Tolerance of Five Genotypes of Lentil to NaCl-Salinity Stress

Abd El-Monem, M. Sharaf

Botany & Microbiology Dept. Fac. of Sci. Al-Azhar Univ. Cairo Egypt.

Sharaf5858@yahoo.com

Abstract: Five Egyptian lentil cultivars, Giza(4), Giza (9), Giza (51), Giza (370) and Sinai (1), were tested for their tolerance to different levels of NaCl (0, 50, 100, 150, 200 and 250 mM). Seed germination, vegetative growth, and activities of certain metabolites were investigated. The inhibitory effects of NaCl differed, depending on the genotypes tested. In Giza (4), Giza (51) and Giza (370), cultivars with reduced germination percentage and lower relative water content, the increase in NaCl concentration resulted in the decrease in growth, levels of photosynthetic pigments, total soluble sugars, total soluble proteins, proline, and activities of the main enzymes involved in the germination process. In contrast, Giza (9) and Sinai(1) cultivars, in response to salt stress accumulated higher proline, total soluble sugar and total soluble proteins concentrations which improved their water status and the enzyme activities. K/Na ratio was varied in the different genotypes in response to different levels of NaCl. Giza (9) and Sinai (1) exhibited highest values of K/Na ratio in their shoots than the other cultivars. [New York Science Journal. 2008;1(3):70-80]. (ISSN: 1554-0200).

Introduction:

The amount and quality of irrigation water available in many of the arid and semi-arid regions of the world are the main limiting factors to the extension of the agriculture (Munns, 2002). The progressive salinization of land is one of the most severe world wide problems in agriculture production (Pandey and Thakares, 1997). Soil salinity affected plants at seedling stage much higher than other plant growth stage; because seed germination usually occurs in the uppermost soil layers which accumulate soluble salts as a result of evaporation and capillary rise of water Almansouri *et al.* (2001). Soil salinity designates a condition in which the soluble salt content of the soil reaches a level harmful to crops through the reduced osmotic potential of the soil solution and the toxicity of specific ions. These soluble salts may be from those present in the original soil profile or transported to the profile by irrigation water containing an unusual high concentration (Ates and Tekeli, 2007). All these factors manifest themselves by morphological, physiological and metabolic modifications in plant such as decrease in seed germination, decrease in shoot and root length (Arshi *et al*., 2002), alterations in the integrity of cell membranes, changes in different enzymatic activities and photosynthesis (Arshi *et al.*, 2002 and Sairam and Tyagi, 2004).

The response of plants to excess sodium chloride (NaCl) is complex and involves changes in their morphology, physiology and metabolism (Parida and Das, 2005).germination is one of the most critical periods for a crop subjected to salinity. In this respect, Fowler (1991) and Puppala *et al.* (1991) reported that germination failures on saline soils are often the results of high salt concentrations in the seed planting zone because of upward movement of soil solution and subsequent evaporation at the soil surface .These interfere with seed germination and crop establishment. Lentil is considered a strategic crop under agronomic and food point of view, because of its role as possible component of the cropping systems in the Mediterranean areas and as a protein source for human and animal consumption (Kurdali, *et al.*, 1997and Katerji, *et al.*, 2001).

Selection for salinity resistance appears as a laborious and hazardous task and plant breeders are, therefore, seeking for quick, cheap and reliable ways to assess the salt-resistance of selected material. Determination of germination potential of seeds in saline conditions could appear as a simple and useful parameter for several reasons. First, salinity resistance at this stage was shown to be a heritable trait which could be used as an efficient criterion for the selection of salt-resistant populations Ashraf (1994), although it is a polygenic character linked to a complex genetic basis Mano and Takeda (1997). Second, seeds and young seedlings are frequently confronted by much higher salinities than vigorously growing plants because germination usually occurs in surface soils which accumulate soluble salts as a result of evaporation and capillary rise of water.

Several studies demonstrated that the evolution of salinity resistance is not the same for all cultivars of a given species (Lutts *et al.*, 1995 and Almansouri *et al.*, 2001). The aim of this work was to study the effect of salinity on five lentil cultivars for evaluation of their salt tolerance. This was carried out on the basis of magnitude of seed germination, early vegetative growth and activities of some metabolites as well as contents of sodium and potassium during the early stage of growth .

Materials and Methods:

Seeds of lentil Giza (4), Giza (9), Giza (51), Giza (370) and Sinai (1) were obtained from Agricultural Research Centre, Ministry of Agriculture, Giza, Egypt.

A pot experiment was carried out in Botanical farm, Fac. Of Sci., Al-Azhar Univ. seeds were sown in pots 45 cm diameter. Each pot was filled with 10kg of clay loamy soil (40% clay, 35% silt, 25% sand) with 10 seeds/pot. Thinning was performed after 1 weak later of germination leaving five plants per pot. The pots were divided into five sets representing the five lentil cultivars. Each set composed of five groups representing the following irrigation salinity levels: 0.0, 50, 100, 150, 200 & 250 mM NaCl.

The relative water content was estimated according to Turner (1981) and was evaluated from the equation:

RWC% = (FW – DW)/(TW)X100, where FW is the fresh weight of the shoots, TW is the weight at full turgor, measured after floating the shoots for 24 hours in water in the light at room temperature and DW is the weight estimated after drying the shoots at 70ºC until a constant weight is achieved.

