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INFLUENCE OF SALT STRESS ON THE GROWTH, ELEMENTS CONTENTS AND ANTIOXIDATIVE ENZYMES ACTIVITIES OF PEA (*Pisum sativum L.*) PLANT GROWN IN SAND CULTURE.

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ABSTRACT

Pot experiment was carried out at Faculty of Agriculture, El Bostan, Alexandria University, in order to evaluate the effect of irrigation with NaCl solutions on the growth characters, elements contents and antioxidative enzymes activities of pea (*Pisum sativum* variety Master B) plant grown in sand culture.

Split plot layout, in randomized completely block design, experiment with six replicates was carried out. The concentrations of NaCl in irrigation water were 00, 25, 50, 75 and 100 mM prepared in a base nutrient solution.

Seeds of pea were sown in a plastic pot containing 2 kg prewashed sand. Two samples of plants (3 for each) were collected at 20 and 27 days after sowing (DAS) for determination of the growth characters, the concentrations of Na^+, K^+, Mg^{2+} and Ca^{2+} and the activities of antioxidative enzymes: catalase (CAT), pyroxidase (POD) and ascorbate peroxidase (APX) in leaves and root of the plant.

The obtained results showed significant decrease in the fresh and dry weights of leaves and root, plant height and leaf area with increasing NaCl concentration treatments. In addition, the concentrations of Na^+, K^+ and Mg^{2+} were lower in leaves than in root while those of Ca^{2+} were higher in leaves than in root of both 20 and 27 DAS plants.

The results showed significant reductions in the activity of CAT enzyme in leaves and root of 20 and 27 DAS plants with increasing salinity. The lowest significant CAT activities were found with 100 mM NaCl treatment which represented values of relative reduction of 20.2 and 10.4% in leaves and 24.3 and 34.2% in root, respectively.

There were significant increase in the activity of POD in leaves of 20 DAS plants and no significant change in leaves of 27 DAS plants with increasing salinity. The highest significant level of POD activity in leaves of 20 DAS plants (132.3 µM H_2O_2 min^{-1}. mg^{-1} protein) was found with 100 mM NaCl treatment which represented a relative increase of 16.6%. However, for 20 DAS plant, there were no significant variations in POD activity in plant root due to NaCl treatments while for 27 DAS plants, there were significant decreases in POD activity with increasing salinity. The lowest significant level of POD activity in root of 27 DAS plants (1778 µM H_2O_2 min^{-1} mg^{-1} protein) was found with 100 mM NaCl treatment which represented a relative reduction in POD activity of 35.3%.

There were significant decreases in the activity of APX enzyme in leaves and root of 20 and 27 DAS plants with increasing salinity. The lowest significant levels were found with 100mM NaCl treatment which represented values of relative reduction in APX activity of 33.9 and 41.5% in leaves and 27.0 and 33.1% in root of 20 and 27 DAS plants, respectively.

Keywords: Antioxidative enzymes, catalase, peroxidase, Ascorbate peroxidase, salt stress, pea plant.

INTRODUCTION

Salinity in soil and water of irrigation is one of the major abiotic stress that severely limit crop production. It is mainly due to high Na^+ concentration which would lead, beside Na^+ toxicity in plant, to the inhibition of either some nutrients uptake by plant, such as K, P, Fe and Zn, water uptake and the growth of root or both (Tester and Davenport, 2003).

In salt-sensitive plants, the growth of shoot and root is permanently reduced (Munns, 2002 and Elsokkary *et al*., 2010). The deleterious effects of salinity on plant growth is associated with low osmotic potential, nutritional imbalance, specific effect of ion (salt stress) or a combination of these factors (Marschner, 1990; Munns, 2002 and Tester and Davenport, 2003).

Salt stress may lead also to the formation of reactive oxygen species (ROS) such as superoxide radicals ($O_2^{\cdot-}$), hydrogen peroxide (H_2O_2), hydroxyl radical ($^{\cdot}OH$) and single oxygen ($^1O_2$). These cytotoxic ROSs can destroy normal cellular membranes and plant tissues via oxidation damage of lipids, proteins and nucleic acids (Hernandez *et al*., 2001). In order to decrease the damage of oxidation, plants employ enzymatic mechanisms to scavenge ROS and to prevent and limit its toxicity. In this concern, plants possess a number of antioxidative enzymes such as catalase (CAT), peroxidase (POD) and ascorbate peroxidase (APX) and others which can limit and prevent the toxicity of ROS (Dalton *et al*., 1993 and Tejera *et al*., 2004).

There are several reports about increasing the activity of antioxidative enzymes in various plant species under saline conditions (Hernandez *et al*., 1999; Meneguzzo *et al*., 1999; Rio Gonzalez *et al*., 2002; Mittler 2002 and Ahmadi *et al*., 2009). It has been also reported that a positive relationship exists between the activities of antioxidative enzymes and salt tolerance of plants (Wang and Han, 2009). Mittova *et al*. (2002) found that higher salt tolerant of wild tomato (*Lycopesicon pennelli*) as compared to the salt-sensitive cultivated tomato (*L. esculentum*) was correlated with increased activity of antioxidative enzymes; POD and APX and also with high Na^+ concentration in leaves of the plant.

Wang and Han (2009), in their study on two alfalfa (*Medicago sutiva L*.) cultivars: slat tolerant and salt sensitive, found that salt stress led to a significant increase in CAT, POD and APX activities in plant leaves of both cultivars. They also found that the percent increases of antioxidative enzymes activities were considerably more in salt-sensitive cultivar than salt-tolerant cultivar. They suggested that salt tolerant alfalfa cultivar may mainly employ APX enzyme for detoxification H_2O_2 in leaves of plant under salt stress, and that salt-sensitive alfalfa cultivar may mainly employ POD enzyme for scavenging of H_2O_2 in leaves of plant under salt-stress.

The objectives of the present study, therefore, were to evaluate the effect of salt stress on the growth, elements concentrations and the activities of the antioxidative enzymes in leaves and root of pea (*Pisum sativum L*.) plant grown in sand culture.

