1.0 mM $MgSO_4$, 0.2 mM KH_2PO_4, 1.0 µM H_3BO_3, 0.5 µM $MnSO_4$, 0.2 µM $CuSO_4$, 0.02 µM $(NH_4)_6Mo_7O_{24}$, 1.0 µM $ZnSO_4$, and 150 µM Fe-EDTA.

After 6 days' preculture in nutrient solution, NaCl was applied as 50 mM increments every 9 h until a final concentration of 250 mM. The plants were grown for 6 days under the NaCl treatment. At harvest, plants were 17 days old and were scored according to the severity of symptoms (chlorosis and necrosis) on older leaves caused by 250 mM NaCl using a 1-5 symptom scale (1: no or very slight, 2: slight, 3: mild, 4: severe, and 5: very severe). Plants were harvested as shoot and root parts separately. The roots were rinsed first in tap water and then in deionized water. All plant samples were dried at 70 °C for 48 h, and the dry matter production was determined. After being ground, plants samples were dry-ashed at 500 °C for 5 h and dissolved in 3.3% (v/v) HCl, and analyzed for Na, K, and Ca by atomic absorption spectrometry (Varian FS 220). Reference leaf samples from the National Institute of Standards and Technology (Gaithersburg, MD, USA) were used for checking Na, K, and Ca measurements. The error found in measurements was very low and varied between 1% and 5%. All the values for each parameter are means of 4 independent replications. For all investigated parameters, the data were subjected to analysis of variance, and significant differences among the means and treatments were compared by LSD test at 5% level using the MSTAT-C software package.

Results

Two-way analysis of variance

The results of two-way analysis of variance (ANOVA) showed that salt stress affected dry weights and Na, K, and Ca concentrations of shoots and roots very significantly ($P < 0.001$). Similarly, the varieties significantly ($P < 0.001$) differed in the measured parameters. With the exception of root dry matter production, the salt x variety interaction was very significant ($P < 0.001$) for the parameters mentioned above.

Leaf Symptoms and Dry Matter Production

The maize varieties showed a large genotypic variation in their tolerance to 250 mM NaCl treatment on the basis of severity of leaf symptoms and decreases in shoot dry matter production (Table 1). Among the varieties, C.7993 and RX.9292 were particularly affected by the NaCl treatment with respect to severity of leaf symptoms caused by salt toxicity. These varieties showed very severe leaf symptoms, such as necrotic brown patches on older leaves. The highest reductions in shoot dry matter production caused by the NaCl treatment was around 71% and 69% in C.7993 and MF.714, respectively (Table 1). The variety Maverik was the most tolerant to the NaCl treatment as judged from the slight development of leaf symptoms caused by NaCl toxicity. The variation in decreases of the shoot dry matter production by salt stress was relatively low and ranged between 52% (Premier 626) and 71% (C.7993) (Table 1). Therefore,

Table 1. Severity of leaf symptoms (necrotic patches on older leaves) caused by the NaCl treatment, shoot and root dry matter production and % decrease in shoot and root dry matter production of nineteen 17-day-old maize varieties grown in nutrient solution with (250 mM) and without NaCl treatment. Data represent means of 4 independent replications.

Variety	Symptom Score*	Shoot		Root		Decrease	
		-NaCl	+ NaCl	-NaCl	+ NaCl	Shoot	Root
		mg plant^{-1}				%	
Maverik	1.3	615 bc	238 g-i	139	75	61.1	45.8
Trebbia	1.5	618 b	241 g-i	175	87	60.9	48.4
RX.770	1.5	411 f	165 i-k	93	58	59.7	37.2
DK.6022	1.5	466 ef	184 h-k	102	70	60.2	29.3
P.3394	1.5	585 b-d	231 g-j	134	73	60.2	44.8
P.3223	1.5	644 ab	298 g	156	106	53.6	31.1
Sele	1.5	621 ab	280 g	132	99	55.9	24.2
P.31 G 98	1.5	638 ab	276 g	146	92	55.4	35.6
Tempra	1.5	657 ab	258 gh	143	106	60.8	26.2
Tector	2.5	605 bc	239 g-i	121	83	59.8	29.2
MF.714	2.5	417 f	124 k	88	52	69.0	41.8
Premier 626	3.0	301 g	144 k	87	54	51.6	38.2
Pegaso	3.0	286 g	130 k	83	51	53.8	38.1
Korduna	3.0	446 ef	147 jk	102	63	66.5	37.5
Doge	3.0	708 a	291 g	169	98	58.8	41.9
Konsur	4.0	397 f	131 k	104	61	65.2	38.4
DK.626	4.0	290 g	127 k	87	55	56.3	37.5
RX.9292	4.5	530 c-e	176 h-k	129	95	66.2	25.9
C.7993	4.8	504 de	145 k	141	66	71.1	52.7
Mean	2.5	513 A	201 B	122 A	76 B	60.3	37.0
$LSD_{0.05}$ (NaCl)		19.6		7.1			
$LSD_{0.05}$ (Var.)		60.4		22.0			
$LSD_{0.05}$ (Int.)		85.4		ns			

