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ORIGINAL ARTICLE

Evaluation of Salt Tolerance (NaCl) in Tunisian Chili Pepper (*Capsicum frutescens* L.) on Growth, Mineral Analysis and Solutes Synthesis

Zhani Kaouther[1], Hermans Nina[2], Ahmad Rezwan[2] and Hannachi Cherif[1]

[1]*University of Sousse, Department of Horticulture and Landscape, Higher Institute of Agronomy. 4042 Chott Mariem, Tunisia*
[2]*Natural Products and Food - Research and Analysis, Department of Pharmaceutical Sciences, University of Antwerp, Belgium.*

*E-Mail: ***kaoutherzheni@yahoo.fr***

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Every year, more and more land becomes non-productive due to salinity which adversely affects the productivity and quality of most crops that is why salinity becomes a concern to be studied more to understand the mechanisms included and select the tolerant genotypes. In this context, this investigation was carried out to study the impact of NaCl on growth, mineral analysis and solutes synthesis in five Tunisian chili pepper (*Capsicum frutescens* L.) cultivars: Tebourba (Tb), Somaa (Sm), Korba (Kb), Awald Haffouzz (Aw) and Souk jedid (Sj). Thus, an experiment took place under greenhouse at Higher Institute of Agronomy, Chott Meriem, Tunisia and stress was induced during two months in water by NaCl (0, 2, 4, 6, 8, 10 and 12 g/l). Results showed that increasing salinity stress, for all cultivars, decreases the height and biomass (dry and fresh weight) of plant in addition to the relative water content. Also, a decline in K^+ and Ca^{2+} amounts in roots and K^+/Na^+ ratio was recorded. However, Na^+ content in roots and the biosynthesis of soluble sugars and soluble proteins in leaves increased. Awlad Haffouzz and Korba cultivars succefully tolerated highest salinity level by accumulating more K^+, Ca^{2+} in roots and containing the highest concentrations of soluble sugars and soluble protein in their leaves contrary to Souk jedid cultivar, considered as the sensitive cultivar.

Key words: Capsicum frutescens; height; mineral nutrition; NaCl; soluble proteins; soluble sugars; weight

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Key words: Capsicum frutescens; height; mineral nutrition; NaCl; soluble proteins; soluble sugars; weight

Chili pepper (*Capsicum frutescens* L.) belongs to family Solanaceae, is one of the most widely grown vegetable in the world. World production of pepper is estimated at 26,537 million tones and the first producer is China with 7.072 million tones nearly 27% (FAOSTAT, 2010). In Tunisia, pepper is widely grown in all regions both on open air and for sericulture and occupies the fourth largest area planted by gardening. In 2010, the area for growing peppers in Tunisia was approximate 17450 ha and production reached 280000 tones corresponding to an average yield of 16.04 t/ha. This production

allows the country to rank second place in the African production after Algeria (317500 tones) and the 15th rank in the world (FAOSTAT, 2010). However this production showed a decrease in the last seasons because peppers were exposed to many biotic (virus, champignon) and abiotic conditions especially salinity which had negative effect on pepper growth (Ibn Maaouia-Houimli *et al.,* 2008), yield and fruit quality (Ibn Maaouia-Houimli *et al.,* 2011) since pepper is a sensitive salt-tolerant crop (2 g/l).

In fact, the quality of irrigation water available in many of the arid and semi-arid regions of the world is the main limiting factor to the extension of the agriculture (Munns, 2002) especially when it is salty. Salinity in soil or water is one of the most damaging abiotic stress factors limiting crops (Debez *et al.,* 2006). It declines yield for most major crop plants by more than 50% and affects more than 10% of arable land (Bray *et al.,* 2000). Transpiration and evaporation from the soil surface, low precipitation (Neumann, 1995), salt load in irrigation water, over use of fertilizers and lack of proper drainage can be the main factors that contribute to this problem. Thus, high concentration of salts in the soils immediately imposes on plants the osmotic stress effect due to lower soil water potential leading to retardation of water uptake (Niu *et al.,* 1995). When exposed for longer period and to higher level of salts, salinity entails ionic stress when plants absorb and accumulate toxic level of Na^+ and Cl^- in the cytoplasm especially Na^+ known by its impact on membrane disorganization, inhibition of cell division and expansion (Deivanai *et al.,* 2011) . Salinity also induces secondary stresses such as nutritional imbalance and oxidative stress (Zhu, 2002). For worldwide crop production, the detrimental effects of salinity on plant growth are associated with low osmotic potential of soil solution (water stress), nutritional imbalance, specific ion effect (salt stress), or a combination of these factors (Levitt, 1980). All of these agents cause adverse effects on plant growth and development at physiological and biochemical levels (Munns, 2002), molecular level (Tester and Davenport, 2003).