Photosynthetic pigments were estimated using the method of Vernon and Selly (1966). Contents of soluble carbohydrates were measured according to the method of Umbriet *et al.* (1969). Contents of soluble proteins were estimated according to the methods of Lowery *et al.* (1951).

For determination of proline contents seeds were hand-homogenized in 3% of sulfosalicylic acid and centrifuged at 3000g at 4°C for 10 min. The supernatants were used for proline estimation (Bates *et al*., 1973).

Activities of amylases were determined using the method of Afifi *et al.* (1986). Proteases activities were estimated using the method of Ong and Guacher (1972). Catalases activity were measured according to the method of Chen *et al.* (2000).

A portion of shoot samples (each 0.5 g) were digested with di-acid mixture of HNO_3 and $HClO_4$ (3:1) for K^+, Na^+ analysis (Yoshida *et al.*, 1976). The concentration of K^+ and Na^+ in the digest was estimated by flamephotometery.

Statistical Analysis:

All the obtained results were statistically analyzed using least significant difference test (L.S.D) and (T. Test) at 5% level of probability (Snedecor and Cochran, 1982).

Results and Discussion:

Germination :

Results of the present study (table 1) revealed that germination of all cultivars began from Two days after sowing, reaching 100% germination percentage in control seeds. Increasing salinity caused delay and a gradual decrease in germination. This was valid among the different cultivars. At the seventh day after sowing (before thinning the plants in pots) the final germination percentage for each of the studied cultivars was calculated. The seeds were not germinated by the 250 mM salinity level. However, at 200 mM NaCl, germination percentage was significantly reduced in Giza-4 (50%) ,and Giza-51 which retched 39% and 10% in Giza-370. At the same salinity level (200 mM NaCl) cultivars Giza-9 and Sinai-1 germinated more than the other ones (77 and 64%), exhibiting a fair degree of salt tolerance. In general the obtained results show a decrease in germination percentage in all studied genotypes, with increasing salinity. In the cultivars Giza-4 ,Giza-370 and, in minor extent, in Giza-51 the magnitudes of such decrease were more as compared to that of cultivars Giza-9 and Sinai-1, in particular way when high salinity concentrations were used. Inhibition of germination due to salinity has been reported (Alyari *et al.*, 2004 and Redondo *et al.,* 2004). Sidari *et al.* (2008) reported that genetic variability within a species offers a valuable tool for studying mechanism of salt tolerance. Germination process can be considered in terms of three sequential steps: imbibition, metabolism leading to initiation of radical growth and radicle growth leading to radical emergence.

Table 1 : Germination percentage (%) of five lentil cultivars under different levels of NaCl.Each value is a mean of three replicates ± standard error of mean .

Cultivar	NaCl Concentrations (mM)					
	0.0	50	100	150	200	250
Giza-4	100.0 ± 0.0	100.0 ± 0.0	71.25 ± 0.04**	56.39± 0.06**	50.21 ± 0.06**	_
Giza-9	100.0 ± 0.0	100.0 ± 0.0	92.34 ± 0.06*	82.13 ± 0.04**	77.05± 0.03**	_
Giza-51	100.0 ± 0.0	97.12 ± 0.10	73.24 ± 0.07**	51.52± 0.06**	39.11± 0.08**	_
Giza-370	100.0 ± 0.0	94.08 ± 0.06	68.27± 0.06**	48.64 ± 0.04**	10.00 ± 0.07**	_
Sinai-1	100.0 ± 0.0	100.0 ± 0.0	87.21 ± 0.04**	76.27 ± 0.05**	64.13± 0.07**	_

* Significant at 5% confidence level;
** Significant at 1% confidence level.

Growth and metabolic responses:

Depending on the obtained results as regards the percent of germination, four levels of salinity (0.0, 50, 100 &150 mM NaCl) were tested for growth and metabolic responses of the five cultivars of lentil.

Data presented in table 2 showed that salinity decreased plant height, as well as plant-fresh and dry weights of the tested cultivars. The magnitude of decrease was increased with increasing salinity level. Statistically, Giza (9) and Sinai (1) cultivars showed insignificant responses as regard the aforementioned growth parameters. This was the case up to100 mM NaCl then they were significantly decreased. Other cultivars showed either significant or highly significant decreases in growth characters in response to all applied levels of salinity except for 50 mM NaCl, they were insignificantly affected.

Results presented in table 2 showed that increasing salinity level led to a gradual decrease in the RWC in all cultivars. The highest water content was detected in cultivar Giza-9 followed by Sinai-1, while the lowest contents were detected in Giza-4, Giza-51 and Giza-370 respectively. This was the case throughout the different levels of NaCl. Relative water content (RWC) was used as a measure of drought (McCaig and Romagosa, 1991). Inhibition of germination due to salinity as suggested in previous reports (Ates and Tekeli, 2007 and Sidari *et al.*, 2008) is attributed to a decrease water content, that affect the synthesis of hydrolytic enzymes limiting the hydrolysis of food reserves from storage tissues as well as to impaired translocation of food reserves from storage tissue to developing embryo axis (Dubey, 2003).

