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ORIGINAL ARTICLE

Impact of salt stress (NaCl) on growth, chlorophyll content and fluorescence of Tunisian cultivars of chili pepper (*Capsicum frutescens* L.)

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Salinity is considered as the most important abiotic stress limiting crop production and plants are known to be able continuing survive under this stress by involving many mechanisms. In this content, the present study was carried out to evaluate the impact of NaCl on some physiological and biochemical parameters 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 of five months was carried out under greenhouse at Higher Institute of Agronomy, Chott Meriem, Tunisia and stress is induced by NaCl at 7 concentrations (0, 2, 4, 6, 8, 10 and 12g/l). Results showed that increasing salinity stress, for all cultivars, had a negative impact on roots (length, fresh and dry weights) and leaves (number and area). Also, chlorophyll (a and b) amount in addition to quantium yield (Fv/Fm) decreased significantly. However, biosynthesis of proline in leaves is activated. Awlad Haffouzz and Korba cultivars succefully tolerated highest salinity level by accumulating more proline in leaves and maintaining usually higher values in all parameters in opposition to Souk jedid cultivar. Taken together, our data partly explain the mechanism used to ovoid salt stress by pepper plants when excessive in the culture medium.

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Key words: chlorophyll content, leaves, NaCl, pepper, proline, quantium yield, roots

Salinity is a major environmental factor determining plant productivity and plant distribution. It affects more than 10 percent of arable land and salinization is rapidly increasing on a global scale, declining average yield for most major crop plants by more than 50 percent (Bray et *al.* 2000). Salt stress occurs in areas where soils are naturally high in salt and precipitation is low (Neumann, 1995) and/or where irrigation, hydraulic lifting of salty underground water, or invasion of sea water in coastal areas brings salt to the surface soil that inhabit plants. Globally 20% of irrigated land and 2.1% of dry land agriculture suffers from the salt problem and NaCl is the predominant salt causing salinization (Munns and Tester 2008). Salinity adversely affects germination, growth, physiology and productivity by reducing the ability of plants to take up water causing imbalance in osmotic potential; ionic equilibrium and nutrient uptake (Niu *et al*. 1995). Further, it facilitates severe ion toxicity by depositing high concentration of Na^+ which causes membrane disorganization, inhibition of cell division and expansion. In addition, it impairs a wide range of cellular metabolism including photosynthesis, protein synthesis and lipid metabolism (Alia-Mohanty and Saradhi, 1992; Ashraf, 1994). Lichtenthaler *et al*. (2005) found that salt stress was responsible for decreased biosynthesis of chlorophyll and inefficiency of photosynthesis (Munns, 2002) all of which ultimately leading to lowered economic productivity. The decline in photosynthesis due to salinity stress could be due to lower stomata conductance, depression in carbon uptake and metabolism, inhibition of photochemical capacity or a combination of all these factors (Mundree *et al.* 2009). Chlorophyll fluorescence has proved particularly useful in salinity-tolerance screening programs (Jimenez *et al.* 1997) because the effects of salt damage can be detected prior to visible signs of deterioration (West, 1986).

To perceive the incoming stresses and rapidly regulate their physiology and metabolism, plants evolved mechanisms that allow them to cope with them (Zhang et *al.* 2006) by synthesis and accumulation of a number of compatible solutes called “osmolytes”. These osmolytes include proteins, carbohydrates, amino acids and quaternary ammonium compounds (Ashraf, 2004) which are accumulated in plants at high concentrations to alleviate enzyme inactivity or loss of membrane integrity due to water deficiency (Schwab and Gaff, 1990). Proline is a key osmolyte which helps plants to maintain cell turgor (Hsu *et al.* 2003, Seki *et al.* 2007). A large number of plant species accumulate proline in response to salinity stress and this accumulation may play a role in defense against salinity stress that is why in some cases higher proline content could be correlated with abiotic stress tolerance (Premachandra *et al.* 1992). The role of proline in cell osmotic adjustment, membrane stabilization and detoxification of injurious ions in plants exposed to salt stress is widely reported (Hare *et al*. 1999; Kavi Kishor *et al*. 2005; Ashraf and Foolad, 2007).

Pepper is one of the most widely grown vegetable in the world. World production of pepper is estimated at 23.2 million tones and the largest producer is China with 11.5 million tons, or nearly 50% (FAO, 2003). In Tunisia, pepper is widely grown in all regions both in the field and under greenhouse and occupies the 4th largest area planted by gardening. In 2009, the area for growing peppers in Tunisia is approximate 18900 ha and production reached 296000 tones corresponding to a yield of 16 t / ha. This production allows the country to rank second place in the African export after Morocco and the fourth in the world after Spain, Hungary and Germany. However in the harvest season, peppers are 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).

