Tolerance of Five Genotypes of Lentil to NaCl-Salinity Stress.

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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 factors manifest themselves 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 acrop 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 fiveplants 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 70°C 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-51which 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 (77and 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 Sinaiwhen high salinity 1,in particular way concentrations were used. Inhibition 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 replicats + standard error of mean.

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

^{*} Significant at 5% 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 aforemintioned growth parameters. This was the case up to 100 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.

Reults 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 troughout 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 hydrolitic 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).

Reults 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 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 troughout 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, salttolerant genotypes exhibited strong affinity for K⁺ over Na⁺ and maintained an 8-fold higher K⁺/Na⁺ ratio as compared to salt-sensitive genotypes.

^{**} Significant at 1% confidence level.

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 replicats \pm standard error of mean.

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

^{*} Significant at 5% 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.

^{**} Significant at 1% confidence level.

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 replicats + standard error of mean.

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

^{*} Significant at 5% 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-51and 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, fructans) and starch accumulate under salt stress (Parida et al., 2002). Their major osmoprotection, functions are osmotic adjustment, carbon storage, and radical scavenging. Salt stress increases reducing sugars (glucose, fructose), sucrose, and fructans in a

number of plants (Kerepesi and Galiba, 2000; Khatkar and Kuhad, 2000; 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 nonreducing 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 increaced in shoots of Giza-9 and sinai-1

^{**} Significant at 1% confidence level.

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, waterlogging, 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 prteins (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 replicats \pm standard error of mean.

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

^{*} Significant at 5% confidence level;

^{**} Significant at 1% confidence level .

Reults 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 concetration of NaCl . In Giza-9 and,to more extent, in sinai-1, proline contants were increased with increasing salinity level. However in other cultivars, contants 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 concetration 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-51and 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 outcom 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.), prtease (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 replicats \pm standard error of mean.

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

^{*} Significant at 5% confidence level;

^{**} Significant at 1% confidence level.

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