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ROOT AND SHOOT RESPONSE OF COMMON VETCH (*Vicia sativa* L.), FORAGE PEA (*Pisum sativum* L.) AND CANOLA (*Brassica napus* L.) TO SALT STRESS DURING EARLY SEEDLING GROWTH STAGES

Uğur BİLGİLİ[1] Emine BUDAKLI ÇARPICI[*1] Bülent Bariş AŞIK[2] Necmettin ÇELİK[1]

[1] Uludag University, Faculty of Agriculture, Department of Field Crops, Turkey
[2] Uludag University, Faculty of Agriculture, Department of Soil Science and Plant Nutrition, Turkey
**Corresponding author: ebudakli@uludag.edu.tr*

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ABSTRACT

The objective of the present study was to identify the most salt-tolerant species among common vetch (*Vicia sativa* cv. Uludag), forage pea (*Pisum sativum* cv. Kirazli) and canola (*Brassica napus* cv. Bristol). To this end, the effects of salinity on the seedlings were determined, and four salt concentrations (0, 50, 100 and 150 mM NaCl) were evaluated. Seedlings were exposed to salinity stress for 45 days, and the seedling height, root length, shoot and root dry weight, leaf number per seedling, leaf area per seedling and Na, K and Ca content of the shoots and roots were determined. In addition, the K/Na and Ca/Na ratios were calculated. For all of the species, salt treatments significantly altered several characteristics of the shoots and roots. For instance, the Na content of the roots and shoots increased with an increase in the salt concentration, and the K and Ca content of the roots and shoots decreased. Furthermore, the results indicated that *Pisum sativum* cv. Kirazli was more resistant to salt stress than the other cultivars and can be cultivated on salty soils containing less than 100 mM NaCl.

Key Words: Canola (*Brassica napus* L.), common vetch (*Vicia sativa* L.), forage pea (*Pisum sativum* L.), salt stress, shoot and root weights.

INTRODUCTION

Salinity is a significant problem that can affect crop productivity, especially in countries where irrigation is essential for crop production. Currently, 275 million hectares of arable land is irrigated, and approximately 20% of irrigated land is affected by salinity (Ghassemi et al., 1995). In addition, marginal lands that were not previously cropped because of a high degree of salinity are now being cultivated due to an increase in the demand for food, especially in developing and under-developed countries (Flowers and Yeo, 1995). In particular, salinity is a significant problem that threatens crop production in Turkey. For instance, approximately 1.5 million hectares of land in Turkey are affected by salinity and sodicity (FAO, 2000). However, cropping on saline lands is risky; thus, salt-tolerant crops must be introduced.

Salinity has three potential effects on plants, including reduced water potential, specific ion toxicity (sodium and chloride) and interference with the uptake of essential nutrients. However, because plants have nutrient reserves that can be mobilized, the latter effect may not be observed (Flowers and Flowers, 2005). The response of plants to salinity is a two-phase process (Munns, 1993). In the first phase, the external water potential decreases due to salt present outside of the root. The second phase includes the senescence of leaves due to the accumulation of ions in older leaves, and the effects of salt tolerance can be clearly observed. Sensitive species or cultivars accumulate ions more quickly than tolerant species, leading to leaf death and the progressive death of the plant (Munns, 2002).

The mechanism for survival under saline conditions is identical for all plants; however, different adaptations in glycophytes may be observed (Flowers and Flowers, 2005). Variations in the salt tolerance of glycophytes occur between and among species, and have been quantified for many crops (Mass and Hoffman, 1976; Francois and Mass, 1994; Flowers and Yeo, 1981). Rapid screening procedures for various crops in early growth stages have been adopted by many researchers (Ashraf et al., 2002; Munns et al., 2002; Eker et al., 2006; Khan et al., 2006).

In previous studies, salt-tolerant varieties have been identified and improved, and the uptake, transport and accumulation of Na, K and Ca have been evaluated (Colmer et al., 2006; Munns et al., 2006). The concentration of Na, K and Ca, as well as the relative ratios of ions (K/Na and Ca/Na), are used as screening parameters for salt tolerance.

Lands that are unproductive due to high salt concentrations may be utilized by growing salt-tolerant crops or reclaiming the soil. However, reclamation is an expensive and time-consuming process. Alternatively, the growth of salt-tolerant forage crops on saline lands is useful for meeting the forage demands of livestock and the reclamation of soils.