MATERIALS AND METHODS

Experimental Layout:

Sand culture pot experiment (Hewitt, 1966) was used to evaluate the effect of irrigation with NaCl solution on the growth and antioxidative enzymes activity of pea (*Pisum sativum L.*) plant. In order to achieve these objectives, split plot layout, in randomized completely block design with six replicates, was carried out in the greenhouse of Faculty of Agriculture, El Bostan, Alexandria University, Egypt in Jan. 2006.

Irrigation solution: The irrigation treatments included NaCl concentrations of 00, 25, 50, 75 and 100 mM in a base solution of half strength modified Hoagland and Arnon nutrient solution (Hewitt, 1966). The concentrations of $N\text{-}NO_3$, $N\text{-}NH_4$, P, K, Ca, Mg and S were 112.65, 14.52, 31.00, 197.20, 72.35, 23.90 and 32.00 mg l^{-1}, respectively and those of B, Mn, Cu, Zn, Fe and Mo were 0.25, 0.25, 0.01, 0.025, 0.30 and 0.025 g l^{-1}, respectively.

Plant sowing: Seeds of pea (*Pisum sativum L.*) variety Master B were surface sterilized by soaking in a solution of H_2O_2 (10%) for 10 mins, then washed thoroughly by tap water followed by distilled water (Hewitt, 1966). Ten seeds were sown in a plastic pot of 16 cm inside diameter and 13 cm depth containing 2 kg prewashed sand (Hewitt, 1966). Each pot was irrigated daily by 200 ml distilled water for one week, then the plants in each pot were thinned to four seedlings per pot and irrigated by 200 ml NaCl irrigation treatment once time every two days.

Plant sampling: Plants were collected two times: at 20 days after sowing (3 replicates) and at 27 days after sowing (3 replicates). These plants were washed by tap water then by distilled water (Hewitt, 1966), separated into leaves and root and their fresh weights were measured. One third of these fresh plant organs were preserved in the refrigerator for biochemical analysis and the other two-thirds were oven-dried at 65 $^{\circ}C$ for 48 hrs and their weights were measured. The oven-dried plant materials were ground using stainless steel mill and preserved for analysis.

Plant Analysis:

Growth characters: The shoot height of plant was measured and the result was expressed as cm $plant^{-1}$. The leaf area was measured, using the fresh plant, by disk method (Radford, 1967) and the result was expressed as cm^2 $plant^{-1}$. The relative growth rate (RGR) was calculated according to Radford (1976).

Elements analysis: One-tength gram oven-dried plant material was subjected to wet digestion (H_2SO_4/H_2O_2) according to Cottonie (1980). The concentrations of Ca^{2+} and Mg^{2+} were determined by versenate method (Chapman and Pratt, 1961) and the concentrations of Na^+ and K^+ were measured by flame photometer (Chapman and Pratt, 1961).

Antioxidative enzyme assay: Leaves and root, of the fresh materials (0.5 gram), were homogenized, using tissue homogenizer, in 4ml ice-cold 100 mM K-phosphate buffer (pH = 7.0) containing 0.1 mM ethylene diamine tetra acetic acid (EDTA) and 1% polyvinylpyrrolindone (PVP). The homogenate was filtered through muslin cloth and centrifuged at 16000 rpm for 15 mins.

The supernatent fraction was used as a crude extract for measuring enzyme activity. All the analytical steps were carried out at 4ºC (Azevedo-Neto *et al*., 2006). The concentration of the crude extract was measured by the method described by Bradford (1976).

Total catalase (EC.1.11.1.6) activity was assayed by adding 50 µl enzymatic extract to 3 ml solution of 50 mM K-phosphate buffer of pH 7 and 20 mM H_2O_2. The decrease in absorbance at 240 nm was measured for 1 min. at 30 ºC (Havir and Mchale, 1987). The enzyme activity was calculated using the molar extinction coefficient of 36 $M^{-1}cm^{-1}$ and expressed as µM H_2O_2 $min^{-1}mg^{-1}$ protein.

Total guaiacol peroxidase (EC.1.11.1.7) activity was assayed by adding 37 µl enzyme extract to 3ml reaction mixture containing 50 mM K-phosphate buffer of pH 6.8, 20 mM guaiacol and 20 mM H_2O_2. The enzyme activity was quantified using the tetraguaiacol molar extinction coefficient (26.6 $mM^{-1}cm^{-1}$)and the result was expressed as µM H_2O_2 $min^{-1}mg^{-1}$ protein (Plewa *et al*., 1991).

Total ascorbate peroxidase (EC.1.11.1.11) activity was assayed according to Nakano and Asada (1981). The reaction mixture (3 ml) contained 50 mM K-phosphate buffer (pH = 7.0), 0.1 mM EDTA, 0.5 mM ascorbate, 1.2 mM H_2O_2 and 0.1 ml enzyme extract. The enzyme activity was quantified using the molar extinction coefficient for ascorbate (2.8 $mM^{-1}cm^{-1}$) and the result was expressed as µM H_2O_2 $min^{-1}mg^{-1}$ protein (Mckersie and Leshem, 1994).

Statidtical Analysis:

The data obtained were statistically analyzed for the least significant difference (LSD) using SAS statistical analysis software (SAS Inst., 1985).

RESULTS AND DISCUSSION

Growth Characters:

Plant weight: Table 1 showed significant decrease in the fresh and dry weight (F.W. and D.W.) of leaves and root of 20 and 27 days after sowing (DAS) pea plant with increasing NaCl concentration treatments. As shown in Table 2, the values of the relative decrease in the F.W. of 20 DAS plants were almost higher in root than in leaves at each level of NaCl treatment. This indicates higher relative reduction in the growth of root than of leaves as a result of increasing salinity at this plant age. For 27 DAS plants, these values on F.W. basis were higher in root than in leaves while on D.W. basis these values were higher in leaves than in root except with 100 mM NaCl treatment.

