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Variations in Response to Salt Stress among Field Pea Genotypes (*Pisum sativum sp.arvense* L.)

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Abstract: This study examined the responses to salt stress in 11 pea genotypes and two pea cultivars. Pea genotypes were selected from among wild pea and white flowered pea cross-breed varieties from different regions in Anatolia. In general, root fresh weight (fw) shoot fw, leaf fw, leaf number and chlorophyll contents were found to decrease with increases in salinity, whereas root and leaf MDA ratios increased with increases in salinity. Genotypes 1 (10431), 9 (101917) and 10 (1103220) were found to be the most salt-tolerant. Genotypes 6 (110121) and 7 (B-6) were found to be the most sensitive.

Key words: Field pea genotypes, salt stress, chlorophyll, lipid peroxidation

INTRODUCTION

Excessive salinity has been considered the most significant edaphic factor limiting the distribution of plants in certain natural habitats, as well as the cause of severe agricultural problems in large parts of the world (Alfocea *et al*., 1993). The development of salt-tolerant crop cultivars represents a practical alternative to expensive engineering approaches to bring saline marginal lands under cultivation (Quresh *et al*., 1990; Hollington, 1998). Indeed, observations of inter-specific (Phills *et al*., 1979; Maas, 1986) and more importantly, intra-specific variations in salt tolerance in many crop plants, such as barley (Epstein *et al*., 1980) and bean suggest that selection and breeding offer the potential for improving salt tolerance in cultivated species. Besides, it is well-known that field Pea play important role for animal nutrition as a valuable protein source.

In general, salinity reduces the growth rate in plants, resulting in smaller leaves, shorter stature and sometimes fewer leaves. The initial and primary effects of salinity, especially at low to moderate salt concentrations, are a result of osmosis (Munns and Termaat, 1986; Pessarakli and Tucker, 1988).

Malondialdehyde (MDA) accumulation and chlorophyll retention are two oxidative stress indicators that are tested tools for determining salt tolerance in plants (Luna *et al*., 2000). The generation of active oxygen species (AOS) is a common response to environmental stress conditions, such as salinity, high and low temperatures, metal toxicity and air pollution (Hernandez *et al*., 1993; Luna *et al*., 1994; Gosset *et al*., 1994). AOS cause chlorophyll degradation and membrane lipid peroxidation. MDA, an end-product of lipid peroxidation, shows greater accumulation under salt stress (Gosset *et al*., 1994).

The pea plant grows in temperate climates, with the largest sowing area found in Asia and the greatest production and yield found in Europe. Following bean, pea is the most widely sown plant crop globally. In Turkey, 1,100 ha are devoted to pea cultivation and the annual pea production is 2,500 tons, giving an average yield of 2,272 kg ha^{-1} (Alan, 1984).

The purpose of this study was to determine the effects of salinity on root, shoot and leaf growth in a number of different pea varieties.

MATERIALS AND METHODS

Plant material: Eleven pea genotypes and two pea cultivars from different region of Anatolia were used in the study.

Plant growth and treatments: Pea seeds were germinated in a growth chamber at 20±2°C and 70% humidity with a 16 h photoperiod. Seeds were placed in plastic pots (40×25×5 cm) filled with pumice and seedlings were irrigated with Hoagland nutrient solution following the emergence of the first true leaves. Following the emergence of the second true leaf, seedlings were

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transplanted to plastic developing dishes (25×25×5 cm) for hydroponics culture using a Hoagland solution replaced at weekly intervals.

Seedlings were grown in control conditions until emergence of the fourth true leaf, at which time salt stress treatment was initiated. Salt treatment consisted of adding 25 mM NaCl daily until a concentration of 75mM NaCl was attained. The experiment used a randomized design of 15 plants per genotype with 4 replications. Fourteen days after the initiation of salt treatment, 6 plants were randomly harvested from each genotype, separated into root, shoot and leaf components and the fresh weights of each component measured.

Chlorophyll contents: Flame photometry was used to determine the chlorophyll contents in 250-mg samples taken from the first two leaves above the shoots. Leaf samples were rinsed in 80% ethanol and placed in a water bath for 20 min and absorbance readings taken from alcohol extracts. Chlorophyll content was calculated according to Tetley and Thimann (1974) as $1000\times A_{654}/(39.8\times$ sample fresh weight).

Lipid peroxidation: Lipid peroxidation was determined by estimating the malondialdehyde (MDA) content in 250-mg fresh weight samples of leaf according to Lutts *et al.* (1996). MDA concentrations were calculated from the absorbance at 532 nm, using an extinction coefficient of 155 mM^{-1} cm^{-1}. Absorbance at 600 nm was subtracted to correct for unspecific turbidity.

RESULTS AND DISCUSSION

Findings showed that salt stress generally resulted in a significant reduction in the fresh root, shoot and leaf weights as well as leaf numbers of plants (Table 1).