The aim of the present work was to identify the most salt-resistant species among common vetch, forage pea and canola. Each species was represented by a newly released variety, and the ability to survive under salty conditions was determined. For this purpose, the growth characteristics of the shoots and roots were evaluated, and the content and relative ratio of Na, K and Ca in the shoots and roots were determined.

MATERIALS AND METHODS

The current investigation was carried out to determine the resistance of common vetch (*Vicia sativa* cv. Uludag), forage pea (*Pisum sativum* cv. Kirazli) and canola (*Brassica napus* cv. Bristol) to salt stress. The experiment was conducted in pots in Bursa, Turkey, and the plants were grown in 5.5-L perlite-filled plastic pots. The seeds were graded, and large seeds with a uniform shape were employed. Prior to seeding, the seed surface was sterilized with 2% sodium hypochlorite for 10 min. After sterilization, the seeds were washed with distilled water three times. In total, six seeds were sown in each pot. The open surface of the pots was covered with aluminum foil to prevent the growth of algae. After germination, the aluminum foils were removed, and the seedlings were thinned to four plants per pot. The pots were irrigated twice a day with a nutrient solution prepared according to the method described by Maas et al., (1986). Specifically, the solution contained 2.5 mM $Ca(NO_3)_2$, 3.0 mM KH_2PO_4, 1.5 mM $MgSO_4$, 0.1 mM KNO_3, 0.1 mM Fe-EDTA, 0.023 mM H_3BO_3, 0.005 mM $CuSO_4$ and 0.01 mM $H_{24}Mo_7N_6O_{24}.4H_2O$. A randomized plot design with three replicates was applied.

Salt solutions containing 0, 50, 100 and 150 mM NaCl were added to the nutrient solutions prior to seeding. The electrical conductivity (EC) of salt solutions containing 0, 50, 100 and 150 mM NaCl was 1.37 (nutrients alone), 7.53, 12.58 and 17.37 dS m^{-1}, respectively. Seedlings were grown in a greenhouse with a 15-h photoperiod and day/night temperatures of 24/17°C. After 45 days of growth, the seedlings were uprooted, and the seedling height, root length, shoot dry weight, root dry weight, leaf number per seedling and leaf area per seedling were measured. The leaf area was measured using a digital leaf area meter (LI-3000 Portable Area Meter Produced by LI-COR Lincoln, Nebraska, USA). Four plants from each pot were harvested. The shoot and root materials were dried at 70°C for 48 h and then weighed, and digested with a mixture of HNO_3 + $HClO_4$ (4:1). Na, K and Ca were determined with a flame emission spectrophotometer (Horneck and Hanson, 1998).

The data were subjected to an analysis of variance (ANOVA) using MINITAB (University of Texas, Austin) and MSTAT-C (Version 2.1 Michigan State University, 1991) software. Significant differences in treatments, primary effects and interactions were determined at a probability level of 0.05 and 0.01 by conducting an F-test. Differences among the means of salt levels, species and their interactions were determined by applying an LSD method at a probability level of 0.05.

RESULTS AND DISCUSSION

The seedling height, root length, shoot dry weight, root dry weight, leaf number and leaf area per seedling of the species are shown in Table 1.

The results indicated that the salinity treatments had strong effects on the growth performance of the seedlings. However, the response of each species to salinity differed with respect to the measured parameter. Nevertheless, all of the growth performance parameters decreased with an increase in the concentration of salt. The negative effect of the highest salt concentration was so high that common vetch and forage pea seedlings did not survive. Therefore, the values of the parameters obtained at the highest salt concentration were excluded, and only the parameters obtained at the other salt levels (0, 50 and 100 mM NaCl) were used in the statistical analysis.

The seedling height of all of the species decreased gradually, depending on the concentration of salt. Thus, the greatest seedling heights were obtained in unsalted pots, and the lowest heights were obtained in pots treated with the highest concentration of salt (Table 1). The observed reduction in the seedling height was greater in forage pea than common vetch and canola. However, canola plants survived at every salt concentration, whereas forage pea and common vetch seedlings died upon treatment with the highest level of salt.