* Symptom Score; 1: no or very slight, 2: slight, 3: mild, 4: severe and 5: very severe.
Means followed by the same letter are not significantly different at 5%.

in the present study, the classification of varieties for their tolerance to salt stress was based on the genotypic differences found in the severity of leaf symptoms and shoot Na concentrations (see below). Regarding the severity of the decreases in shoot dry matter production caused by the NaCl treatment, the variety Premier 626 showed the lowest decrease in shoot dry matter production when exposed to salt stress. Compared to the varieties RX.9292 and C.7993, the shoot dry matter production of Maverik was less affected (Table 1). Most of the maize varieties tested showed an intermediate tolerance to NaCl toxicity regarding the severity of leaf symptoms. Based on the average value of all varieties, the root dry matter production of the varieties was less affected by NaCl (around 37%) than the shoot dry matter production (around 60%) (Table 1). These results indicate that, under salt stress, measurement of the shoot growth may be a more reliable and useful parameter than the root growth in ranking varieties for their tolerance to NaCl toxicity.

Concentrations of Na, K and Ca

As expected, applying NaCl at 250 mM resulted in significant increases in shoot (Table 2) and root (Table 3) concentrations of Na. When NaCl was not applied, the shoot concentration of Na showed little variation between the varieties (Table 2). The amount of Na measured in the control plants can probably be derived from Fe-EDTA that contains Na and was applied at a rate of 0.15 mM (see Materials and Methods). In the case of NaCl treatment, varieties showed a significant variation in the shoot concentration of Na (Table 2). The shoot concentrations of Na varied from 14.5 mg g^{-1} dry weight (Maverik) to 56.1 mg g^{-1} dry weight (C.7993), with an average value of 33.7 mg g^{-1} dry weight (Table 2).

There were significant differences in the shoot concentration of K among the varieties in both the control and 250 mM NaCl treatments (Table 2). However, when compared to Na, the genotypic differences in K concentrations were considerably low. For example, in the 250 mM NaCl treatment, the shoot concentration of K varied 1.7-fold among the varieties, while this variation was nearly 4-fold in the case of shoot Na concentrations

Table 2. Shoot Na, K and Ca concentration of nineteen 17-day-old maize varieties grown in nutrient solution with (250 mM) and without NaCl treatment. Data represent means of 4 independent replications.

Variety	Concentration in Shoot (mg g^{-1} dry weight): Na -NaCl	Na +NaCl	K -NaCl	K +NaCl	Ca -NaCl	Ca +NaCl
Maverik	4.9 k	14.5 j	86.6 a-c	68.5 hi	5.9 g	2.3 op
Trebbia	4.6 k	28.4 fg	80.0 d-g	41.7 r	9.0 a	3.1 l-n
RX.770	5.6 k	32.4 ef	82.9 c-f	50.3 op	7.0 ef	3.9 i-k
DK.6022	4.4 k	27.4 fg	91.8 a	62.2 j-l	6.9 f	3.7 i-k
P.3394	4.9 k	17.7 ij	88.3 a-c	59.6 k-m	5.4 gh	2.5 n-p
P.3223	5.8 k	21.1 hi	86.6 a-c	58.8 k-m	5.0 h	2.0 p
Sele	5.5 k	34.4 de	82.9 c-g	47.1 p-r	7.1 d-f	3.4 j-l
P.31 G 98	6.7 k	24.0 gh	77.0 g	55.0 m-o	5.4 gh	2.7 n-p
Tempra	5.3 k	34.7 de	84.4 b-e	55.3 m-o	7.2 d-f	3.4 j-m
Tector	4.0 k	31.0 ef	78.9 e-g	51.6 n-p	7.6 b-d	3.5 j-l
MF.714	4.5 k	52.9 a	83.8 c-f	51.7 n-p	6.8 f	4.0 ij
Premier 626	4.5 k	41.8 bc	78.4 fg	50.4 op	7.1 d-f	3.9 ij
Pegaso	5.5 k	42.0 bc	90.2 ab	44.1 qr	7.8 bc	3.3 k-m
Korduna	4.8 k	39.4 cd	87.6 a-c	48.4 pq	7.2 d-f	3.6 i-l
Doge	4.5 k	19.0 h-j	79.6 e-g	70.4 h	7.5 b-e	2.8 m-o
Konsur	4.4 k	45.5 b	88.6 a-c	48.7 pq	8.0 b	4.2 i
DK.626	5.5 k	35.5 de	85.5 b-d	63.6 i-k	6.7 f	3.4 j-m
RX.9292	5.5 k	43.4 bc	87.9 a-c	56.9 l-n	7.3 c-f	3.5 j-l
C.7993	6.2 k	56.1 a	85.7 b-d	68.0 h-j	7.1 d-f	4.2 i
Mean	5.1 B	33.7 A	84.6 A	55.4 B	7.0 A	3.3 B
$LSD_{0.05}$ (NaCl)	1.37		1.36		0.14	
$LSD_{0.05}$ (Var.)	4.21		4.19		0.44	
$LSD_{0.05}$ (Int.)	5.96		5.93		0.63	