It is also clear from Table 2 that the values of the relative decrease in leaves and root, on F.W. and D.W. basis, were almost higher in older than younger plants. These data reveal that the magnitude of reduction in the growth of pea plant, due to salt stress, would be increased with proceeding plant age.

Table 1: The weight of leaves and root (g. plant^{-1}) shoot height (cm plant^{-1}) and leaf area (cm^2 plant^{-1}) of pea plant at 20 and 27 DAS as influenced by NaCl concentration treatments.

NaCl (mM)	Leaves		Root		L/R ratio		Shoot height	Leaf area
	F.W.	D.W.	F.W.	D.W.	F.W.	D.W.		
20 DAS								
0	0.96a	0.075a	1.48a	0.070a	0.65c	1.07a	6.2a	28.77a
25	0.84b	0.071a	1.16b	0.063a	0.72a	1.13a	5.6b	24.66b
50	0.72b	0.059b	0.97b	0.053b	0.74a	1.11a	5.1c	20.25c
75	0.69b	0.052b	1.00b	0.045b	0.69b	1.16a	4.8c	176.74d
100	0.44c	0.039c	0.62	0.036c	0.71a	1.08a	4.2d	11.87e
$LSD_{0.05}$	0.15	0.015	0.21	0.010	0.06	0.15	0.6	2.44
27DAS								
0	2.19a	0.214a	3.41a	0.165a	0.64b	1.30b	10.3a	73.56a
25	1.75b	0.149b	2.47b	0.124b	0.71a	1.20c	8.4b	51.55b
50	1.34c	0.113c	2.01c	0.094c	0.67a	1.20c	7.1c	38.03c
75	1.14c	0.086d	1.64c	0.071c	0.70a	1.21c	6.3d	29.69d
100	0.86d	0.060d	1.22d	0.042d	0.71a	1.43a	5.5e	20.06e
$LSD_{0.05}$	0.21	0.015	0.24	0.026	0.05	0.18	0.6	7.39

Table 2: The relative decrease (%) in the weight of leaves and root, shoot height and leaf area of pea plant at 20 and 27 DAS as influenced by NaCl concentration treatment.

NaCl (mM)	Leaves		Root		Shoot height	Leaf area
	F.W.	D.W.	F.W.	D.W.		
20 DAS						
25	12.50	5.33	21.62	10.00	9.68	14.27
50	25.00	21.33	34.46	24.29	17.74	29.61
75	28.13	30.61	32.43	35.71	22.58	38.33
100	54.17	48.00	58.11	48.57	32.26	58.72
Mean	29.95	26.32	36.66	29.64	20.57	35.23
27 DAS						
25	20.09	30.37	27.57	24.85	18.45	18.64
50	38.81	47.20	41.06	43.03	31.07	38.89
75	47,95	59.81	51.91	56.97	38.83	47.29
100	60.73	71.96	64.22	74.55	46.60	60.09
Mean	41.90	52.34	46.19	49.85	33.74	41.23

Table 1 showed significant increase in the values of L/R ratio of 20 and 27 DAS plants, on F.W. and D.W. basis, with increasing salinity. These ratios, on F.W. basis, were almost less than unity which indicate higher relative root F.W. than leaves F.W. On the other hand, on D.W. basis, there were no significant variations in the values of L/R ratio of 20 DAS plant with increasing salinity, while for 27 DAS plants these; values significantly increased with increasing salinity from 1.30 (the control plant) to 1.43 (100 mM NaCl treated plant).

It is clear from Table 1 that L/R, on D.W. basis, were almost higher in older than younger plants. These data point out to relatively higher reduction in the growth of root than that of leaves with both increasing salinity and proceeding plant age. This indicates higher sensitivity of root to salinity than leaves and this sensitivity increased with proceeding plant age.

Table 3 showed significant decrease in the values of relative growth rate (RGR) of leaves and root, on both F.W. and D.W. basis, with increasing

NaCl concentration treatments. It is also clear that, on D.W. basis, the RGR was markedly higher in leaves that in root at each level of NaCl treatment.

Table3: The relative growth rate (g.g^{-1} day) of leaves and root of pea plant as influenced by NaCl concentration treatments.

NaCl (mM)	Leaves		Root	
	F.W.	D.W.	F.W.	D.W.
0	0.18	0.022	0.32	0.014
25	0.13	0.011	0.19	0.009
50	0.09	0.008	0.15	0.006
75	0.06	0.005	0.09	0.004
100	0.06	0.003	0.09	0.001
Mean	0.10	0.010	0.17	0.007

It can be reported that, on D.W. basis, the lower value of RGR of root (relative decrease of 92.9%) with 100 mM NaCl treatment as compared to that of leaves (relative decrease of 86.4) denotes that the growth of root has been reduced by salinity more than leaves. This reveals that the root of pea plant is more sensitive to salinity than leaves at each level of NaCl treatment.

Moisture content: Table 4 showed marked decrease in moisture content in leaves and root of 20 and 27 DAS plants with increasing NaCl concentration treatments. Also, the levels of moisture content were almost higher in root than in leaves and were higher in those of older than younger plants, at each level of NaCl treatment. These data point out to a negative relationship between moisture content in both leaves and root of pea plant and the levels of NaCl treatments. Thus, there is a positive relationship between water deficit in plant organs and salinity of the growth medium. This can be achieved by studying the data of the relative decrease in moisture content with salinity as given in Table 4. These data clearly showed that the relative decrease in moisture content was higher in root than in leaves and was higher in older than younger plant.

Table 4: The moisture content (g. plant^{-1}) and its relative decrease (%) in leaves and root of pea plant at 20 and 27 DAS as influenced by NaCl concentration treatments.

NaCl (mM)	Leaves		Root	
	F.W.	D.W.	F.W.	D.W.
20 DAS				
0	0.885	1.410	-	-
25	0.769	1.097	13.11	22.20
50	0.661	0.917	25.31	34.97
75	0.638	0.955	27.91	32.27
100	0.401	0.584	54.69	58.58
27 DAS				
0	1.976	3.245	-	-
25	1.601	2.346	18.98	27.90
50	1.227	1.916	37.91	40.96
75	1.054	1.569	46.66	51.65
100	0.800	1.178	59.51	63.70

These results point out to the dehydration of plant organs with increasing salinity. This data can provide an explanation about the progressive reduction in the growth of pea plant under salt-stress.