For all of the species, the root length was negatively affected by salt treatment, and significant reductions in the root length were observed, depending on the salt concentration (Table 1). Canola presented greater reductions in the root length than forage pea and common vetch after exposure to low levels (50 mM NaCl) of salt. However, canola showed poor tolerance to the highest salt level (150 mM NaCl), and the other species did not survive. The root length of common vetch, forage pea and canola decreased by 19.2%, 43.5% and 51.7%, respectively, as the salinity level decreased from 0 to 100 mM NaCl. The seedling roots are the first organ exposed to salinity, and root growth is particularly sensitive to increased salt concentrations. As a result, root growth is prevented or rapidly reduced by salinity (Cramer et al., 1988). Under saline conditions, the depletion of O_2 deprives plants of their primary energy source, and high levels of internal ethylene are accumulated, which inhibits root elongation (Koning and Jackson, 1979) by reducing root growth.

An inverse relationship in the dry weight of the shoots and roots of all of the species and the salt concentration was observed (Table 1). Thus, the highest values were obtained from control pots, and the lowest values were observed at NaCl concentrations of 100 mM. The overall reduction in the shoot dry weight was 80%, 64% and 73% for common vetch, forage pea and canola, respectively. Therefore, the results indicated that the salt treatments had a stronger effect on common vetch and canola than on forage pea. Moreover, similar results were observed for the root dry weight of all of the species. Another striking result obtained in the present study is that salt stress inhibited shoot growth more than root growth in all of the species. Similar findings have been

Table 1. Average of seedling height (cm), root length (cm), shoot dry weight (g seedling^{-1}), root dry weight (g seedling^{-1}), leaf number seedling^{-1} and leaf area (cm^2 seedling^{-1}) at harvest for species grown under different NaCl levels (mM)

NaCl (mM)	Common vetch	Forage pea	Canola	Means (NaCl)
	Seedling Height (cm)			
0	65.7 b	86.6 a	38.5 d	63.5 a
50	51.6 c	48.4 c	28.5 e	42.8 b
100	32.6 de	31.9 e	19.6 f	28.0 c
150	-	-	9.0	-
Means (Species)	50.0 b	55.6 a	28.8 c	
	Root Length (cm)			
0	18.2 ab	20.7 a	20.5 a	19.80 a
50	16.4 bc	18.9 ab	13.3 de	16.20 b
100	14.7 cd	11.7 ef	9.9 f	12.11 c
150	-	-	6.4	-
Means (Species)	16.5 a	17.1 a	4.6 b	
	Shoot Dry Weight (g seedling^{-1})			
0	1.07 b	1.17 b	2.11 a	1.45 a
50	0.48 d	0.72 c	1.02 b	0.74 b
100	0.21 e	0.42 d	0.58 cd	0.40 c
150	-	-	0.23	-
Means (Species)	0.59 c	0.77 b	1.24 a	
	Root Dry Weight (g seedling^{-1})			
0	0.12 b	0.08 bc	0.32 a	0.17 a
50	0.05 c	0.10 bc	0.12 b	0.09 b
100	0.05 c	0.07 bc	0.05 c	0.06 b
150	-	-	0.03	-
Means (Species)	0.07 b	0.08 b	0.16 a	
	Leaf Number Seedling^{-1}			
0	27.0 a	13.9 bc	7.3 e	16.1 a
50	16.4 b	13.3 c	5.9 e	11.9 b
100	10.5 d	10.5 d	5.5 e	8.8 c
150	-	-	4.0	-
Means (Species)	18.0 a	12.6 b	6.3 c	
	Leaf Area (cm^2 seedling^{-1})			
0	192.7 c	178.9 cd	355.0 a	242.2 a
50	68.7 f	126.8 e	238.5 b	144.7 b
100	21.9 g	57.3 fg	148.8 de	76.0 c
150	-	-	43.1	
Means (Species)	94.4 c	121.0 b	247.4 a	

Means followed by the same letter for each components are not statistically different by LSD at 0.05 level.

reported for barley (*Hordeum vulgare* L.) (Huang and Redmann, 1995), pigeon pea (*Cajanus cajan*) (Subbarao et al., 1991), tepary bean (*Phaseolus acutifolius*) (Goertz and Coons, 1991) and tomato (*Lycopersicon*) (Foolad, 1996). In addition, Jeannette et al. (2002) demonstrated that the weight of the shoots and roots of *Phaseolus* decreased dramatically as salt stress increased. In these types of studies, the relative salinity tolerance of a species or a variety is defined as the salt level that equates to a 50% reduction in shoot yield (Mass, 1986). This parameter is accepted as the threshold value of salinity and represents a significant risk for plant production. In the current study, the threshold value for shoot yield was 50 mM NaCl for common vetch (55 %), < 100 mM NaCl for forage pea and 50 mM NaCl for canola (52 %). These results indicate that canola and common vetch reach the threshold salinity earlier than forage pea.