Results presented in table 2 showed also that increasing salinity level led to a decrease in the K/Na ratio in all cultivars. Progressive decrease in the K/Na ratio were observed in Giza-4, Giza-51 and Giza-370 respectively. This was more obvious at the high levels of NaCl. On the other hand, highest levels of K/Na ratio was detected in cultivar Giza-9 followed by Sinai-1. This was the case throughout the different levels of NaCl. K^+/Na^+ ratio is a good indicator to assess plant tolerance to salt stress (Ashraf *et al.*, 2007). They reported that reduction in K^+/Na^+ ratio of sugarcane genotypes under salt stress was attributed to increased absorption of Na^+ and decreased absorption of K^+ by sugarcane genotypes. However, salt-tolerant genotypes exhibited strong affinity for K^+ over Na^+ and maintained an 8-fold higher K^+/Na^+ ratio as compared to salt-sensitive genotypes.

Table 2: Effect of different levels of NaCl on plant height (cm.), F.wt. (gm./plant), D.wt. (gm./plant), RWC (%/plant) and K^+/ Na^+Per plant of five lentil cultivars. Each value is a mean of three replicates ± standard error of mean .

Cultivars	NaCl (mM)	plant height (cm)	F.wt. gm/plant	D.wt. gm/plant	RWC% Per plant	K/Na ratio Per plant
Giza-4	0.0	24.11 ± 0.12	3.25 ± 0.10	1.17 ± 0.02	47.06 ± 1.02	3.29 + 0.01
	50	23.98 ± 0.14	3.09 ± 0.01*	1.14 ± 0.03	46.10 ± 1.12	3.01 + 0.04
	100	18.24 ± 0.12**	2.17 ± 0.12**	1.09 ± 0.02*	33.13 ± 0.19**	2.36 + 0.02**
	150	13.27 ± 0.24**	2.01 ± 0.04**	1.03 ± 0.02**	31.21 ± 0.54**	0.57 + 0.02**
Giza-9	0.0	26.25 ± 0.16	3.69 ± 0.16	1.18 ± 0.01	48.49 ± 0.35	3.31 + 0.04
	50	26.02 ± 0.13	3.38 ± 0.08	1.11 ± 0.05	50.56 ± 0.84	3.24 + 0.06
	100	23.17 ± 0.10	3.01 ± 0.07	1.09± 0.01	47.91 ± 0.73	3.19 + 0.03
	150	21.91 ± 0.15*	2.98 ± 0.23	1.08 ± 0.02	48.63 ± 0.29	2.93 + 0.07
Giza-51	0.0	25.36 ± 0.13	3.18 ± 0.15	1.14 ± 0.03	47.23 ± 0.53	3.16 + 0.04
	50	22.40 ± 0.08	2.57 ± 0.06*	1.10 ± 0.01	40.57 ± 0.81	2.58 + 0.06
	100	17.14 ± 0.13**	2.45 ± 0.07**	1.07 ± 0.01*	39.27 ± 0.09**	1.27 + 0.03**
	150	15.39 ± 0.13**	2.26 ± 0.05**	1.08 ± 0.01*	35.61 ± 0.38**	0.97 + 0.08**
Giza-370	0.0	25.37 ± 0.20	3.41 ± 0.07	1.11 ± 0.04	50.98 ± 0.94	3.12 + 0.02
	50	22.34 ± 0.14*	3.16 ± 0.04	1.13 ± 0.01	47.54 ± 0.82	2.09 + 0.04*
	100	19.30 ± 0.10**	2.70 ± .03**	1.09 ± 0.03	43.66 ± 0.37*	1.20 + 0.06**
	150	14.21 ± 0.08**	1.89 ± 0.04**	1.00 ± 0.02**	30.79 ± 0.77**	0.57 + 0.09**
Sinai-1	0.0	25.69 ± 0.14	3.51 ± 0.08	1.09 ± 0.02	52.60 ± 0.18	3.24 + 0.04
	50	25.02 ± 0.12	3.47 ± 0.05	1.04 ± 0.00	53.46 ± 1.06	3.05 + 0.01
	100	21.95 ± 0.16	3.42 ± 0.07	1.04 ± 0.01	53.33 ± 0.38	2.81 + 0.04
	150	20.34 ± 0.17*	3.00 ± 0.10	1.03 ± 0.03	47.25 ± 0.11	2.79 + 0.08

* Significant at 5% confidence level;

** Significant at 1% confidence level.

Results of the present study(table 3) showed a great variations as regards the contents of photosynthetic pigments depending on the type of lentil cultivar and the applied level of NaCl. Cultivars of Giza-4, Giza-51 and Giza-370 showed, in most cases, highly significant decreases in the contents of chlorophyll a, chlorophyll b and carotenoids especially at the high salinity levels. In cultivar Giza-9 followed by Sinai-1, contents of chlorophyll a, chlorophyll b as well as carotenoid contents were generally insignificantly affected in response to different applied levels of salinity.