Means followed by the same letter are not significantly different at 5%.

(Table 2). Under the NaCl treatment, the most sensitive (C.7993) and the most tolerant (Maverik) varieties did not differ in terms of the shoot concentration of K.

As expected, applying NaCl resulted in significant decreases in shoot (Table 2) and root (Table 3) concentrations of K. The decrease in K concentration of plants with the NaCl treatment was more pronounced in the roots. In all treatments, the tolerant variety, Maverik, contained more K in the roots than the sensitive variety, C.7993 (Table 3). However, in the case of other varieties, there was no consistent relationship between the salt sensitivity and root K concentrations. As found with K, the concentration of Ca in roots and shoots was also decreased by the NaCl treatment. The varieties showed distinct differences in the shoot and root concentrations of Ca under the NaCl treatment, but the extent of these differences was rather small when compared to the differences found for Na (Table 2 and 3).

As shown in Table 4, the highest K/Na ratio (4.83) under NaCl supply was found in Maverik, 4-fold higher

Table 3. Root Na, K and Ca concentration of nineteen 17-day-old maize varieties grown in nutrient solution with (250 mM) and without NaCl treatment. Data represent means of 4 independent replications.

	Concentration in Root					
	Na		K		Ca	
Variety	-NaCl	+NaCl	-NaCl	+NaCl	-NaCl	+NaCl
	(mg g^{-1} dry weight)					
Maverik	6.1 gh	66.6 bc	63.7 a	11.5 j-l	5.9 i-k	3.8 o-q
Trebbia	5.5 gh	67.0 bc	58.2 b	7.3 j-n	6.2 i	3.4 pq
RX.770	6.8 gh	65.4 b-d	51.5 c-f	6.7 l-n	8.1 fg	3.3 q
DK.6022	5.1 gh	66.6 bc	50.1 d-f	9.1 j-n	9.8 cd	4.5 m-o
P.3394	5.4 gh	58.4 ef	54.0 b-d	11.1 j-m	9.2 c-e	5.2 j-m
P.3223	5.5 gh	62.8 c-e	41.7 hi	11.7 j-l	12.1 a	6.0 ij
Sele	4.3 h	66.5 bc	51.1 c-f	7.2 j-n	7.8 f-h	4.3 n-p
P.31 G 98	6.3 gh	62.7 c-e	47.3 e-g	6.3 mn	6.1 ij	3.7 o-q
Tempra	4.7 h	62.5 c-f	52.2 c-e	12.3 j	4.7 l-n	3.7 o-q
Tector	5.4 gh	66.5 bc	48.1 e-g	6.9 l-n	9.1 de	4.9 l-n
MF.714	7.6 gh	65.2 b-d	49.5 d-f	4.7 n	8.5 ef	4.2 n-p
Premier 626	7.9 gh	61.7 d-f	41.0 i	7.5 j-n	7.6 gh	4.5 m-o
Pegaso	4.2 h	63.2 cd	46.4 f-h	7.5 j-n	9.1 c-e	4.3 n-p
Korduna	4.9 gh	68.7 b	44.2 g-i	8.1 j-n	10.9 b	4.9 l-n
Doge	4.0 h	58.1 f	50.1 d-f	9.5 j-n	7.7 f-h	4.9 l-n
Konsur	4.5 h	69.7 b	50.7 d-f	8.0 j-n	10.0 c	5.5 i-l
DK.626	9.4 g	63.0 cd	55.9 bc	11.9 jk	7.2 h	5.1 k-n
RX.9292	4.3 h	76.5 a	50.4 d-f	6.8 l-n	10.0 c	4.9 l-n
C.7993	4.6 h	63.6 cd	51.8 c-e	6.9 k-n	8.1 f-h	4.4 m-o
Mean	5.6 B	64.9 A	50.4 A	8.5 B	8.3 A	4.5 B
$LSD_{0.05}$ (NaCl)	1.05		1.17		0.20	
$LSD_{0.05}$ (Var.)	3.24		3.60		0.63	
$LSD_{0.05}$ (Int.)	4.58		5.09		0.89	