Shoot height: Table 1 showed significant decrease in the shoot height of both 20 and 27 DAS plants with increasing salinity. As shown in Table 2, there were higher values of the relative decrease in shoot height of 27 DAS plant than that those of 20 DAS plant at each level of NaCl treatment. In addition, the values of the relative decrease in shoot height had increased with increasing salinity for both 20 and 27 DAS plants. These data indicate that pea plant grown under salt-stress is usually stunted and shows dwarfism morphological appearance as compared to plant grown under non-salt-stress.

Leaf area: Table 1 showed significant decrease in leaf area per plant with increasing NaCl concentration treatments for both 20 and 27 DAS plants. The relative reductions in leaf area were almost higher in older than younger plant at each level of NaCl treatment (Table 2). It has been reported that reduction in leaf, due to salt stress, is eventually associated with low rate of net assimilation of CO_2 and consequently low plant biomass (Marschner, 1990).

Element Concentration:

Sodium: The concentration of Na^+ in leaves and root of 20 and 27 DAS plants has been increased with increasing NaCl concentration treatments and was higher in older than younger plant (Table 5). In the case of 20 DAS plant, the values of the relative increase in Na^+ concentration were lower in leaves (67.2, 103.2, 125.4 and 162.7% with a mean value of 114.6%) than in root (74.5, 110.2, 142.3 and 148.2% with a mean value of 118.8%) while those of 27 DAS plant were opposite, and were higher in leaves 223.5, 329.4, 313.2 and 358.8% with a mean value of 306.2%) than in toot (105.3, 173.7, 210.5 and 236.1% with a mean value of 181.4%) with 25, 50,75 and 100 mM NaCl concentration treatments, respectively. These data point out to increasing Na^+ concentration in leaves with proceeding plant age. In addition, there was a positive relationship between the relative increase of Na^+ concentration in plant organs and the relative decrease of D.W. of these organs.

Table 5: The concentration of Na, K, Mg and Ca ($mg.g^{-1}$) in leaves (L) and root (R) of pea plant at 20 and 27 DAS as influenced by NaCl concentration treatments.

NaCl (mM)	Na		K		Mg		Ca	
	L	R	L	R	L	R	L	R
20 DAS								
0	6.7c	13.7c	25.4a	37.8ab	6.4a	19.4a	20.0a	11.7a
25	11.2bc	23.9b	20.7b	41.6a	4.4b	14.2bc	18.0	10.0b
50	13.6ab	28.8ab	15.4c	36.9ab	4.4b	15.6b	15.3c	8.0c
75	15.1ab	33.2a	18.9bc	36.5b	4.6b	13.8bc	13.0d	5.7d
100	17.6a	34.0a	17.7bc	26.0c	5.6ab	11.6c	13.3d	4.0e
$LSD_{0.05}$	4.6	5.8	3.9	5.1	1.5	2.7	1.3	1.3
27 DAS								
0	6.8c	13.3d	26.0a	47.4ab	9.8a	20.8a	28.7a	18.3a
25	22.0	27.3c	21.3b	48.8a	9.8a	14.4b	22.0b	19.0a
50	29.2a	36.4b	17.7c	46.1ab	5.6b	14.2b	18.0c	20.7a
75	28.1a	41.3a	17.7c	41.0b	4.6b	14.8b	15.0d	11.0b
100	31.2a	44.7a	20.8b	32.1c	6.4b	15.0b	14.0d	7.0c
$LSD_{0.05}$	4.3	4.2	2.8	7.8	3.0	2.0	2.6	3.6

Potassium: Table 5 showed a significant decrease in the concentrations of K^+ in leaves and root of 20 and 27 DAS plant with increasing salt stress. It is also clear that the concentration of K^+ was markedly higher in root than in leaves and was higher in older than younger plant at each level of NaCl treatment.

Magnesium: The concentrations of Mg^{2+} in leaves and root, of 20 and 27 DAS plants, significantly decreased with increasing NaCl concentration treatments (Table 5). Also, Mg^{2+} concentration was almost higher in root than in leaves and was higher in older than younger plant.

Calcium: Table 5 showed a significant decrease in the concentrations of Ca^{2+} in leaves and root with increasing salinity and was also markedly higher in older than younger plant. It is clear from Table 5 that Ca^{2+} concentration was almost higher in leaves than in root at each level of NaCl treatment. This trend of Ca^{2+} distribution between leaves and root is opposite to that of Na^+, K^+ and Mg^{2+} (Table 5).

The increased concentration of Na^+ and the decreased concentrations of K^+, Mg^{2+} and Ca^{2+} in leaves and root of pea plant would lead to elements-imbalance and consequently disorders of the biochemical processes in plant. This would lead to deficiency of some nutrients and reduction in plant growth (Marschner, 1990). It has been also reported that high Na^+ concentration and low K^+ concentration in plant are considered to be one of the important physiological factors contributing to the tolerance of many plant species to salt stress (Marschner, 1990).

Na_L/Na_R ration: There were no significant differences in Na_L/Na_R ratio with all levels of NaCl treatments for 20 DAS plants. However, this ratio significantly increased, relative to the control, for 27 DAS plant even under low salt stress (Table 6). In general, these ratios were almost less than unity which indicate lower proportion of Na^+ absorbed by plant has been transported from root to leaves than that accumulated in root. In addition, the higher Na_L/Na_R in older than younger plant reveal that Na^+ transport from root to leaves increased with proceeding plant age.

Table 6: Leaves/root (L/R) ratio of Na, K, Mg and Ca of pea plant at 20 and 27 DAS as influenced by NaCl concentration treatments.