Several investigators reported that chlorophyll and total carotenoid contents of leaves decrease, in general, under salt stress. The ability of plants to tolerate salt is determined by multiple biochemical pathways that facilitate retention and/or acquision of water, protect chloroplast function and maintain ion homeostasis (Parida and Das, 2005). In Grevilea, protochlorophyll, chlorophylls, and carotenoids are significantly reduced under NaCl stress, but the rate of decline of protochlorophyll and chlorophyll is greater than that of Chl-a and carotenoids (Kennedy and De Fillippis, 1999). In leaves of tomato, the contents of total chlorophyll (Chl.a + b), Chl-a, and b carotene decrease by NaCl stress (Khavarinejad and Mostofi, 1998). Under salinity stress, leaf pigments studied in nine genotypes of rice reduce in general, but relatively high pigment levels are found in six genotypes (Alamgir and Ali, 1999). Salinity causes significant decreases in Chl-a, Chl-b, and carotenoid in leaves of *B. parviflora* (Parida *et al.*, 2002). Decrease in carotenoids lead to degradation of B-carotene and formation of Zeaxanthins, which are apparently involved in protection against photoenhibition (Sharma and Hall, 1991). However, Wang and Nil (2000) have reported that chlorophyll content increases under conditions of salinity in *Amaranthus* plants.

Table 3: Effect of different levels of NaCl on contents of Chlo.a, mg/g. F.wt., Chlo.b, mg/g. F.wt. and contents of Carotenoids mg/g. F.wt. of five lentil cultivars. Each value is a mean of three replicates ± standard error of mean.

Treatments Cultivars	NaCl (mM)	Chlo.a,mg/g. F.wt.	Chlo.b,mg/g. F.wt.	Carotenoids mg/g F.wt.
Giza-4	0.0	3.21 ± 0.03	3.09 ± 0.01	2.21 ± 0.01
	50	3.11 ± 0.02	2.89 ± 0.04	2.14 ± 0.05
	100	2.61 ± 0.05*	1.39 ± 0.03**	1.64 ± 0.03**
	150	2.01 ± 0.02**	1.09 ± 0.01**	1.07± 0.02**
Giza-9	0.0	3.35 ± 0.04	3.19 ± 0.01	2.33 ± 0.05
	50	3.33 ± 0.01	3.09 ± 0.06	3.33 ± 0.01**
	100	3.19 ± 0.05	2.89 ± 0.02	2.17± 0.05
	150	2.94 ± 0.03	2.81 ± 0.05	2.24 ± 0.07
Giza-51	0.0	3.14 ± 0.02	3.12 ± 0.03	2.84 ± 0.02
	50	3.01 ± 0.04	3.09 ± 0.01	3.02 ± 0.06
	100	2.13 ± 0.04**	2.19 ± 0.04**	2.74 ± 0.03
	150	1.47 ± 0.02**	2.09 ± 0.06**	1.74 ± 0.02**
Giza-370	0.0	3.09 ± 0.04	3.06 ± 0.01	2.09 ± 0.01
	50	3.01 ± 0.02	3.00± 0.08	3.01 ± 0.02*
	100	2.31 ± 0.05*	2.10 ± 0.02**	1.28 ± 0.04**
	150	1.11 ± 0.02**	2.04 ± 0.05**	1.17 ± 0.02**
Sinai-1	0.0	3.40 ± 0.05	3.10 ± 0.03	2.40 ± 0.04
	50	3.01 ± 0.02	3.06 ± 0.01	3.00 ± 0.02*
	100	3.01 ± 0.03	2.79 ± 0.02	3.01 ± 0.02*
	150	2.84 ± 0.02	2.09 ± 0.04*	2.94 ± 0.03*

* Significant at 5% confidence level;
** Significant at 1% confidence level.

The obtained results (table 4) revealed that contents of soluble carbohydrates in shoots of different cultivars were greatly affected in response to different levels of salinity. In Giza-4, Giza-51 and Giza-370, contents of soluble carbohydrates in both shoots and roots were increased at the lower level of NaCl (50mM), then they were significantly decreased at the higher levels (150mM NaCl). However, in Giza-9 followed by Sinai-1, an opposite trend was observed, where contents of soluble carbohydrates in shoots and roots were increased with increasing salinity level. Statistically, the observed increases were found to be significant up to 100 mM of NaCl then they were insignificantly affected at 150 mM NaCl. Carbohydrates such as sugars (glucose, fructose, sucrose and fructans) and starch accumulate under salt stress (Parida *et al.*, 2002). Their major functions are osmoprotection, osmotic adjustment, carbon storage, and radical scavenging. Salt stress increases reducing sugars (glucose and fructose), sucrose, and fructans in a number of plants (Kerepesi and Galiba, 2000, Khatkar and Kuhad, 2000 and Singh *et al.*, 2000). In Vicia faba salinity decreases soluble and hydrolyzable sugars (Gadallah, 1999). Sugar content increases in some genotypes of rice but also decreases in some genotypes (Alamgir and Ali, 1999). Under salinity, the starch content in roots of rice plants declines and remains unchanged in shoots. A decrease in starch content and an increase in both reducing and non-reducing sugar have been reported in leaves of *Bruguiera parviflora* (Parida *et al.*, 2002). Sidari *et al.* (2008) in lentil, indicate a lower content of total soluble sugar and proline in presence of the highest salt concentration in Castelluccio and Eston cultivars compared to Ustica and Pantelleria cultivars, suggesting that salt tolerance ability of these two last landraces appears to be associated to the accumulation of osmolytes which improved their water status.