Mostly, to survive in hyper-saline soil, plants evolved strategies that contribute to the adaptation to osmotic and ionic stresses caused by high salinity and maintain growth. This strategies range from morpho-anatomical to physiological and biochemical in nature (Cheesemann, 1988; Zhu, 2001). In fact, plants cope up with salinity by osmotic adjustment which is usually established by intake of inorganic ions as well as accumulation of compatible solutes (also known as Osmoprotectants). Inorganic ions are sequestered in the vacuole, while organic solutes are compartmentalized in the cytoplasm to balance the low osmotic potential in the vacuole (Yancey *et al.,* 1982, Rontein *et al.,* 2002, Garg *et al.,* 2002). These osmoprotectants include proteins, carbohydrates, amino acids and quaternary ammonium compounds (Ashraf, 2004), whose accumulation may protect plants against damage, helping maintain protein structure or scavenge reactive oxygen species besides maintaining balance in osmotic potential in the cytosol (Smirnoff and Cumbes, 1989; Parvanova *et al.,* 2004) and to alleviate enzyme inactivity or loss of membrane integrity due to water deficiency (Schwab and Gaff, 1990).

Comparing the response of cultivars of one species to salinity provides a convenient and useful tool for unveiling the fundamental mechanisms

involved in salt tolerance on the first hand and allows us to identify the plant genotypes capable of increased tolerance to salt on the second hand and as consequence the incorporation of these genotypes selected into culture will be interesting to reduce the effect of salinity on productivity.

In this context, this study was initiated in the objective of evaluating the effects of salt stress on growth, ion accumulation, soluble sugars and soluble proteins of five Tunisian accessions of chili pepper (Tebourba, Korba, Somaa, Awlad haffouzz and Souj Jdid) watered by seven level of NaCl (0, 2, 4, 6, 8, 10 and 12 g/l) in order to better understand their differences on salt stress tolerance and select the tolerant accession.

MATERIALS AND METHODS

Experiment

In Higher Institute of Agronomy, Chott Meriem, Tunisia, the study was carried out under greenhouse characterized by an area of 170 m² (20 m*8.5 m) and 25°C/18°C day/night temperature. This greenhouse is covered with plastic film (low density polyethylene) and cemented by its side. Seeds of five accessions: Tebourba (Tb), Somaa (Sm), Korba (Kb), Awald Haffouzz (Aw) and Souk jedid (Sj) were sterilized for 20 mn in sodium hypochloride solution (5%) and then rinsed 3 times with distilled water. Ten seeds for each cultivar (Figure 1) were sown on February 15th 2012, at depth of 2 cm, in plastic pot (20 cm diameter and 25 cm height) filled with peat, sand and topsoil (1/3:1/3:1/3). Pots were left in greenhouse on bricks. Once the seeds have germinated, we kept a single plant on which the trial continued (Figure 2). For two months (April and May), plants were watered with saline water at seven levels of NaCl concentrations (0, 2, 4, 6, 8, 10 and 12 g/l). During culture, plants were not fertilized but were processed by Talastar (80 cc/hl) preventively and curatively against aphids using a Knapsack sprayer. Salinity stress effect was studied by measuring plant height, fresh and dry weights of whole plant, relative water content, K^+, Na^+, Ca^{2+} amounts in roots and soluble sugars and soluble protein content in leaves.

Plant material was dried at 80 °C for 48 h and then dry weights are measured. Relative water content (RWC) was calculated as follows (Zheng et al., 2008):

RWC (%) = [(Plant Fresh Weight – Plant Dry Weight)/(Plant Fresh Weight)] x100

For ion determination, fresh samples of root were extracted in concentrated 0.1 N nitric acid. The extraction of ions took place at ambient laboratory temperature for at least 48 h. After filtration, all cations (K^+, Na^+ and Ca^{2+}) were determined with a flame emission spectrophotometer (JENWAY PFP7).

Soluble sugars estimation

Sugars were extracted from the leaves according to Dubois *et al.,* (1956) as follows: 500 mg (FW) of sample were homogenized with 2 ml of 80% ethanol solution in a mortar and pestle. After heating the homogenate in a water bath at 75°C for 10 min, the insoluble residue was removed by centrifuging at 5000×*g* for 10 min. The precipitate was re-extracted with 2 ml of 80% ethanol at 75°C and re-centrifuged. The supernatants were pooled and dried under a stream of hot air, and the residue was resuspended in 1 ml of water and desalted through a column of ion-exchange resin (Amberlite MB3). The filtrate was used for soluble sugar determinations. Total soluble sugars were determined by the phenol-sulphuric acid method using glucose as standard. Optical density was

recorded at 625 nm on a UV spectrophotometer (PG instruments T60).

Soluble protein estimation

Total soluble protein content was measured according to Bradford (1976) using bovine serum albumin (BSA) as a protein standard. Fresh leaf samples (100 mg) were homogenized with 4 ml Na-Phosphate buffer (pH 7.2) and then centrifuged at 13000 ×g for 4.5 min at 4°C. 1 ml of Supernatant is added to the Bradford reagent (5 ml) and the mixture was incubated thereafter in the dark for 15 min. Then, it was pipetted in spectrophotometer cuvettes and absorbance were measured using a UV spectrophotometer (PG instruments T60) at 595 nm. It was evaluated in milligrams of protein per gram of dry material (mg/g DW)

Pots were disturbed in completely randomized design with three replications and data analysis was done using "SPSS software 13.00". Duncan's Multiple Range test was used to compare between means and determine significance between variables ($P < 0,01$).