Fresh root weight: The Genotype 3 (B-8) Control Treatment was found to have the highest fresh root weight (2.15 g), whereas the Genotype 10 (1103220) NaCl Treatment had the lowest fresh root weight (0.84 g). Fresh root weights were found to have decreased as a result of NaCl treatment for most genotypes tested. This decrease was significant ($p<0.05$) in Genotypes 3, 4, 6 and 7. In contrast, the fresh root weight of Genotype 10 increased significantly ($p<0.05$) as a result of NaCl treatment.

Hasson and Poljakoff-Mayber (1981) observed greater root aging in the salt-resistant Dan variety of Alaska peas. In a study examining the fresh and dried root weights and root diameters in rice cultivated in saline conditions, Demiral and Türkan (2005) found that root weight decreased by 43 percent in the Pokkali variety as a result of salt stress. Atanassova *et al.* (1997) found a 20% decrease in the root development of pea and corn plants over a 10-day period as a result of salt stress. The results of the present study are in line with the results of these earlier studies.

Fresh shoot weight: Fresh shoot weight tended to decrease with salinity, with the greatest decrease seen in Genotype 2, followed by Genotype 6. Stress did not effect Genotypes 1 or 4.

Lacerda *et al.* (2000) investigated root and shoot systems in 2 sorghum varieties and found that salinity caused decreases in dry matter as well as in the length of roots and shoots. Atanassova *et al.* (1997) stated that salinity led to a 50% decrease in shoot development of corn and pea plants. The present results are in line with these findings.

Fresh leaf weight: With the exception of Genotype 9, fresh leaf weights in all genotypes decreased as a result of NaCl stress, with the greatest decreases found in Genotypes 2 and 7. Bandeoglu *et al.* (2004) found that salinity treatments hindered leaf development more than root development in lentil varieties treated with 200 nM NaCl. The results of the present study are partially in line with these results.

Leaf number: Leaf number decreased with salinity treatments in all genotypes except for Genotype 10 (Table 1) No information on the effects of salinity on leaf number were found in the literature.

Chlorophyll content: In response to NaCl treatment, chlorophyll content decreased in Genotypes 1, 5, 8 and 11, whereas it increased in Genotype 9. It is possible that Genotype 9 has a high antioxidant content that may protect it against chlorophyll degradation.

Luna *et al.* (2000) found chlorophyll degradation in Chloris gayana when plants were subjected to drastic salt treatment, but no degradation when the plants were gradually subjected to treatment.

In a study on salinity stress in cotton, Meloni *et al.* (2003) examined the lipid peroxidation rates and chlorophyll content of cotton varieties exposed to 50, 100 and 200 moL m^{-3} increasing over a 21-day period. While findings showed restricted photosynthesis in both the Guazuncho and Pora varieties, decreases in chlorophyll content were greater in the sensitive Guazuncho when compared to the Pora variety.

Table 1: Results of duncan's test showing mean values for fresh root, shoot and leaf weights and leaf numbers for different pea genotypes

Genotype name	Genotype number	Fresh root weight (g)		Fresh shoot weight (g)		Fresh leaf weight (g)		Leaf number	
		Control	NaCl	Control	NaCl	Control	NaCl	Control	NaCl
10431	1	1.13 fg A	1.25 c A	0.87 d A	0.96 b A	1.33 de A	1.22 bc A	9.33 c A	9.00 b A
1121918	2	1.26.0 efA	0. 85 e B	0.71 e A	0.54 fg B	1.27 e A	0.68 f B	8.67 c A	6.00 d B
B-8	3	2.15 aA	1.05 d B	1.43 b A	0.70 de B	1.97 a A	1.08 cd B	9.00 c A	8.33 bc A
1084222	4	1.76 bc A	1.78 a A	1.20 c A	1.12aA	1.89 a A	1.35 b B	11.00 b A	8.67 b B
1101545	5	1.96 ab A	1.48 b B	1.54 a A	0.83 c B	1.61 b A	0.90 de B	14.00 a A	10.33 a B
110121	6	1.91 ab A	0.89 e B	0.87 d A	0.55 fg B	1.71 b A	1.04 cd B	12.00 b A	7.667 c B
B-6	7	0.9 g A	0.7 f A	0.75 e A	0.53 fg B	1.47 c A	0.71 ef B	11.33 b A	10.00 a B
1131556	8	1.46 ed A	1.47 b A	0.86 d A	0.75 cd B	1.31 de A	1.21 bc A	11.67 b A	10.00 a B
101917	9	1.71 bc A	1.76 a A	0.89 d A	0.60 ef B	1.42 cd B	1.71 a A	11.00 b A	10.00 a A
1103220	10	0.84 g B	1.08 d A	0.56 f A	0.54 fg A	1.01 g A	1.13 c A	8.67 c B	10.00 a A
110121-1	11	1.08 fg A	0.84 e B	0. 69 e A	0.46 g B	1.45 c A	0.78 ef B	11.33 b A	10.33 a A
Winner	12	0.93.0 g A	0.77 ef A	0.40 g A	0.31 h A	0.89 g A	0.78 ef A	5.33 d A	5.00 e A
Karina	13	1.53.0 ce A	1.51 b A	0. 37 g A	0.44 g A	1.12 f A	1.11 c A	6.33 d A	6.00 d A