The leaf number per seedling of all of the species was inversely related to the salt concentration (Table 1). The observed reduction in the leaf number seedling^{-1} of common vetch was greater than that of forage pea and canola. As the salt concentration increased from 0 mM NaCl to 100 mM NaCl, the leaf number of common vetch, forage pea and canola decreased by 61%, 24% and 24%, respectively (Table 1).

For all of the species, salt treatments significantly affected the leaf area per seedling. For instance, as the salt concentration increased from 0 mM NaCl to 100 mM NaCl, the leaf area per seedling decreased by 89% in common vetch, 68% in forage pea and 58% in canola. These results suggested that canola was the least sensitive to salt stress, followed by forage pea and common vetch (Table 1). Similar results were obtained in a study conducted by Seema et al. (2003). Moreover, Wang and Nil (2000) stated that the immediate response of plants to salt stress was a reduction in leaf surface expansion. Grieve et al. (1999) and Meena et al. (2003) reported similar results and demonstrated that the leaf area per seedling decreased with an increase in salinity.

Table 2 shows the Na, K and Ca concentration of the shoots and roots of each species under different salt treatments. For all of the species, the Na content of the shoots increased linearly with an increase in the salt concentration and reached a maximum value at 100 mM NaCl. Although the Na content of the shoots of all of the species were similar in the absence of additional NaCl, common vetch, forage pea and canola seedlings contained approximately 32-fold, 32-fold and 11-fold higher Na concentrations in their shoots compared to growth in the absence of excess salt. This result indicates that the salt-avoiding mechanism of canola is stronger than that of common vetch and forage pea. Moreover, at the highest salt concentration, some canola seedlings survived, and the highest Na concentrations were observed. Alternatively, common vetch and forage pea did not survive (Table 2). Significant differences in the K concentrations of the shoots were observed among species (Table 2). Canola possessed higher K concentrations than the other two species, which possessed K concentrations equal to the control pots. As the salt concentration increased, the K concentration of canola shoots decreased; however, this effect was not observed in common vetch and forage pea, indicating that NaCl has an antagonistic effect on K uptake in canola. The Ca content of the shoots was significantly different among species, and canola presented higher Ca contents at each salt level than the other two species. A reduction in the Ca content of the seedlings of common vetch and forage pea was not observed; however, the Ca content of canola seedlings decreased significantly with an increase in the NaCl concentration, indicating that NaCl prevented Ca uptake by canola seedlings.

Table 2. Shoot and root Na^+, K^+, Ca^{2+} (mg g^{-1}) determined at harvest for species grown under different NaCl levels (mM)

NaCl (mM)	Common vetch	Forage pea	Canola	Means (NaCl)
		Shoot		
		Na (mg g^{-1})		
0	1.36 f	0.78 f	3.28 f	1.81 c
50	21.14 d	10.50 e	27.54 c	19.73 b
100	43.14 a	24.40 cd	35.74 b	34.43 a
150	-	-	51.00	-
Means (Species)	21.88 a	11.89 b	22.19 a	
		K (mg g^{-1})		
0	25.50 cd	27.30 bc	58.50 a	37.10 a
50	25.90 c	31.30 bc	32.58 b	29.93 b
100	19.16 d	28.16 bc	26.68 bc	24.67 c
150	-	-	20.26	-
Means (Species)	23.52 c	28.92 b	39.25 a	
		Ca (mg g^{-1})		
0	14.48 cd	12.34 d-f	20.34 a	15.72 a
50	12.78 c-e	11.52 ef	17.86 b	14.05 b
100	10.18 f	10.26 f	15.04 c	11.83 c
150	-	-	14.80	-
Means (Species)	12.48 b	11.37 b	17.75 a	
		Root		
		Na (mg g^{-1})		
0	5.97 d	2.27 d	2.86 d	3.70 b
50	33.65 a	25.05 b	12.32 c	23.67 a
100	31.71 a	25.73 b	13.29 c	23.58 a
150	-	-	17.66	-
Means (Species)	23.78 a	17.69 b	9.49 c	
		K (mg g^{-1})		
0	38.18 b	55.75 a	22.78 c	38.90 a
50	12.50 d	21.79 c	15.50 cd	16.60 b
100	11.67 d	17.93 cd	11.21 d	13.60 b
150	-	-	10.71	
Means (Species)	20.78 b	31.83 a	16.50 b	
		Ca (mg g^{-1})		
0	8.96 cd	7.84 cd	48.90 a	21.90 a
50	7.90 cd	8.82d	24.98 b	12.44 b
100	8.31 cd	4.44 cd	13.68 c	10.27 b
150	-	-	12.92	-
Means (Species)	8.39 b	7.03 b	29.19 a	

Means followed by the same letter for each components are not statistically different by LSD at 0.05 level.