RESULTS

Growth

Salt stress exerted a significant impact on the plant height of the five chili pepper studied. Thus, in control plant, the length of pepper plant varied from 36.4 cm for Sj cv to 39.2 cm for Kb cv (Table 1). When NaCl is added to the irrigation water, the plant height decreased by increasing salt stress level. At the highest concentration (12 g/l), we observed the most significant decline: 70, 73, 76, 81 and 86 % respectively in Aw, Kb, Sm, Tb and Sj cv.

Results for fresh and dry weights of the five cv of pepper grown in 0 to 12 g/l showed that fresh and dry biomass of all cv reduced significantly with increasing NaCl concentration. Treatement interaction was also highly significant ($P<0,01$). Thus, at 12 g/l NaCl, fresh (Table 2) and dry weight (Table 3) decreased respectively upto 97 and 94% for Sj cv. Calculating Relative water content (RWC), we found that it showed similar trend as biomass and it declined as well as NaCl concentration increased in root medium (Table 4). At the highest level stress, the relative water content decreased to 8, 25, 31, 41 and 42 % respectively in Kb, Tb, Sm, Aw and Sj cv.

Mineral nutrition

Salt stress affected significantly the nutrient content in roots, in all pepper cv in study. Results showed, on the first hand, that in control medium, the roots contain low amounts of sodium ranging from 0.52 (Sm cv) to 0.76 mg/gDW (Tb cv) (Table 5). With addition of NaCl, the Na^+ concentration increased significantly to 4.802 mg/gDW in Sj cv. On the second hand, K^+ uptake in stressed plants decreased as compared to the control plants and the lowest amounts were observed at the highest level of salt stress where K^+ concentration decreased till 98 % in Sj and Tb cultivars (Table 5). At this concentration (12 g/l), the highest accumulation was observed in Aw cv (0.375 mg/gDW) and the lowest one in Sj cv (0.056 mg/gDW). In consequence, the ration K^+/Na^+ was also significantly influenced by high NaCl application and salt treatment resulted in the decline of the K^+/Na^+ ratio in all the accessions (Figure 3). Generally, high ratios were found in Aw cv (0.105) and Kb cv (0.052) whereas the lowest value in Sj cv (0.011).

According to Figure 4, all saline stressed plants showed lower Ca^{2+} content in their roots compared to the control group and there was significant difference between the cultivars at $P < 0.01$. So, Ca^{2+} concentration decreased significantly especially

at the highest NaCl concentration: the amounts ranged from 0.007 (Sj cv) to 0.022 mg/gDW (Tb cv) whereas at the control, it ranged from 0.824 (Kb cv) to 0.925 g/gDW (Sj cv).

Solutes synthesis

Data for total soluble sugars (Table 4) indicates that salt stress caused an increase in accumulation of total soluble sugars in leaves of all the pepper cv. The maximum concentration was recorded under higher salinity treatment (12 g/l) as compared to control. Thus, the increase was 1.11 , 1.12 , 1.13, 1.19 and 1.23 times the level measured in controlled plants respectively for Sj, Tb, Sm, Kb and Aw cv. At the highest level stress, Kb and Aw cv had the highest amount of soluble sugars while lower levels were in leaves of Sj cv.

The measurement of soluble proteins content in leaves showed that the salt stress induces a significant amelioration in amounts of soluble proteins as NaCl concentration increases, in all the pepper cv (Table 5). So, at the highest stress level, we observed the highest values of soluble proteins content which ranged from 33.17 (Sj cv) to 40.09 mg/gFW (Aw cv) corresponding to a respective increase of 29 and 50% compared to the control.

Figure 1: Pepper seeds sown in pots

Figure 2: Plants of chili pepper grown in pots

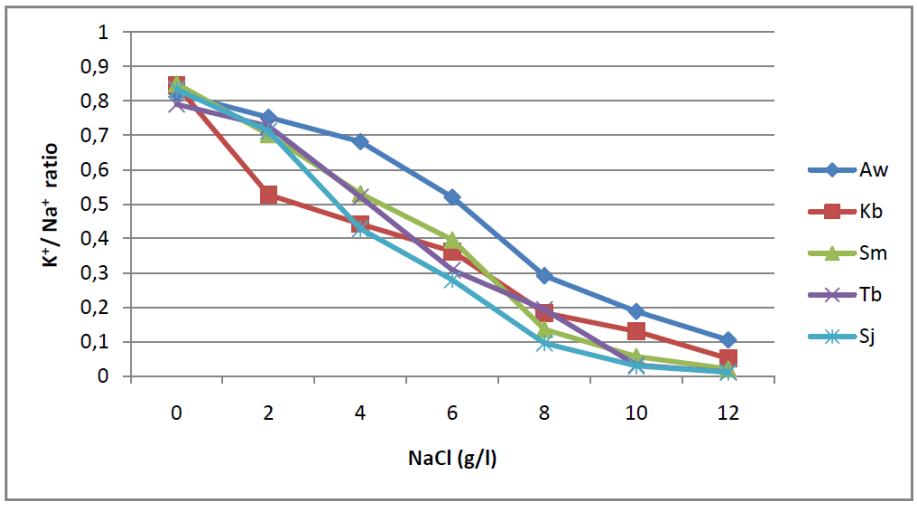

Figure 3: K^+/Na^+ ratio in roots of five Tunisian chili pepper cultivars watered during 60 days with NaCl (0, 2, 4, 6, 8, 10 and 12 g/l)