Data presented in table 4 revealed that contents of soluble protein were significantly increased in shoots of Giza-9 and sinai-1 cultivars in response to all applied concentrations of NaCl. In Giza 51, contents of soluble proteins were increased only at the lower level of NaCl (50mM), then they were significantly decreased at 100 & 150 mM NaCl. Highly significant decreases in protein contents were detected in shoots of Giza-4 and Giza 370 cultivars. This was the case throughout all the applied levels of NaCl. Several salt-induced proteins have been identified in plants species and have been classified into two distinct groups (Mansour, 2000), salt stress proteins which accumulate only due to salt stress, and stress associated proteins, which also accumulate in response to heat, cold, drought, water logging, and high and low mineral nutrients. Proteins that accumulate in plants grown under saline conditions may provide a storage form of nitrogen that is re-utilized when stress is over (Singh *et al*., 1987) and may play a role in osmotic adjustment. Proteins may be synthesized de novo in response to salt stress or may be present constitutively at low concentration and increase when plants are exposed to salt stress (Pareek *et al*., 1997). A higher content of soluble proteins has been observed in salt tolerant than in salt sensitive cultivars of barley Hurkman *et al*., 1989, finger millet (Uma *et al*., 1995), and rice (Lutts *et al*., 1996). In wheat, Ashraf and O'Leary (1999) reported that total soluble proteins increased due to salt stress in all cultivars tested but that this increase was more marked in a salt sensitive cultivar and low in a salt tolerant. In contrast, in lentil, Ashraf and Waheed (1993) reported that leaf soluble proteins decreased due to salt stress in all lines, irrespective of their salt tolerance. Ashraf and Fatima (1995) found that salt tolerant and salt sensitive accessions of safflower did not differ significantly in leaf soluble proteins. Similarly, comparison of salt tolerant wild populations with cultivated populations of *Melilotus indica* and *Eruca sativa*, Ashraf (1994) showed that the salt tolerant populations did not differ from salt sensitive populations in soluble protein content of their leaves at varying salt levels of the growth medium.

Table 4: Effect of different levels of NaCl on contents of Soluble carbohydrates (mg/g. D.wt.), Soluble proteins (mg/g D.wt.) and contents of Proline (mg/g. D.wt.) in shoots of five lentil cultivars. Each value is a mean of three replicates ± standard error of mean.

Treatments		Soluble	Soluble	Proline
Cultivars	NaCl (mM)	carbohydrates mg/g D.wt.	prteins mg/g D.wt.	mg/g D.wt.
Giza-4	0.0	56.27 ± 0.12	61.95 + 0.24	0.53 ± 0.01
	50	54.19 ± 0.14	66.32 + 0.28*	0.79 ± 0.02*
	100	43.28 ± 0.21**	55.09 + 0.30**	0.52 ± 0.01
	150	41.91 ± 0.15**	53.27 + 0.16**	0.34 ± 0.05**
Giza-9	0.0	57.28 ± 0.13	63.05 + 0.24	0.61 ± 0.02
	50	62.27 ± 0.14*	67.91 + 0.22*	0.87 ± 0.04*
	00	74.09 ± 0.14**	73.68 + 0.17**	0.93 ± 0.03**
	50	74.60 ± 0.20**	77.15 + 0.24**	0.94 ± 0.02**
Giza-51	0.0	55.92 ± 0.15	60.58 + 0.14	0.56 ± 0.04
	50	51.36 ± 0.18*	61.24 + 0.12	0.67 ± 0.01*
	100	43.50 ± 0.15**	54.10 + 0.16**	0.41 ± 0.03*
	150	39.20 ± 0.14**	49.88 + 0.15**	0.37 ± 0.04**
Giza-370	0.0	56.38 ± 0.18	61.05 + 0.17	0.58 ± 0.04
	50	57.25 ± 0.15	58.20 + 0.14	0.55 ± 0.05
	100	42.60 ± 0.30**	49.26 + 0.25**	0.41 ± 0.01**
	150	36.81 ± 0.15**	45.57 + 0.34**	0.35 ± 0.04**
Sinai-1	0.0	54.96 ± 0.17	62.28 + 0.17	0.56 ± 0.02
	50	66.10 ± 0.14**	67.24 + 0.23*	0.61 ± 0.06*
	100	73.31 ± 0.18**	70.34 + 0.19**	0.67 ± 0.03**
	150	75.90 ± 0.12**	70.94 + 0.16**	0.78 ± 0.02**

* Significant at 5% confidence level;
** Significant at 1% confidence level .

Results in table 4 showed that contents of proline in shoots of the tested cultivars were varied greatly according to the type of cultivar and the applied concentration of NaCl. In Giza-9 and, to more extent, in sinai-1, proline contents were increased with increasing salinity level. However, in other cultivars, contents of proline were, generally, decreased with increasing salinity level. Accumulation of some compatible solutes (proline and free amino acids) in stressed plants produced lower solute potential, which allows plant cell to maintain a higher water content than the corresponding control. These solutes play an important role in plants under stress conditions, where major functions of sugars are osmoprotection and/or osmotic adjustment as reported by Parida *et al*. (2002).