NaCl (mM)	$Na_{L/NaR}$	$K_{L/KR}$	$Mg_{L/MgR}$	$Ca_{L/CaR}$
20 DAS				
0	0.49a	0.67a	0.33b	1.71c
25	0.47a	0.50b	0.31b	1.80c
50	0.47a	0.42b	0.28b	1.91c
75	0.46a	0.52b	0.33b	2.28b
100	0.52a	0.68a	0.48a	3.33a
$LSD_{0.05}$	0.27	0.13	0.14	0.32
27 DAS				
0	0.51b	0.55ab	0.47abc	1.57ab
25	0.81a	0.44bc	0.68a	1.16bc
50	0.84a	0.38c	0.39ab	0.87c
75	0.68a	0.43bc	0.31c	1.36bc
100	0.70a	0.65a	0.43bc	2.00a
$LSD_{0.05}$	0.16	0.13	0.22	0.56

K_L/K_R ratio: Table 6 showed that the values of K_L/K_R ratio were less than unity for both 20 and 27 DAS plants. This points out to low relative transport of K^+ from root to leaves and that higher proportion of K^+ absorbed by plant has been accumulated in root.

Mg_L/Mg_R ratio: Table 6 showed that the values of Mg_L/Mg_R ratio were less than unity for both 20 and 27 DAS plants. This indicates that higher proportion of Mg^{2+} absorbed by plant has been accumulated in root rather than transported to leaves.

Ca_L/ Ca_R: The values of Ca_L/Ca_R were generally more than unity which indicate that higher proportion of Ca^{2+} absorbed by plant has been transported from root to leaves (Table 6). There were also significant higher Ca_L/Ca_Rratios (3.33 and 2.00) with the highest NaCl treatment (100 mM NaCl) relative to those of the control (1.71 and 1.57) for both 20 and 27 DAS plants, respectively. It is also clear from Table 6 that the values of Ca_L/Ca_R ratio were higher in younger than in older plants which indicate relatively low transport of Ca^{2+} from root to leaves with proceeding plant age.

K/Na ratio: Table 7 showed marked decrease in the values of K/Na ratio in leaves and root of 20 and 27 DAS plants with increasing NaCl concentration treatments. These values were almost more than unity for younger plant except in root with 100 mM NaCl treatment and also were higher in leaves than in root at each NaCl treatment. These values out that higher proportion of K^+ absorbed plant has been transported from root to leaves relative to that which is accumulated in root. This trend was opposite for 27 DAS plant since higher K/Na ratios were found in root than in leaves at each level of NaCl treatment. It is also clear that K/Na ratios in leaves of 27 DAS plants treated with 25-100 mM NaCl were less than unity. This reveals that lower proportion of K^+ absorbed by plant has been transported from root to leaves relative to that of Na^+ with increasing both NaCl concentration treatments and plant age.

Table 7: The ratio of K/Na, Mg/Na and Ca/Na in leaves (L) and root (R) of pea plant at 20 and 27 DAS as influenced by NaCl concentration treatments.

NaCl (mM)	K/Na		Mg/Na		Ca/Na	
	L	R	L	R	L	R
20 DAS						
0	3.79	2.76	0.96	1.42	2.99	0.85
25	1.88	1.74	0.39	0.59	1.61	0.42
50	1.13	1.28	0.32	0.54	1.13	0.28
75	1.25	1.10	0.31	0.42	0.86	0.17
100	1.01	0.77	0.32	0.34	0.76	0.12
27 DAS						
0	3.82	3.56	1.44	1.65	4.22	1.38
25	0.97	1.79	0.46	0.53	1.00	0.70
50	0.61	1..27	0.19	0.39	0.62	0.57
75	0.63	0.99	0.16	0.36	0.53	0.27
100	0.66	0.72	0.21	0.34	0.45	0.16

Plants grown under non-salt-stress (the control plant) have the highest K/Na ratio in both leaves and root of 20 and 27 DAS plants as compared to those of plants grown under salt-stress (Table 7). These values

were more than unity and also were higher in leaves (3.79 and 3.82) than in root (2.76 and 3.36) of 20 and 27 DAS plants, respectively. This could be due mainly to selective absorption of K^+ by plant relative to that of Na^+ especially for plants grown under non-salt stress. It has been reported by Tester and Davenport (2003) that high K/Na ratio is concomitant with high growth of leaves and root than low K/Na ratio.

Mg/Na ratio: Table 7 showed marked decrease in the values of Mg/Na ratio in leaves and root of 20 and 27 DAS plants with increasing NaCl concentration treatments. These ratios were less than unity which indicate low Mg^{2+} transport from root to leaves relative to that of Na^+. However, pea plant grown under non-salt stress have Mg/Na ratio more than unity except that of leaves of 20 DAS plant. This reveals that under non-salt stress condition, leaves and root would contain relatively higher Mg^{2+} concentration than Na^+. The higher Mg/Na ratio in root than in leaves, at each NaCl concentration treatment, points out to lower transport of Mg^{2+} from root to leaves.

Ca/Na ratio: There was a decrease in the values of Ca/Na in leaves and root of 20 and 27 DAS plants with increasing salinity (Table 7). These ratios were almost higher in leaves than in root, at each level of NaCl treatment, which indicate relatively higher transport of Ca^{2+} from root to leaves relative to its accumulation in the root. Also, Ca^{2+} transport from root to leaves, of plants under salt-stress, were more observed in younger than in older plants as indicated by the higher Ca/Na ratio. In addition, for younger plants, the Ca/Na ratios were more than unity with 25 and 50 mM NaCl and also were higher in younger than older plants. This indicates relatively higher transport of Ca^{2+} to leaves of younger than older plants as compared to that of Na^+.

Plant grown under non-salt stress had higher Ca/Na ratio than that grown under salt-stress. This ratio was higher in leaves than in root and also was higher in older than younger plants. This indicates that plant grown under non-salt stress would contain relatively higher level of Ca^{2+} than of Na^+.