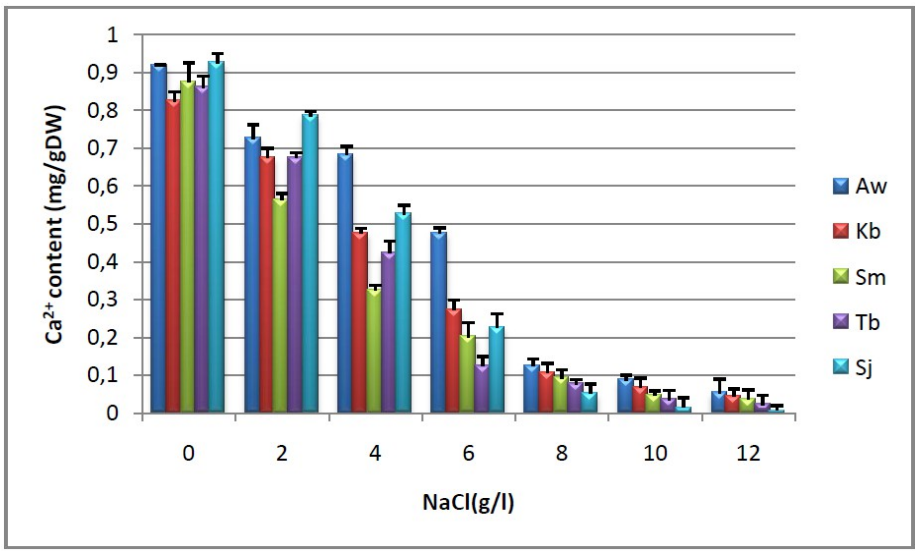

Figure 4: Ca^{2+} amount (mg/gDW) in roots of five Tunisian chili pepper cultivars watered during 60 days with NaCl (0, 2, 4, 6, 8, 10 and 12 g/l)

Table 1. Plant length (cm) of five cultivars of Tunisian chili pepper watered during 60 days with NaCl (0, 2, 4, 6, 8, 10 and 12 g/l)

	NaCl (g/l)						
	0	**2**	**4**	**6**	**8**	**10**	**12**
Tebourba	38.50^{a}	34.10^{ab}	29.30^{bc}	23.10^{c}	17.20^{cd}	10.70^{e}	7.30^{f}
Somaa	38.10^{a}	36.00^{b}	34.10^{bc}	30.80^{c}	23.70^{d}	14.50^{e}	9.30^{f}
Korba	39.20^{a}	36.70^{b}	32.40^{bc}	27.30^{cd}	20.20^{e}	13.30^{fg}	10.60^{g}
Awlad haffouzz	37.60^{a}	35.20^{ab}	30.30^{cd}	26.60^{d}	20.50^{e}	15.30^{f}	11.10^{g}
Souk jedid	36.40^{a}	32.70^{ab}	27.30^{c}	19.50^{d}	12.10^{e}	9.350^{f}	5.20^{g}

Means followed by the same letter are not significantly different at 5% level according to Duncan test.

Table 2. Plant fresh weight (g) of five cultivars of Tunisian chili pepper watered during 60 days with NaCl (0, 2, 4, 6, 8, 10 and 12 g/l)

	NaCl (g/l)						
	0	**2**	**4**	**6**	**8**	**10**	**12**
Tebourba	48.51^{a}	42.12^{ab}	37.18^{c}	32.12^{d}	22.12^{d}	13.14^{e}	7.11^{f}
Somaa	46.75^{a}	43.45^{b}	38.10^{c}	30.10^{cd}	20.13^{d}	10.12^{e}	7.50^{f}
Korba	58.14^{a}	50.12^{b}	42.86^{c}	31.81^{d}	25.19^{e}	16.31^{f}	9.04^{g}
Awlad haffouzz	49.56^{a}	42.18^{b}	36.20^{c}	24.76^{de}	20.12^{e}	15.51^{f}	10.05^{g}
Souk jedid	39.20^{a}	29.53^{b}	20.41^{c}	13.17^{d}	6.21^{e}	3.69^{f}	1.21^{g}

Means followed by the same letter are not significantly different at 5% level according to Duncan test.