Results presented in table 5 revealed that activities of amylases, proteases and catalases in shoots of the tested cultivars were varied greatly according to the type of cultivar and the applied concentration of NaCl. In Giza-9 and, to more extent, in sinai-1, activities of amylases and proteases were mostly insignificantly affected in response to the different levels of salinity, while activities of catalases were significantly increased. In other cultivars, Giza-4, Giza-51 and Giza-370, activities of amylases, proteases and catalases were decreased. The magnitude of the decreases was increased with increasing salinity level. The activities of the antioxidative enzymes such as catalase (CAT), increase under salt stress in plants and a correlation of these enzyme levels and salt tolerance exists (Lee *et al.*, 2001, Mittova *et al.*, 2003 and Parida and Das, 2005). Vardhini and Rao (2003) observed that CAT activity decreased in susceptible sorghum and maize varieties but increased in resistant varieties as compared to unstressed control plants.

Conclusion:

Although lentil is considered a very sensitive species to salinity, much more than other legumes such as broad bean, chick pea and soybean, from the outcome of the obtained results, it could be identified, at least , two cultivars, Giza (9) and Sinai (1) that could be utilized not only in breeding programs to improve the saline resistance of the species but also to be cultivated in environments where salinity of the soils is a frequent constraint.

Table 5: Effect of different levels of NaCl on contents of amylase (mg/g. F.wt.), protease (mg/g. F.wt.) and contents of catalase (mg/g F.wt.) in shoots of five lentil cultivars. Each value is a mean of three replicates ± standard error of mean.

Treatments		Amylase	Protease	Catalase
Cultivars	NaCl (mM)	mg/g F.wt.	mg/g F.wt.	mg/g F.wt.
Giza-4	0.0	3.21 ± 0.03	3.09 ± 0.01	2.21 ± 0.01
	50	3.11 ± 0.02	2.89 ± 0.04	2.14 ± 0.05
	100	2.61 ± 0.05*	1.39 ± 0.03**	1.64 ± 0.03**
	150	2.01 ± 0.02**	1.09 ± 0.01**	1.07± 0.02**
Giza-9	0.0	3.35 ± 0.04	3.19 ± 0.01	2.33 ± 0.05
	50	3.33 ± 0.01	3.09 ± 0.06	3.33 ± 0.01**
	100	3.19 ± 0.05	2.89 ± 0.02	2.87± 0.05*
	150	2.94 ± 0.03	2.81 ± 0.05*	2.84 ± 0.07*
Giza-51	0.0	3.14 ± 0.02	3.12 ± 0.03	2.84 ± 0.02
	50	3.01 ± 0.04	3.09 ± 0.01	3.02 ± 0.06
	100	2.13 ± 0.04**	2.19 ± 0.04**	2.74 ± 0.03
	150	1.47 ±0.02**	2.09 ± 0.06**	1.74 ±0.02**
Giza-370	0.0	3.09 ± 0.04	3.06 ± 0.01	2.09 ± 0.01
	50	3.01 ± 0.02	3.00± 0.08	3.01 ± 0.02
	100	2.31 ± 0.05*	2.10 ± 0.02**	1.28 ± 0.04**
	150	1.11 ± 0.02**	2.04 ± 0.05**	1.17 ± 0.02**
Sinai-1	0.0	3.40 ± 0.05	3.10 ± 0.03	2.40 ± 0.04
	50	3.01 ± 0.02	3.06 ± 0.01	3.00 ± 0.02*
	100	3.01 ± 0.03	2.79 ± 0.02	3.01 ± 0.02*
	150	2.84 ± 0.02	2.09 ± 0.04*	2.94 ± 0.03*

* Significant at 5% confidence level; ** Significant at 1% confidence level.

Corresponding author: Abd El-Monem M. Sharaf
Sharaf5858@yahoo.com

References:

Afifi, W.M.; Zeinab, A. and Abd El-Hameid, M.F. (1986): Effect of gamma-irradiation on pea seedlings. Ann. Agric., Moshtohor, 24(4), 2047-2057.

Agastian, P.; Kingsley, S.J. and Vivekanandan, M. (2000): Effect of salinity on photosynthesis and biochemical characteristics in mulberry genotypes. Photosynthetica, 38, 287-290.

Alamgir, A.N. and Ali, M.Y. (1999): Effect of salinity on leaf pigments, sugar and protein concentrations and chloroplast ATPAase activity of rice (*Oryza sativa* L.). Bangladesh J. Bot., 28, 145-149.

Almansouri, M., Kinet, J.M. and Lutts, S. (2001): Effect of salt and osmotic stresses on germination in durum wheat (*Triticum durum* Desf.). Plant Soil, 7, 243-254.

Alyari, H.; Shekari, F.; Motallebi, A.; Shekari F. and Golipoor, M. (2004): Effects of different salt concentrations on seed germination and seedling growth of Iranian alfalfa (*M. sativa L.*) populations Turk. J. Field Crops, 9, 22-34.

Arshi, A., M.Z. Abdin and M. Iqbal, (2002): Growth and metabolism of senna as affected by salt stress. Biol. Plant., 45, 295-298.