Element Uptake:

Na^+ uptake: Table 8 showed significant increase in the amounts of Na^+ uptake in leaves and root with NaCl concentration treatment up to 100 mM NaCl for 20 DAS plants and up to 75 mM NaCl for 27 DAS plants relative to the control treatment. The amounts of Na^+ uptake were higher in root than in leaves at each level of NaCl treatment.

K^+ uptake: It is clear from Table 8 that the amounts of K^+ uptake in leaves and root, of 20 and 27 DAS plants, significantly decreased with increasing NaCl concentrations treatments. These amounts were almost higher in root than in leaves at each level of NaCl treatment and were also higher in 27 DAS plants than 20 DAS plants. It is also clear from Table 8 that the highest levels of K^+ uptake were found in leaves and root of 20 and 27 DAS plants when grown under non-salt-stress as compared with those grown under salt stress.

Mg^{2+} uptake: The amounts of Mg^{2+} uptake in leaves and root of 20 and 27 DAS plants decreased significantly with increasing NaCl concentration treatments (Table 8). These amounts were almost higher in root than in

leaves, and were also higher in older than younger plants at each level of NaCl treatment.

Table 8: The amount of Na, K, Mg and Ca uptake (mg.plant^{-1}) in leaves (L) and root (R) of pea plant at 20 and 27 DAS as influenced by NaCl concentration treatments.

NaCl (mM)	Na		K		Mg		Ca	
	L	R	L	R	L	R	L	R
20 DAS								
0	0.50c	0.96c	1.91a	2.65a	0.48a	1.36a	1.50a	0.82a
25	0.80a	1.51a	1.47b	2.62a	0.31b	0.89b	1.28b	0.63b
50	0.80a	1.53a	0.91c	1.96b	0.26b	0.83b	0.90c	0.42c
75	0.99a	1.49a	0.98c	1.64b	0.24c	0.62bc	0.68d	0.26d
100	0.69b	1.22b	0.66d	0.94c	0.21c	0.42c	0.49e	0.14e
$LSD_{0.05}$	0.10	0.30	0.35	0.50	0.10	0.23	0.12	0.08
27 DAS								
0	1.46c	2.20c	5.56a	7.82a	2.10a	3.43a	6.15a	3.02a
25	3.28a	3.39a	3.17b	6.05b	1.40b	1.39b	3.28b	2.36b
50	3.30a	3.42a	2.00c	4.33c	0.64c	1.35b	2.03b	1.95c
75	2.42b	2.93b	1.52d	2.91d	0.40c	1.05bc	1.29c	0.78d
100	1.87c	1.88c	1.24d	1.35e	0.38c	0.63c	0.84c	0.29e
$LSD_{0.05}$	0.65	0.44	0.88	0.95	0.46	0.72	0.65	0.42

Ca^{2+}uptake: Table 8 showed a significant decrease in the amounts of Ca^{2+} uptake in leaves and root of 20 and 27 DAS plants with increasing NaCl concentration treatments. These amounts were higher in leaves than in root and were higher in older than younger plants, at each level of NaCl treatment.

Antioxidative Enzyme Activity:

Catalase (EC.I.11.1.6): Table 9 showed no significant differences between the levels of catalase (CAT) activity in leaves of 20 or 27 DAS plants treated with 25, 50 and 75 mM NaCl and that of the control treatment. However, the lowest significant levels of CAT activity were found in leaves of 20 and 27 DAS plants (12.73 and 16.70 µM H_2O_2. min^{-1}. mg^{-1} protein, respectively) with 100 mM NaCl treatment, which represented values of relative decrease of 20.2 and 10.4%, respectively.

Tables 5 and 9 showed a negative relationship between Na^+ concentration and CAT activity in leaves of either 20 or 27 DAS plants. This reveals that salinized leaves, of pea plant, exihibit relatively lower CAT activity than non-salinized leaves.

The study carried out by Tejera Garcia *et al*. (2007) showed significant reduction in CAT activity in leaves of salt-sensitive bean (*Phaseolus vulgaris*) with increasing salinity. They reported that the relative reductions in CAT activity were 40 and 75% with 25 and 50 mM NaCl treatments, respectively. Also, Kant and Turan (2011) found a significant decrease in CAT activity in root of bean (*Phaseolus vulgaris L.*) with increasing salinity up to 120 mM NaCl. On the other hand, Wang and Han (2009) reported that CAT activity did not significantly change in leaves of salt-sensitive alfalfa (*Midicago sativa*) cultivar subjected to 70 and 140 mM NaCl

for 7 days, relative to that in leaves of the control plant. However, they found a significant reduction in leaves CAT activity with higher concentration of NaCl up to 210 mM.

Table 9: The activity of CAT, POD and APX enzymes (μM H_2O_2. $min.^{-1}mg^{-1}$ protein) in leaves (L) and root (R) of pea plant at 20 and 27 DAS as influenced by NaCl concentration treatments.

NaCl (mM)	CAT		POD		APX	
	L	R	L	R	L	R
20 DAS						
0	15.95a	9.42a	102.5b	1785a	28.31a	45.89a
25	14.52a	8.52ab	95.9b	1543b	24.21b	39.83b
50	15.05a	10.99a	71.1c	1601b	27.19a	42.64a
75	15.74a	8.67ab	119.5a	1865a	22.82bc	39.48b
100	12.73c	7.13c	132.3a	1894a	18.72c	33.48c
$LSD_{0,05}$	2.54	1.40	17.3	166	3.82	5.14
27 DAS						
0	18.64a	12.15a	103.4b	2749a	24.18a	58.14a
25	18.82a	10.96ab	105.3b	2176b	20.31b	51.11b
50	19.30a	11.75a	117.1a	2106b	23.79a	58.47a
75	18.29a	8.62bc	111.1ab	1864c	17.85bc	45.26b
100	16.70c	7.99c	100.0b	1778c	14.05c	38.88c
$LSD_{0.05}$	2.60	1.55	12.0	175	2.52	6.15

It is clear from Table 10 that the mean level of CAT activity was significantly higher in leaves of older than younger plant. However, Tejera Garcia *et al*. (2007) found a decrease in the activity of CAT in leaves of bean (*Phaseolus vulgaris*) with increasing age of plant up to 30 DAS which is opposite to our finding in the present study.