Table 3. Plant dry weight (g) of five cultivars of Tunisian chili pepper watered during 60 days with NaCl (0, 2, 4, 6, 8, 10 and 12 g/l)

	NaCl (g/l)						
	0	**2**	**4**	**6**	**8**	**10**	**12**
Tebourba	12.13^{a}	10.56^{a}	9.78^{b}	8.52^{b}	6.71^{cd}	4.76^{d}	3.14^{d}
Somaa	18.76^{a}	17.95^{a}	16.21^{b}	13.24^{c}	8.81^{d}	4.81^{ef}	4.42^{f}
Korba	17.85^{a}	15.81^{b}	13.11^{bc}	10.23^{c}	8.36^{d}	5.81^{de}	3.27^{e}
Awlad haffouzz	13.47^{a}	12.41^{b}	11.12^{c}	10.71^{c}	9.81^{d}	7.91^{e}	5.72^{f}
Souk jedid	13.73^{a}	10.71^{ab}	7.82^{b}	4.76^{c}	2.33^{d}	1.84^{e}	0.75^{f}

Means followed by the same letter are not significantly different at 5% level according to Duncan test.

Table 4. Pant relative water content in plant of five Tunisian chili pepper cultivars of watered during 60 days with NaCl (0, 2, 4, 6, 8, 10 and 12 g/l)

	NaCl (g/l)						
	0	**2**	**4**	**6**	**8**	**10**	**12**
Tebourba	74.995^{a}	74.929^{b}	73.695^{bc}	73.474^{c}	69.665^{d}	63.775^{d}	55.873^{e}
Somaa	59.872^{a}	58.454^{b}	57.454^{c}	56.013^{d}	56.234^{d}	52.470^{e}	41.066^{f}
Korba	69.298^{a}	68.456^{a}	69.412^{a}	67.871^{b}	66.812^{b}	64.377^{c}	63.827^{c}
Awlad haffouzz	72.821^{a}	70.578^{b}	69.282^{b}	56.745^{c}	51.242^{d}	49.000^{e}	43.084^{f}
Souk jedid	64.974^{a}	63.732^{ab}	61.685^{b}	63.857^{ab}	61.866^{b}	50.135^{c}	38.016^{d}

Means followed by the same letter are not significantly different at 5% level according to Duncan test.

Table 5. Effect of NaCl on Na^{+} and K^{+} content (mg/gDW) in roots of five cultivars of Tunisian chili pepper watered during 60 days with NaCl (0, 2, 4, 6, 8, 10 and 12 g/l)

		NaCl (g/l)						
		0	**2**	**4**	**6**	**8**	**10**	**12**
Na^{+}	**Tebourba**	0.765^{f}	0.876^{e}	1.481^{de}	1.963^{d}	2.185^{c}	3.827^{b}	4.753^{a}
	Somaa	0.518^{g}	0.741^{f}	0.963^{e}	1.123^{d}	2.704^{c}	3.074^{b}	4.025^{a}
	Korba	0.506^{e}	1.852^{d}	2.210^{c}	2.592^{c}	3.074^{b}	3.605^{a}	3.802^{a}
	Awlad haffouzz	0.555^{g}	0.777^{f}	0.876^{e}	1.407^{d}	2.308^{bc}	2.802^{b}	3.172^{a}
	Souk jedid	0.555^{g}	0.712^{f}	0.951^{e}	1.617^{d}	2.716^{c}	3.704^{b}	4.807^{a}
K^{+}	**Tebourba**	2.883^{a}	2.325^{ab}	1.608^{c}	0.875^{d}	0.525^{de}	0.127^{f}	0.059^{g}
	Somaa	2.925^{a}	1.750^{b}	1.087^{bc}	0.737^{d}	0.425^{e}	0.187^{f}	0.087^{g}
	Korba	2.750^{a}	2.062^{b}	1.750^{c}	1.475^{cd}	0.815^{d}	0.555^{e}	0.207^{ef}
	Awlad haffouzz	2.562^{a}	2.362^{ab}	1.875^{c}	1.525^{cd}	0.951^{e}	0.653^{f}	0.375^{g}
	Souk jedid	2.770^{a}	1.762^{b}	0.712^{c}	0.629^{c}	0.287^{d}	0.112^{de}	0.056^{f}

Means followed by the same letter are not significantly different at 5% level according to Duncan test.

Table 6. Soluble sugars content (µg/gFW) in leaf of five cultivars of chili pepper watered during 60 days with NaCl (0, 2, 4, 6, 8, 10 and 12 g/l)

	NaCl (g/l)						
	0	**2**	**4**	**6**	**8**	**10**	**12**
Tebourba	1049^{de}	1071^{d}	1112^{c}	1139^{c}	1171^{bc}	1191^{b}	1205^{a}
Somaa	1071^{e}	1093^{d}	1103^{d}	1154^{bc}	1184^{b}	1201^{a}	1235^{a}
Korba	1025^{f}	1081^{e}	1116^{d}	1167^{c}	1194^{c}	1241^{ab}	1267^{a}
Awlad haffouzz	1043^{d}	1072^{d}	1113^{c}	1148^{c}	1171^{c}	1203^{b}	1241^{a}
Souk jedid	1012^{d}	1021^{c}	1035^{c}	1041^{bc}	1089^{b}	1114^{a}	1131^{a}

Means followed by the same letter are not significantly different at 5% level according to Duncan test.