Means followed by the same letter are not significantly different at 5%.

Table 4. Shoot K/Na and Ca/Na ratios of nineteen 17-day-old maize varieties grown in nutrient solution with 250 mM NaCl treatment. Data represent means of 4 independent replications.

Variety	Shoot			
	K/Na		Ca/Na	
Maverik	4.832	a	0.162	a
Trebbia	1.500	e-h	0.111	ef
RX.770	1.617	e-g	0.123	c-e
DK.6022	2.268	d	0.136	b-d
P.3394	3.384	bc	0.143	a-c
P.3223	2.888	c	0.100	fg
Sele	1.376	e-h	0.101	e-g
P.31 G 98	2.352	d	0.114	d-f
Tempra	1.649	ef	0.100	fg
Tector	1.675	ef	0.113	ef
MF.714	0.993	h	0.077	h
Premier 626	1.250	f-h	0.096	f-h
Pegaso	1.056	h	0.078	gh
Korduna	1.227	f-h	0.092	f-h
Doge	3.735	b	0.147	ab
Konsur	1.102	gh	0.093	f-h
DK.626	1.895	de	0.100	fg
RX.9292	1.321	f-h	0.080	gh
C.7993	1.215	f-h	0.076	h
Mean	1.965		0.107	
$LSD_{0.05}$	0.5222		0.0228	

Means followed by the same letter are not significantly different at 5%

than the ratio found in C.7993 (1.22). With the exception of Maverik, Doge, P.3394, P.3223, P.31 G 98, and DK.6022, all varieties had similar K/Na ratios in shoots under the NaCl supply. Maverik had also the highest Ca/Na ratios among all other varieties. The lowest Ca/Na ratios were found in the varieties C.7993, Pegaso, and MF.714. The remaining varieties were similar in Ca/Na ratios in the shoot (Table 4). In contrast to the large variation in shoots, the K/Na and Ca/Na ratios showed little variation in roots, and therefore the results are not presented.

Discussion

The response of the maize varieties to NaCl treatment was variable during the early vegetative stage. Based on the severity of the leaf symptoms caused by the NaCl application, the variety Maverik was identified as the most tolerant and the variety C.7993 as the most sensitive to salt toxicity (Table 1). Beside these varieties, RX.9292 and MF.714 can be classified as sensitive and P.3394 and P.3223 as tolerant varieties based on the severity of leaf symptoms and shoot Na concentrations. Most of the remaining varieties responded to salt stress moderately. Such important variability in tolerance to NaCl has also been shown in other maize varieties by Maiti et al. (1996), Rao and McNeilly (1999) and Khan et al. (2003).

The most sensitive (C.7993) and the most tolerant (Maverik) maize varieties based on the severity of leaf symptoms (Table 1) and shoot Na concentrations (Table 2) did not differ in terms of their shoot concentrations of K under NaCl treatment (Table 2). The sensitive variety C.7993 contained 4 times higher Na in shoot than the tolerant variety Maverik (Table 2). These indicate that lower accumulation of Na in shoots rather than enhanced K accumulation is the main mechanism contributing to higher salt tolerance in the maize varieties tested. This suggestion is in close agreement with the correlation coefficients between the shoot concentrations of Na and K and the shoot dry matter production and severity of leaf symptoms of the salt-affected 19 maize varieties. Significant correlations were found between the severity of leaf symptoms and shoot Na concentration ($r = 0.628^{***}$), and shoot dry matter production and shoot Na concentration ($r = -0.683^{***}$). In the case of the shoot K concentrations, there was no significant relation to the shoot dry matter production and severity of leaf symptoms. Furthermore, in the roots, like in the shoots, there were significant (but rather lower) relationships between root Na concentrations and severity of leaf symptoms or shoot dry matter production under NaCl treatment.