Table 10: The mean value of the activity of CAT, POD and APX enzymes (μM H_2O_2 . min^{-1} mg^{-1}protein) in leaves (L) and root (R) of pea plant as influenced by plant age.

Plant age (DAS)	CAT		POD		APX	
	L	R	L	R	L	R
20	14.39b	9.03b	104.2a	1735b	24.05a	40.16b
27	18.13a	10.29a	107.4a	2135a	19.72b	50.39a
$LSD_{0.05}$	2.27	1.25	15.3	214	3.42	5.80

Table 9 showed that the highest significant levels of CAT activity were found in root of 20 or 27 DAS plant with 50 mM NaCl treatment (10.99 or 11.75 μM H_2O_2. min^{-1}. mg^{-1} protein, respectively) and in root of the control plant (9.42 or 12.15 μM H_2O_2. min^{-1}.mg^{-1} protein, respectively). On the other hand, the lowest significant levels of CAT activity were found in root of 20 and 27 DAS plants (7.13 and 7.99 μM H_2O_2. min^{-1}. mg^{-1} protein, respectively) with 100 mM NaCl treatment, which represented values of relative reduction in CAT activity of 24.3 and 34.2%, respectively. These data point out to relatively higher reduction in the activity of CAT enzyme in root of pea plant with both high salinity and proceeding plant age.

Tables 5 and 9 showed a negative relationship between Na^+ concentration and CAT activity in root of 20 or 27 DAS plants.

The data presented in Tables 9 and 10 showed higher levels of CAT activity in leaves than in root. This can be clearly noticed at each level of NaCl treatment (Table 9).

The study carried out by Tejera Garcia *et al*. (2007) suggested that CAT activity in salt-sensitive legumes would be deminished under salt stress and that the detoxifying role of the enzyme should be limited under such conditions. Thus, the data obtained in the present study, therefore, may support the assumption concerning the possibility of direct inhibition of CAT enzyme by NaCl in salt-sensitive plant.

Peroxidase (EC.1.11.1.7): Table 9 showed that the highest significant levels of peroxidase enzyme (POD) activity in leaves of 20 DAS plant were found with 75 and 100 mM NaCl treatments (119.5 and 132.3 µM H_2O_2. min^{-1}. mg^{-1} protein, respectively). These levels represented relative increases in POD activity of 16.6 and 29.1%, respectively. On the other hand, the lowest significant level of POD activity in leaves was found with 50 mM NaCl treatment (71.1 µM H_2O_2. min^{-1}. mg^{-1} protein) which represented relative reduction of 30.6%.

In the case of 27 DAS plant, the highest significant level of POD activity in leaves occurred with 50 mM NaCl treatment, which represented a relative increase of 13.3%. However, there were no significant variations between POD activities in leaves with 25 and 100 mM NaCl treatments and the control.

Tables 5 and 9 showed a positive relationship between Na^+ concentration and POD activity in leaves of 20 DAS plant while in those of 27 DAS plant this relation was not clear.

It is clear from Table 10 that there was no significant difference between POD activity in leaves of the younger and old plants.

Wang and Han (2009) found a significant increase in POD activity in leaves of salt-sensitive alfalfa cultivar with increasing salinity from 70 to 210 mM NaCl as compared to the control. Also, Kant and Turan (2011) found an increase of POD activity in leaves of bean with increasing salinity up to 120 mM NaCl.

Table 9 showed no significant differences between POD activity in root of 20 DAS plants treated with 75 and 100 mM NaCl and that of the control. However, the lowest significant levels of POD activity in root were found with 25 and 50 mM NaCl treatments which represented relative reductions of 13.6 and 10.3%, respectively. In the case of 27 DAS plant, the lowest POD activities in root were found with 75 and 100 mM NaCl treatments which represented relative reductions of 32.2 and 35.3%, respectively.

Tables 5 and 9 showed a negative relationship between the concentration of Na^+ and the activity of POD in root of 27 DAS plants. These data reveal that high salinity (100 mM NaCl) had inhibited the activity of POD enzyme in the root of 27 DAS plant while it did not significantly affect POD activity in root of 20 DAS plant. The study carried out by Jebara *et al*. (2005), however, showed that under salt stress (50mM NaCl) the POD activity in root of bean (Phaseolus vulgaris genotype PAT 474) was increased relative to that of the control.

Table 10 showed that the POD activity was significantly higher in root of older than younger plant. Also, Tables 9 and 10 showed that the POD activity was markedly higher in root than in leaves, at each level of NaCl treatment.

Ascorbate Peroxidase (EC.1.11.1.11): As shown in Table 9, the lowest significant levels of ascorbate peroxidase (APX) activity, in leaves of 20 and 27 DAS plants, were due to 100mM NaCl treatment. These levels were 18.72 and 14.05 µM H_2O_2. min^{-1}. mg^{-1} protein, and represented values of relative reduction in the enzyme activity of 33.9 and 41.5%, respectively. This points out that high salinity (100 mM NaCl) has inhibited APX enzyme in leaves of pea plant grown under salt stress. Also, there was a high relative reduction in the activity of APX enzyme in leaves with proceeding plant age.

Studies carried out by Hernandez *et al*. (2001) & Hernandez and Almansa (2002) showed that there were no significant changes in the activity of APX in leaves of salt-sensitive pea plant with increasing salinity. However, in earlier study, Hernandez *et al*. (2000) found that the activity of APX in leaves of pea plant had significantly increased with increasing salinity. In addition, Wang and Han (2009) found higher levels of APX activity in leaves of salt-sensitive alfalfa (*Medicago sativa*) cultivar grown under salt stress relative to the control. They reported that salt-sensitive alfalfa cultivar may mainly employ APX for detoxification of H_2O_2 in leaves of plant under salt stress (210 mM NaCl).