Table 7. Soluble proteins content (mg/gFW) in leaf of five cultivars of chili pepper watered during 60 days with NaCl (0, 2, 4, 6, 8, 10 and 12 g/l)

	NaCl (g/l)						
	0	**2**	**4**	**6**	**8**	**10**	**12**
Tebourba	27.523^{ef}	28.132^{e}	28.811^{e}	29.132^{cd}	31.34^{c}	33.738^{b}	36.481^{a}
Somaa	28.245^{e}	30.040^{de}	32.772^{d}	35.016^{bc}	36.921^{b}	37.310^{ab}	38.411^{a}
Korba	25.712^{f}	26.701^{f}	30.511^{e}	31.171^{cd}	33.135^{c}	36.812^{bb}	38.762^{a}
Awlad haffouzz	26.754^{g}	29.145^{ef}	31.215^{e}	34.132^{d}	36.412^{bc}	38.626^{b}	40.092^{a}
Souk jedid	25.632^{d}	27.813^{d}	28.313^{cd}	29.072^{b}	29.785^{b}	30.261^{ab}	33.173^{a}

Means followed by the same letter are not significantly different at 5% level according to Duncan test.

DISCUSSION

In our study, the responses of five Tunisian chili pepper accessions (Tb, Kb, Sm, Aw and Sj) watered for two months by NaCl (0, 2, 4, 6, 8, 10 and 12 g/l) were compared with regard to vegetative growth, ion accumulation and soluble solutes.

The current results shows that high salinity level resulted in significant reduce of plant height, fresh and dry biomass and also in relative water content in roots especially for Sj cv whereas Aw and Kb cv shows resistance to this effect usually upto the highest value. These results are comparable to those reported for different crops such as maize (Cicek and Cakirlar, 2002), soyebean (Essa, 2002; Li *et al.,* 2006; Tunçtürk *et al.,* 2008), canola (Rameeh *et al.,* 2004), sprout (Al Thabet *et al.,* 2004), luzerne (Ibriz *et al.,* 2005), melon (Kusvuran *et al.,* 2007), peanut (Singh *et al.,* 2007), wheat (Khan *et al.,* 2009), carthamus (Abbaspour, 2010) and pistachio (Banakar and Ranjbar, 2010). The deleterious effect of salinity was suggested as a result of water stress, ion toxicities, ion imbalance, or combination of all these factors (Kurth *et al.,* 1986). Some researchers thought that the growth reduction is the consequences of ion accumulation through the changing of membrane permeability (Cramer *et al.,* 1985; Grieve and Fujiyama, 1987). Bandeoglu *et al.,* (2004) indicated also that this retarded growth is due to inhibition of cell elongation due to higher concentration of Na+ which causes membrane disorganization, inhibition of cell division and expansion (Deivanai *et al.,* 2011). Researchs of Rahimi and Biglarifard (2011) showed that accumulation of ions in plant growth environment causes osmotic and pseudo-drought stress leading to decrease of water absorption by plant tissues. Mer *et al.,* (2000) confirmed also the same effect and reported that probably the negative effect of salinity on plants provoked osmotic potential by salt in the culture medium, such that the root cells did not obtain the required water from the medium. Therefore in plants, the uptakes of some mineral nutrients dissolved in water were also restricted. Thus, growth and development of plants were inhibited due to the occurring defect in metabolism. Decrease of tissue water content resulted in reduction of cellular growth and development. Therefore, restriction of water absorption and its consequences for cellular growth and development was one of the most important causes of decreased root growth.

The results for ion content in roots show that there was a competition between Na^+ and K^+ regarding their uptake and so increasing NaCl stress is accompanied by an increase in Na^+ concentration for all the five cv and a decrease at the same time of K^+ uptake. The present result was in agreement with the work of Gucci *et al.,* (1997) in olive, Mezni *et al.,* (2002) in luzene, Kaya *et al.,* (2002) in