Ashraf, M. (1994): Organic substances responsible for salt tolerance in *Eruca sativa*, Biol. Plant., 36, 255-259.

Ashraf, M. and O'Leary, J.W. (1999): Changes in soluble proteins in spring wheat stressed with sodium chloride, Biol. Plant., 42, 113-117.

Ashraf, M. and Waheed, A. (1993): Responses of some local/exotic accessions of lentil (*Lens culinaris* Medic.) to salt stress, J. Agron. Soil Sci., 170, 103-112.

Ashraf, M. and Fatima, H. (1995): Responses of some salt tolerant and salt sensitive lines of safflower (*Carthamus tinctorius* L.), Acta Physiol. Plant., 17, 61-71.

Ashraf, M., Rahmatullah, Kanwal, S., Tahir, M.A., Sarwar, A. and Ali, L. (2007): Differential salt tolerance of sugarcane genotypes. Pakistan J. Agr. Sci., 4, 85-89.

Ates, E. and Tekeli, A.S. (2007): Salinity tolerance of persiam clover (*Trifolium resupinatum* var. Majus Boiss) lines at germination and seedling stage. World J. Agric. Sci., 3(1), 71-79.

Bates, L.S.; Waldren, R.P. and Teare, I.D. (1973): Rapid determination of free praline for water stress studies. Plant Soil, 39, 205-207.

Burton, K., (1956): A study of the conditions and mechanism of the diphenylamine reaction of colorimetric estimation of deoxyribonucleic acid. Biochem. J., 62, 315.

Chen, Y.; Cao, X.X.D.; Lu Y. and Wang, X.R. (2000): Effects of rare earth metal ions and their EDTA complex on antioxidant enzymes of fish liver. Bull. Environ. Contam. Toxicol., 65, 357-365.

Dubey, R.S. (2003): Effect of salinity on nucleic acid metabolism of germinating rice seeds differing in salt tolerance. Plant Physiol. Biochem., 12, 9-16.

Gadallah, M.A.A. (1999): Effects of proline and glycinebetaine on *Vicia faba* response to salt stress. Biol. Plant., 42, 249-257.

Fowler, J.L.(1991): Interaction of salinity and temperature on the germination of crambe. Agron. J., 83, 169-172.

Hernandez, J.A.; Campillo, A.; Jimenez, A.; Alacon, J.J. and Sevilla, F., (1999): Response of antioxidant systems and leaf water relations to NaCl stress in pea plants. New Phytol., 141, 241-251.

Hurkman, W.J. Fornari, C.S. and Tanaka, C.K. (1989): A comparison of the effect of salt on polypeptide and translatable mRNA in roots of a salt tolerant and salt sensitive cultivar of barley, Plant Physiol., 90, 1444-1456.

Katerji, N.; van Hoorn; J.W. Hamdy; A. Mastrorilli; M. Oweis T. and Erskine, W.(2001): Response of two varieties of lentil to soil salinity. Agric. Water Manage., 47, 179-190.

Khavarinejad, R.A. and Mostofi, Y. (1998): Effects of NaCl on photosynthetic pigments, saccharides, and chloroplast ultrastructure in leaves of tomato cultivars. Photosynthetica, 35, 151-154.

Kennedy, B.F. and De Fillippis, L.F., (1999): Physiological and oxidative response to NaCl of the salt tolerant *Grevillea ilicifolia* and the salt sensitive *Grevillea arenaria*. J. Plant Physiol., 155, 746-754.

Kerepesi, I. and Galiba, G. (2000): Osmotic and salt stress-induced alteration in soluble carbohydrate content in wheat seedlings. Crop Sci., 40, 482-487.

Khatkar, D. and Kuhad, M.S. (2000): Short-term salinity induced changes in two wheat cultivars at different growth stages. Biol. Plant., 43, 629-632.

Kurdali, F., Kalifo, K. and Al Schamma, M. (1997): Cultivar differences in nitrogen assimilation, partitioning and mobilization in rain-fed grown lentil. Field Crops Res., 54, 235-243.

Lee, D.H., Kim, Y.S. and Lee, C.B. (2001): The inductive responses of the antioxidant enzymes by salt stress in the rice (*Oryza sativa* L.). J. Plant Physiol., 158, 737-745.

Lowery, O.H.; Rosebrough, N.J.; Farr, A.L. and Randall, R.J. (1951): Protein measurement with the folin reagent. J. Biol. Chem., 193, 265-275.

Lutts, S.; Kinet, J.M. and Bouharmont, J. (1995): Changes in plant response to NaCl during development of rice (*Oryza sativa* L.) varieties differing in salinity resistance. J. Exp. Bot., 46, 1843-1852.

Lutts, S. Kinet, J.M. and Bouharmont, J. (1996): Effects of salt stress on growth, mineral nutrition and proline accumulation in relation to osmotic adjustment in rice (*Oryza sativa* L.) cultivars differing in salinity tolerance. Plant Growth Regul., 19, 207-218.

Mano, Y. and Takeda, K. (1997): Mapping quantitative trait loci for salt tolerance at germination and seedling stage in barley (*Hordeum vulgare* L.). Euphytica, 94, 263-272.