Examining the data in Tables 5 and 9 showed that there was a negative relationship between the concentration of Na^+ and the activity of APX enzyme in leaves of either 20 or 27 DAS plants.

Table 10 showed that the level of APX activity was significantly higher in leaves of older than younger plants. This is clearly observed at each level of NaCl treatment (Table 9).

Table 9 showed that the highest significant levels of APX activity were found in root of the control plants (45.89 and 58.14 µM H_2O_2. min^{-1}. mg^{-1} protein for 20 and 27 DAS plants, respectively). The lowest significant levels of APX activity in root were found with 100 mM NaCl treatment (33.48 and 38.88 µM H_2O_2. $min^{-1}mg^{-1}$ protein for 20 and 27 DAS plants, respectively) which represented values of relative reduction in APX activity of 27.0 and 33.1%, respectively. However, Jebara *et al*. (2005) found that the activity of APX enzyme in root of bean (*Phaseolus vulgaris* genotype BAT 477) had increased when the plant was grown under salt stress (50 mM NaCl) relative to the control plant.

Tables 5 and 9 showed a negative relationship between the concentration of Na^+ and the activity of APX enzyme in root of either 20 or 27 DAS plants.

It is clear also from Table 10 that the activity of APX was significantly higher in root of older than younger plant. This is markedly clear at each level of NaCl treatment (Table 9). It is also clear that APX activity was markedly higher in root than in leaves of pea plant at each level of NaCl treatment (Table 9).

Conclusion

Accumulation of Na^+ in leaves and root of pea (*Pisum sativum*) plant grown under salt stress was associated with low growth of plant and low concentrations of K^+, Mg^{2+} and Ca^{2+}. This elements imbalance led to inhibition of the enzymes (CAT and APX) in both leaves and root of 20 and 27 DAS plants. The relative increases of enzymes inhibition were higher in older than younger plants. On the other hand, the activity of POD enzyme in leaves of the younger plant had increased with increasing salinity but did not change in leaves of older plant. In addition, POD activity did not change in root of younger plant with increasing salinity but decreased in root of older plant.

Several studies suggested that the decrease in the activity of antioxidative enzymes (CAT, POD and APX) usually occurred in plant which can be considered salt-sensitive. This was mostly associated with high concentrations of Na^+ and low concentrations of K^+, Mg^{2+} and Ca^{2+} in leaves and root of the plant.

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تـأثير الإجهـاد الملحـى علـى النمـو ومحتـوى العناصـر ونشـاط إنزيمـات مضـادات الأكسدة لنبات البسلة النامى فى مزرعة رملية.

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أجريت تجربة أصص فى البستان، كلية الزراعة، جامعة الاسكندرية بهدف تقييم تأثير الرى بمحاليل كلوريد الصوديوم على كل من خواص النمو، محتوى العناصر وأنشطة إنزيمات مضادات الأكسدة لنبات البسلة النامى فى مزرعة رملية.

أجريت التجربة فى تصميم القطع المنشقة مع ستة مكررات وإستخدام محاليل كلوريد الصوديوم بتركيزات صفر، 25، 50، 75، 100 مليمول.

بذرت بذور البسلة فى أصص بلاستيكية تحتوى 2 كيلوجرام رمل سبق غسله ثم جمعت عينتين من النبات (3 مكرات لكل عينة) عند عمر 20، 27 يوم من عمر النبات وذلك لتقدير كل من خواص النمو ومحتوى العناصر وأنشطة الإنزيمات المضادة للأكسدة: الكتاليز، البيروكسيديز والأسكوربيت بيروكسيدير فى أوراق وجذور النبات.

أوضحت النتائج المتحصل عليها حدوث نقص معنوى فى أوزان الأوراق والجذور للنبات مع زيادة تركيز ملوحة ماء الرى. كذلك وجد أن تركيز الصوديوم والبوتاسيوم والمغنسيوم كان أقل فى الأوراق عن الجذور بينما كان تركيز الكالسيوم أكبر فى الأوراق عن الجذور.

أوضحت النتائج كذلك حدوث نقص معنوى فى نشاط إنزيم الكتاليزفى أوراق وجذور النبات مع زيادة تركيز كلوريد الصوديوم فى ماء الرى وكان أدنى نشاط عند المعاملة 100 مليمول كلوريد صوديوم والذى أحدث نقص نسبى فى نشاط الإنزيم قدره 20.2، 10.4% فى الأوراق وكذا 34.3، 34.2% فى الجذور للنباتات عند عمر 20، 27 يوم على التوالى.

كذلك تشير النتائج إلى حدوث زيادة معنوية فى نشاط إنزيم البيروكسيديز فى الأوراق للنبات عند عمر 20 يوم بينما لم يحدث تغيير معنوى فى نشاط الإنزيم فى الأوراق للنبات عند عمر 27 يوم. كذلك وجد أن أقصى نشاط للإنزيم فى أوراق النبات عند عمر 20 يوم عند معاملة 100 مليمول كلوريد الصوديوم وهذه أدت إلى زيادة نسبية فى نشاط الإنزيم قدرها 16.6%. كذلك توضح النتائج عدم حدوث تغير معنوى فى نشاط إنزيم البيروكسيدير فى جذور النبات عند عمر 20 يوم بينما حدث نقص معنوى لنشاط الإنزيم فى جذور النبات عند عمر 27 يوم حيث حدث نقص نسبى قدره 35.3% عند معاملة 100 مليمول كلوريد الصوديوم.

توضح النتائج إلى حدوث نقص معنوى فى نشاط إنزيم الأسكوربيت بيروكسيديز فى الأوراق والجذور للنباتات عند عمر 20، 27 يوم مع زيادة تركيز ملوحة ماء الرى. وقد حدث أدنى نشاط للإنزيم عند المعاملة 100 مليمول كلوريد الصوديوم وذلك بقيم نقص نسبى قدرها 33.9، 41.5% فى الأوراق و 27.0، 33.1% فى الجذور للنبات عند عمر 20، 27 يوم على التوالى.

قام بتحكيم البحث

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