strawberry, Sadeghi (2009) in barley, Molazem *et al.,* (2010) in corn, Ben Dkhil and Denden (2010) in okra and Akibarimoghaddam *et al.,* (2011) in wheat in which the authors observed that high saline concentration increased Na^+ content and decreased K^+ content in the affected crops. Bybordi *et al.,* (2010) reported that Teak and Zamin (2008) showed that potassium content decreased due to salinity in sensitive cultivars. It seems that this decrease is due to an antagonistic effect between sodium and potassium. El-Samad and Shaddad (1997) reported also that one of the primary plant responses to salinity is the decrease in K^+ concentration in plant tissues and thus the substitution of K^+ by Na^+ may lead to nutritional imbalances. Both of these ions might compete for entry into plant root cells. Greenway and Munns (1980) confirmed the antagonistic effect between these elements. According to the results, in Aw and Kb cv, Na^+ accumulation was lower but higher for K^+ ion in the roots contrary to Sj cv which demonstrates the highest Na^+ content and the lowest K^+ concentration. It is concluded then that Aw cv is the most salt stress tolerant due to its less Na^+ absorption and more Na^+ accumulation in roots compared with the four others studied cultivars and that Sj cv is the most sensitive. Similar results were reported with different cultivars of soybean (Essa, 2002; Li *et al.,* 2006), green bean (Yasar *et al.,* 2006) and canola (Bandeh-Hagh *et al.,* 2008). Ashraf and Harris (2004) confirmed that sodium accumulation in tolerant cultivars was lower than in sensitive cultivars and potassium concentration was higher in tolerant cultivars. The results for this tolerant accession can be explained in the light of early findings of many scientists that salt tolerant mesophytes generally exclude either Na^+ (Lauchli *et al.,* 1994; Saqib *et al.,* 2005) because Na^+ is the primary cause of ion specific damage, resulting of disorders in enzyme activation and protein synthesis (Tester and Davenport, 2003). Therefore, exclusion of Na^+ and maintenance of high K^+ level are vital for the plants to grow under saline conditions (Munns *et al.,* 2000). Zhu *et al.,* (2001) found also that plants are able to tolerate moderately saline environments with a greater ability to exclude Na^+ from shoot or at least the leaf blade and concurrently maintain high level of K^+.

The ration Na^+/K^+ is also reduced by salt stress and this result is the same with which found in tomato (El-Iklil *et al.,* 2001), legume (Amador *et al.,* 2007) and rapeseed (Farhoudi, 2011). Aw cv maintained also considerably high ratio (0.105) contrary to Sj cv (0.011). Such result explaines more the advantage of this cultivar to present its well responding to salt stress during vegetative growth. Morant-Manceau *et al.,* (2004) and Farhoudi and Tafti (2011) found also K^+/Na^+ ratio was higher respectively in salt tolerance of Triticale and soybean cultivars. This trait has also potential value as selection criterion for salt tolerance (Greenway and Munns, 1980; Ashraf, 2004).

Measurement of Ca^{2+} content demonstrates that it is also affected by salinity and that there is a positive correlation in the accumulation of Ca^{2+} and K^+ and so a negative one with Na^+ content. The lowest amount is obtained at the highest concentration (0.007 mg/gDW in Sj cv). The same result was observed in wheat (A Tammam *et al.,* 2008), rice (Nemati *et al.,* 2011), soyeboen (Amirjani, 2010) and canola (Tunçtürk *et al.,* 2011). In fact, calcium has been shown to play an important role in regulating ion transfer into plant cells growing in saline medium (Ashraf and Naqvi, 1992; Soussi *et al.,* 2001) and in amelioration of the adverse effects of salinity on plants (Amador *et al.,*

2007). Ca^{2+} can also affect membrane stability (Rengel, 1992; Marschner, 1995) and ion translocations (Maas and Grieve, 1987; Cramer, 1992; Unno *et al.,* 2002). It is well known to have regulatory roles in metabolism (Cramer *et al.,* 1985) and sodium ions may compete with calcium ions for membrane binding sites. Therefore, it has been suggested that high calcium levels can protect the cell membrane from the adverse effects of salinity. Calcium was also shown to inhibit Na^+ absorption in beans (Lahaye and Epstein, 1971; Awada *et al.,* 1995), soybeans (Wieneke and Lauchli, 1980) and pigeon pea (Subbarao *et al.,* 1990) and thus may be an important factor in controlling salinity response of legumes. The accumulation of moderate amounts of Ca^{2+} by Aw cv in its roots is less easy to explain in view of the early findings of various workers. Davenport et al., (1997) reported that in wheat, extracellular Ca^{2+} inhibits unidirectional Na^+ influx and also inhibits Na^+ influx through a non selective cation channel, isolated in planar lipid bilayers, suggesting that the effect of Ca^{2+} on Na^+ influx might be direct and cytosolic signaling for modification of ion channel activity is not required.

Soluble sugars content in leaves is also affected by salt treatment with a greater increase as the NaCl concentration was increased. This finding agrees with reporters suggesting that salt stress increase soluble sugars amounts of rice (Dubey and Singh, 1999; Pattanagul and Thitisaksakul, 2008; Siringam *et al.,* 2011; Amirjani, 2011), *Chenopodium* (Prado *et al.,* 2000), sorghum (Gill *et al.,* 2003; Thakur and Dev Sharma, 2005), sugar beet (Khavari-Nejad *et al.,* 2008), alfalfa (Wang and Zang, 2009), potato (Farhad *et al.,* 2011), citrus (Balal *et al.,* 2011), pistachio (Abbaspour *et al.,* 2012) and *Pistacia atlantica* (Ben Hassaini *et al.,* 2012). Sugars content in leaves was the highest in Aw and Kb cv (1241 and 1267 µg/gFW respectively) and the lowest in Sj cv (1131 µg/gFW). Balal *et al.,* (2011) working on citrus rootstocks and Dubey and Singh (1999) working on rice affirmed that the most sugars accumulation was in the salt tolerant. In fact, it is believed that accumulation of total soluble sugars is a common phenomenon under stress condition (William *et al.,* 2000; Murakeozy *et al.,* 2003). It has an important role in osmoregulation (Mohanty *et al.,* 2002; Martino *et al.,* 2003) which reduce the cell osmotic potential to a level to provide high turgor potential allowing the plants to maximize sufficient storage reserves to support basal metabolism under stressed environment (Hurry *et al*., 1995) and maintaining growth (Lacerda *et al.,* 2003; Ashraf and Harris, 2004; Cha-um *et al.,* 2004). Singh (2004) proved then that a greater accumulation of sugars lowers the osmotic potential of cells and reduces loss of turgidity in tolerant genotypes. Another possible role of sugars may be as a readily available energy source. Various experimental approaches have shown that sugars play a key role in this regulatory mechanism by repressing the expression of the photosynthesis genes (Koch, 1996) because excess amount of sugars in cytoplasm seems having a role to repress Rubisco (Sawada *et al.,* 1992).