*Letters a-g and A-B indicate treatment groups between which no significant differences were found

Table 2: Results of duncan's tests showing mean values for chlorophyll content, root MDA and Leaf MDA for different pea genotypes

Genotype name	Genotype number	Chlorophyll content µg mL^{-1}		Root MDA (µL g^{-1} fresh weight)		Leaf MDA (µL g^{-1} fresh Weight)	
		Control	NaCl	Control	NaCl	Control	NaCl
10431	1	2.77 fg A	2.66 c A	1.22 b-d B	4.97 g A	4.30 ab B	14.39 cd A
1121918	2	5.35 ab A	1.41 d B	1.36 bc B	11.06 e A	3.87 bc B	19.13 ab A
B-8	3	5.68 a A	1.92 cd B	1.47 b B	22.66 c A	4.59 ab B	19.49 ab A
1084222	4	4.26 b-e A	2.05 cd B	1.46 b B	29.40 b A	4.41 d B	18.90 ab A
1101545	5	3.09 e-g A	2.37 c A	1.10 b-d B	28.11 b A	4.90 a B	17.84 b A
110121	6	4.53 a-d A	1.29 d B	0.72 ed B	36.51 a A	4.21 ab B	20.14 a A
B-6	7	4.74 a-c A	1.50 d B	0.71 ed B	11.14 e A	4.72 a B	19.60 ab A
1131556	8	3.38 d-g A	4.03 ab A	0.48 e B	9.43 ef A	5.05 a B	13.16 c-e A
1011917	9	3.11 e-g B	4.36 a A	0.87 c-e B	9.45 ef A	4.35 ab B	10.931 f A
1103220	10	3.56 e-f A	3.50 b A	0.96 b-d B	9.43 ef A	4.32 ab B	12.05 ef A
110121-1	11	2.25 g A	2.61 c A	1.05 b-d B	9.98 e A	4.67 ab B	13.69 c-e A
Winner	12	2.20 g A	1.38 d A	0.72 ed B	7.12 fg A	4.75 a B	13.38 c-e A
Karina	13	4.70 a-c A	2.00 cd B	2.40 a B	18.414d A	3.24 c B	15.74 c A

*Letters a-g and A-B indicate treatment groups between which no significant differences were found

As stated by Steduto *et al*. (2000), the decline in productivity observed for many plant species subjected to excess salinity is often associated with a reduction in photosynthetic capacity.
The present results are in line with these studies.

Root MDA: Root MDA increased with salinity. Genotypes 4, 5 and 6 experienced the greatest increases, whereas Genotype 1 had the smallest increase (Table 2).

Numerous studies have looked at root MDA and salinity in a variety of plant species. Meloni *et al*. (2003) determined that MDA was produced as a result of peroxidation of polyunsaturated fatty acids in the membrane. In a study on cotton, they found higher amounts of MDA in the Guazuncho variety than the Pora variety, indicating a higher rate of lipid peroxidation due to salt stress in Guazuncho.

Luna *et al*. (2000) obtained similar results in a study of Chloris gayana that found greater MDA increases at 200 mM NaCl than at 300 mM. Screenivasulu *et al*. (1999) found greater MDA accumulation in salt-sensitive millet cultivars when compared to more resistant cultivars.

Demiral and Türkan (2005) found increased MDA ratios in roots of the salt-sensitive rice variety IR-28, but not in the Pokkali variety.

Leaf MDA: Leaf MDA increased with salinity treatment in all genotypes in the present study. Genotype 6 had the highest value and Genotype 9 the lowest. There have been several studies on different plants showing increases in leaf MDA with salinity treatment.

El-Baky *et al*. (2003) found the rate of lipid peroxidation in onion cultivars, as indicated by increases in MDA content, increased due to salt stress. Lipid peroxidation rates were found to increase gradually with gradual increases in salt stress, especially in sensitive cultivars. MDA accumulation was greater in salt-sensitive cultivars than in salt-tolerant cultivars.

Bandeoglu *et al*. (2004) stated that MDA accumulation was greater in leaf structures than in root structures in lentils subjected to 200 nM NaCl stress.

Luna *et al*. (2000) stated that MDA values are lower in tolerant groups than in sensitive groups.

The results of the present study are in line with the results of the above-mentioned studies.

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