These results indicate that the differential salt tolerance of 19 maize varieties is related to the exclusion capacity of varieties for Na. The genotypic variation for the Na concentration was pronounced in the shoots, but slight in the roots (Tables 2 and 3), indicating that not only the root uptake of Na but also its transport from the roots into shoots is an important trait in the expression of high tolerance to NaCl. The ability of varieties to limit Na transport into the shoots, and thus to reduce the Na accumulation in the rapidly growing shoot tissues, is critically important for maintenance of high growth rates and protection of the metabolic process in elongating cells from the toxic effects of Na. Munns et al. (2006) reviewed several reports showing that reduced Na transport capacity of varieties from the roots into the shoots greatly contributes to high tolerance to NaCl toxicity. The well-documented differences in salt tolerance between durum and bread wheats is related to low rates of Na transport into shoot parts from roots (Gorham et al., 1990; Munns et al., 2003; Husain et al., 2004). Davenport et al. (2005) showed that the rate of Na loading into the xylem channel from root cells is much lower in salt-tolerant durum wheats. Based on these results, it can be speculated that, in the salt-tolerant maize varieties tested in the present study, especially in Maverik, xylem loading of Na for transport into shoots is markedly reduced. This point should be investigated in future studies.

Besides decreased root-to-shoot transport, reduced uptake of Na by roots is a further characteristic of Na-tolerant varieties as shown in different crop species such as maize (Fortmeier and Schubert, 1995) and wheat (Gorham et al., 1990; Munns and James, 2003). *Thellungia halophila* is known as a highly salt-tolerant species, and has a greater salt tolerance capacity than the closely related species *Arabidopsis thaliana* (Gong et al., 2005). Wang et al. (2006) showed that at 100 mM NaCl supply, *T. halophila* exhibited significantly lower Na uptake rate by roots than *A. thaliana*. Limitation of the Na uptake rate by roots and thus low accumulation of Na in plant tissue under saline conditions has been discussed as a major mechanism for high salt tolerance of *T. halophila* (Wang et al., 2006).

Reduced root uptake and root-to-shoot transport of Na will result in high K/Na ratios in shoots as found in many salt-tolerant maize varieties, especially in Maverik (Table 3). Higher K/Na ratios have been repeatedly shown as a reliable parameter for determination of salt tolerance in crop plants such as tomato (Agong et al., 1997; Dasgan et al., 2002), cotton (Ashraf, 2002), rice (Aslam et al., 2003), and wheat (Gorham et al., 1991; Poustini and Siosemardeh, 2004; Colmer et al., 2006).

Calcium nutrition plays an important role in the maintenance of a high growth rate under saline conditions (Marschner, 1995). Several reports show a significant role of Ca in improving the salt tolerance of plants. In studies on the soybean and cucumber, an additional supply of Ca to salt-stressed plants improved the salt tolerance of plants by reducing Na uptake and transport (Dabuxilatu and Ikeda, 2005). According to Husain et al. (2004), the major role of Ca in increasing the salt tolerance of plants is related to its inhibitory effect on the xylem loading of Na and thus decreases in shoot Na concentration. Song et al. (2006) reported that high levels of external Ca are essential for the maintenance of high root uptake and shoot accumulation of Ca and K on saline soils and thus for avoiding salinity damage in plants as shown in rice plants. However, in the present study, the shoot concentrations of Ca did not contribute to salt-stress tolerance (Table 2). There was a positive significant correlation between shoot Ca concentrations and leaf symptoms ($r = 0.477^{***}$). Based on these results, it can be concluded that the level of Ca nutrition of the maize varieties tested in the present study is not involved in the differential expression of salt tolerance.

The present study shows the critical importance of shoot Na concentrations in the differential expression of salt-stress tolerance in maize during early growth. Reductions in root uptake and shoot transport of Na under salt stress will greatly contribute to salt tolerance in maize. New maize varieties have been identified with lower capacity for root uptake and root-to-shoot transport of Na. In contrast to the shoot concentrations of Na, the concentrations of K and Ca in the shoots of 19 maize varieties were only slightly affected by the NaCl treatment. As the genotypic differences in the measured parameters were more distinct in the case of severity of leaf symptoms and shoot Na concentration and thus the K/Na ratio (up to 4-fold), attention has been given to the genotypes exhibiting the highest and the lowest values for those parameters. In conclusion, measurement of Na concentrations and calculation of K/Na ratios in shoot are reliable physiological parameters for ranking maize varieties for their tolerance to salt toxicity based on the severity of leaf damage and decreases in dry matter production under salt stress.

Acknowledgments

This research has been financially supported by the Çukurova University Scientific Research Projects Unit, Project No. ZF 2003 BAP 5.

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