Soluble proteins content in leaves, after examination, shows that it is also touched by salinity stress. Indeed, in all the cultivars studied, biosynthesis of soluble proteins is enhanced as NaCl concentration increases. At the highest level, we observed the highest amelioration: 29, 32, 36, 50 and 51% respectively in Sj,Tb, Sm, Aw and Kb cv. Enhanced soluble proteins concentration induced by NaCl was also reported in barley (Hurkman *et al.,* 1989), sunflower (Ashraf and Tufail, 1995), rice (Lutts *et al.,* 1996; Pareek *et al.,* 1997; Raja babu

and Ramesh, 2007), chick pea (Soussi *et al.,* 1998), wheat (Javed, 2002; Afzal *el al*. 2006; Zerrad *et al.,* 2008; Sen and Alikamanogli, 2011), *Pancratium maritimum* (Khedr *et al.,* 2003), cotton (Jiang *et al.,* 2005), tomato (Amini and Ehsanpour, 2005), sesame (Hukam Gehlot *et al.,* 2005), mulberry (Ahmad *et al.,* 2006; Ahmad and Sharma, 2010), lentil (Abd El-Monem Sharaf, 2008) *Triticum* (Afrasayab *et al.,* 2010), and strawberry (Rahimi *et* Biglarifard*,* 2011). According to Mansour (2000), several salt-induced proteins have been identified in plants species and have been classified into two distinct groups, 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). Among the proteins discovered to be increased in response to salt stress, we quote a 26 kDa protein named as osmotin in tobacco (Singh *et al.,* 1987), osmotin-like protein in salt stressed *Mesembryanthemum* (Thomas and Bohnert, 1957), two 26 kDa polypeptides, not immunologically related to osmotin, identified as germin in barley (Hurkman *et al.,* 1991), a 22 kDa protein in radish (Lopez et al., 1994), a 54 kDa and 23–24 kDa proteins in finger millet (*Eleusine coracana*) (Uma et al., 1995) and 33 kDa protein in wheat (Wimmer *et al.,* 2003). In another study, Cheng et al., (2002) reported that the second-generation of rice transgenic plants subjected to salt stress showed high accumulation of either MA80 or PMA1959 proteins correlated with increased tolerance of transgenic rice plants to salt stresses. While investigating the mechanisms of salt tolerance in a mangrove, *Bruguiera sexangula*, Yamada *et al.,* (2002) found also a specific protein, allene oxide cyclase (AOC) responsible for enhanced salt tolerance. They designated this protein as "mangrin". Furthermore, expression of mangrin in *Saccharomyces cerevisiae* and tobacco cell lines also enhanced salt tolerance in these species. In our study, the highest content of soluble proteins is observed in Aw cv and this proves also that it's the tolerant accession because Hurkman *et al.,* 1989, Uma *et al.,* 1995 and Lutts *et al.,* 1996 reported that this amount are the highest in salt tolerant than in salt sensitive cultivars of barley, finger millet and rice .

CONCLUSION

The assessment of the effects of salinity on the growth and biochemical attributes in the five cultivars of Tunisian chili pepper lead us to conclude that all the considered parameters were significantly affected by the salt stress especially at the highest salt level (12 g/l NaCl). On the first hand, vegetative growth is inhibited as well as NaCl is increasing in the water (plant height, dry and fresh biomass and relative water content). On the second hand, mineral nutrition is affected by the decrease of K^+ and Ca^{2+} uptake and the increase of Na^+ amount in roots. However, biosynthesis of soluble proteins and soluble sugars is enhanced. Aw and Kb cv of chili pepper are classified as the salt tolerant genotypes whereas Sj cv as susceptible genotype based on various parameters studied especially the maintaining of the highest values of K^+ content and K^+/Na^+ ratio in roots and soluble solutes (sugars and proteins) in leaves. Thus, we

can conclude that the synthesis of compatible organic solutes occurs in response to salt stress and that these organic solutes could be used as a biochemical marker for assessing increased salt tolerance in pepper genotypes.

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