For all of the species, salt treatments increased the Na content of the roots compared to the control; however, significant differences among the 50- and 100-mM NaCl treatment were not observed. In the control experiment, forage pea presented the highest Na concentration, followed by common vetch and canola. In all of the species, the K content of the roots decreased dramatically with an increase in the salt concentration. Moreover, reductions in the K content of the roots were similar among species. In the control experiments, forage pea yielded roots with higher K contents than the other species. Significant differences in the effects of the salt concentration on the Ca content of the roots were observed among species. The Ca content of canola roots decreased with an increase in the salt level; however, changes in the Ca content of common vetch and forage pea roots were not observed. Thus, the Ca content of canola roots was significantly restrained by the Na concentration.

The K/Na and Ca/Na ratios of the shoots and roots are presented in Table 3. As shown in the table, the highest K/Na and Ca/Na ratios were observed in the control pots. Salt treatment decreased the K/Na and Ca/Na ratio of the shoots and roots of all species; however, differences between salt levels were not observed. When the plants were not treated with salt, the shoots and roots of forage pea possessed higher K/Na and Ca/Na ratios than the other species. Alternatively, under the same conditions, canola produced roots with higher Ca/Na ratios than the other two species.

Table 3. Shoot and root K/Na, Ca/Na determined at harvest for species grown under different NaCl levels (mM),

NaCl (mM)	Common vetch	Forage pea	Canola	Means (NaCl)
	Shoot			
	K/Na			
0	19.53 b	35.88 a	17.92 b	24.44 a
50	1.24 c	2.99 c	1.18 c	1.80 b
100	0.45 c	1.19 c	0.75 c	0.80 b
150	-	-	0.40	-
Means (Species)	7.07 b	13.36 a	6.62 b	
	Ca/ Na			
0	11.01 b	16.06 a	6.23 c	13.10 a
50	0.61 d	1.10i. d	0.65 d	0.84 b
100	0.24 d	0.44 d	0.42 d	0.65 b
150	-	-	0.30	-
Means (Species)	2.47 b	8.78 a	3.34 b	
	Root			
	K/Na			
0	6.63 b	24.75 a	7.94 b	11.10 a
50	0.38 c	0.88 c	1.26 c	0.79 b
100	0.41 c	0.70 c	0.84 c	0.37 b
150	-	-	0.68	-
Means (Species)	3.96 b	5.87 a	2.43 c	
	Ca/ Na			
0	1.56 bc	3.47 b	17.42 a	7.48 a
50	0.24 c	0.36 c	2.04 bc	0.82 b
100	0.27 c	0.18 c	1.02 bc	0.55 b
150	-	-	0.88	-
Means (Species)	0.69 b	1.34 b	6.83 a	

Means followed by the same letter for each components are not statistically different by LSD at 0.05 level.

K nutrition is disturbed by salt stress due to changes in the transcription level of several K^+ transporter genes (Su et al., 2002), a reduction in the deposition rate of growing cells (Bernstein et al., 1995), a decline in the K^+ content of the xylem (Munns, 1985), shoot (Jaschk and Wolf, 1985) and expanding leaf tissue (Benstein et al., 1995; Lazof and Benstein, 1999) and increased K^+ efflux from the roots (Shabala et al., 2003). Reduced K^+ uptake is also related to competition with Na^+ uptake through Na^+- K^+ co-transporters, which can block K^+ specific transporters in root cells (Zhu, 2002).

CONCLUSION

The results of the present study indicated that *Pisum sativum* cv. Kirazli, which displayed a relatively high K/Na ratio and low reductions in the shoot and root dry weight and leaf number per seedling, were more salt tolerant than common vetch and canola. Thus, forage pea can be cultivated on salty soils containing less than 100 mM NaCl.

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