Mansour, M.M.F. (2000): Nitrogen containing compounds and adaptation of plants to salinity stress. Biol. Plant., 43, 491-500.

McCaig, T.N. and Romagosa, I. (1991): Water status measurements of excised wheat leaves: position and age effects. Crop Sci., 31, 1583-1588.

Mittova, V., Tal, M., Volokita, M., Guy, M. (2003): Up-regulation of the leaf mitochondrial and peroxisomal antioxidative systems in response to salt-induced oxidative stress in the wild salt-tolerant tomato species *Lycopersicon pennellii*. Plant Cell Environ., 26, 845-856.

Munns, R. (2002): Comparative physiology of salt and water stress. Plant Cell and Environment, 25, 239-250.

Ong, P.S. and G.M. Gaucher, (1972): Protease production by thermophillic fungi. Can. J. Microbiology, 19, 129-133.

Pandey, A.N. and Thakares, N.K. (1997): Effect of chloride salinity on survival and growth of *Prosopis chilensis* seedlings. Trop. Ecol., 3, 145-148.

Pareek, A.; Singla, S.L and Grover, A. (1997): Salt responsive proteins/genes in crop plants, in: P.K. Jaiwal, R.P. Singh, A. Gulati (Eds.), Strategies for Improving Salt Tolerance in Higher Plants, Oxford and IBH Publication Co., New Delhi, , pp. 365–391.

Parida, A. and Das, A.B. (2002): NaCl stress causes changes in photosynthetic pigments, proteins and other metabolic components in the leaves of a true mangrove, *Bruguiera parviflora*, in hydroponic cultures. J. Plant Biol., 45, 28–36.

Parida, A. and Das, A.B. (2005): Salt tolerance and salinity effects on plants. Ecotoxicol. Environ. Safety, 60, 324-349.

Puppala, N.; Fowler, J.L.; Poindexter, L. and Bhardwaj, H.L.(1999): Evaluation of salinity tolerance of canola germination. In: Perspectives on New Crops and New Uses. Ed., Janick, J., ASHS Press, pp: 251-253.

Redondo, S.; Rubio-Casal, A.E.; castillo, J.M. Luque, C.J.; Alvarez, A.A.; Luque T. and Figueroa, M.E. (2004): Influences of salinity and light on germination of three *Sarcocornia* taxa with contrasted habitats. Aquatic Botany, 78, 255-264.

Sairam, R.K. and A. Tyagi, (2004): Physiology and molecular biology of salinity stress tolerance in plants. Curr. Sci., 86, 407-421.

Sharma, P.K. and D.O. Hall, (1991): Interaction of salt stress and photoinhibition on photosynthesis in barley and sorghum. J. Plant Physiol., 138, 614-619.

Singh, N.K.; Bracken, C.A.; Hasegawa, P.M.; Handa, A.K.; Buckel, S.; Hermodson, M.A.; Pfankoch, F.; Regnier, F.E. and Bressan, R.A. (1987): Characterization of osmotin. A thaumatin-like protein associated with osmotic adjustment in plant cells, Plant Physiol., 85, 529-536.

Sidari, M.; Muscolo, A.; Anastasi, U.; Preiti, G. and Santonoceto, C. (2008): Variations in four genotypes of lentil under NaCl-salinity stress. Amer. J. of Agr. and Biol. Sci., 3(1), 410-416.

Singh, S.K.; Sharma, H.C.; Goswami, A.M.; Datta, S.P. and Singh, S.P. (2000): In vitro growth and leaf composition of grapevine cultivars as affected by sodium chloride. Biol. Plant., 43, 283-286.

Snedecor, G.M. and Cochran, W.G. (1982): Statistical methods 7 edition, l th owa state Univ., Press, Ames., lowa U.S.A., pp: 325-330.

Turner, N.C. (1981): Techniques and experimental approaches for the measurement of plant water status. Plant and Soil, 58, 339-366.

Uma, S.; Prasad, T.G. and Kumar, M.U. (1995): Genetic variability in recovery growth and synthesis of stress proteins in response to polyethylene glycol and salt stress in finger millet, Ann. Bot., 76: 43-49.

Umbriet, W.W.; Burris, R.H.; stauffer, J.F.; Cohen, P.P.; Johsen, W.J.; Lee G.A., Patter, V.R. and Schneicter, W.C. (1969): Manometric techniques, manual describing methods applicable to the studs of tissue metabolism. Burgess publishing Co., U.S.A., pp: 239.

Vardhini, B.V. and Rao, S.S.R. (2003): Amelioration of water stress by brassinosteroids on seed germination and seedling growth of three varieties of sorghum. Plant Growth Regul., 41, 25-31.

Vernon, L.P. and Selly, G.R (1966): The chlorophylls. Academic press. New York and London.

Wang, Y. and Nil, N.(2000): Changes in chlorophyll, ribulose biphosphate carboxylase–oxygenase, glycine betaine content, photosynthesis and transpiration in *Amaranthus tricolor* leaves during salt stress. J. Hortic. Sci. Biotechnol., 75, 623-627.

Yoshida, S.; Forno, D.A.; Cock, J.H. and Gomez, K.A. (1976): Laboratory Manual for Physiological Studies of Rice. The International Rice Research Institute, Manila, Philippines.