Hence the present study was initiated to evaluate the effect of NaCl treatment in five accessions of chili pepper on growth, chlorophyll content, fluorescence and proline synthesis in order to better understand their differences on salt stress tolerance and select tolerant accession.

MATERIALS AND METHODS

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 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 continues. 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 12g/l). During culture, plants were not fertilized but were processed by Talastar (80cc/hl) preventively and curatively against aphids using a Knapsack sprayer. Salinity stress effect was studied by measuring root length, fresh and dry weights of roots, number of leaves, leaf area, chlorophyll content (a and b), quantum yield (Fv/Fm) and proline amount in leaves. Dry weight was determined after drying into oven at 80°C for 48 h. Leaf area was measured by planimeter (Area Meter 3100). Chlorophyll (a and b) content was determined by Arnon method (1949) at 663 nm and 645 nm according to the following equations:

$$\text{Chl a (mg/gFW)} = 12{,}7\ (\text{OD } 663) - 2{,}63\ (\text{OD } 645)$$

$$\text{Chl b (mg/gFW)} = 22{,}9\ (\text{OD } 645) - 4{,}86\ (\text{OD } 663)$$

Proline content is estimated by Bates et *al.* (1973) method at 520 nm by UV spectrophotometer. Ratio (Fv/Fm) was measured using portable fluometer (FI 1500) when leaves were dark-adapted for 30mn, then dark fluorescence (F0), maximal fluorescence (Fm) and photochemical yield (Fv/Fm) were recorded (Fv=Fm-F0).

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 ($P < 0{,}05$).

RESULTS

Root length

In control, root length varied from 25 cm (Tb cv) to 30.3 cm (Sj cv). The addition of sodium chloride in water reduced root length in all cultivars (Table 1). This reduction becomes more pronounced at highest salt concentration. Thus, at 12 g/l, the root is shorted by 72, 78, 85, 88 and 92 % respectively for Kb, Aw, Sm, Tb and Sj cv.

Roots fresh and dry weights

According to Figure 1, in control plant, pepper root system weights 19.6 g (Aw cv) to 25.9 g (Sj cv). The presence of NaCl is accompanied with a significant reduction in root fresh weight in all cultivars as well as NaCl concentration is increasing. At the highest concentration (12 g/l), the fresh weight decreased till 60% in Aw cv to 82 % in Sj cv. The same decrease is observed for dry weight of roots (Figure 2) where the decrease compared to control was 40, 46, 67, 74 and 85 % respectively for Aw, Kb, Sm, Tb and Sj cv. This impact on roots is more illustrated in Figure 3.

Number of leaves

Number of leaves is significantly affected by salt stress ($P<0.05$); it declines in all cultivars by increasing NaCl concentration (Table 2). At the highest level, pepper plants don't produce more than 3.5 (Tb cv) to 9.15 (Kb cv) leaves; it is a respective decrease of 86 and 69% compared to control.

Leaf area

Salt stress affects negatively leaf surface in plants of pepper of all cultivars in study. Indeed, in control, leaf area ranged from 10.92 cm² for Sj cv to 21.5 cm² for Tb cv. By addition salt (2 g/l), leaf area decreased to 9.55 cm² and 19.14 cm² respectively in Sj and Tb cv (Table 3). This drop increases gradually as one increases the concentrations of NaCl in water. At the highest concentration, leaf area did not exceed 1.01 cm² for Sj cv corresponding to a significant decrease of 91%.

Chlorophyll content

Leaves of control plants contain about 1.44 to 3.65 mg/gFW for chlorophyll a (Table 4) and about 0.89 to 1.84 mg/gFW for chlorophyll b (Ttable 5) respectively for Aw and Sj cultivars. Among the five cultivars of chili pepper in study, increase of NaCl concentration is accompanied by a decline of chlorophyll synthesis. Thus, at 12g/leaves contains only 0.11 mg/gFW of chlorophyll a (Sj cv) and 0.07 mg/gFW of chlorophyll b (Aw cv) corresponding to a respective decrease of 97 and 85%. This very low content results in chlorosis (Figure 4).

Chlorophyll fluorescence (photochemical efficiency)

Salt application affected negatively maximal efficiency of PSII photochemistry (Fv/Fm) measured in the dark adapted leaves of the five cultivars studied. Thus, in absence of NaCl, (Fv/Fm) ratio was in the range of 0.760 to 0.807 respectively for Sj cultivar and Aw cv (Table 6). NaCl addition (2 g/l) declines of about 15% (Sj cv) occurred for plants irrigated with distilled water. This decline was increased by 26% (Tb cv) at the highest NaCl concentration when results showed that Kb cv exhibit the highest quantum yield (0.635) differing with those of Sj cv (0.603).

Proline content

According obtained results in Table 6, leaf proline in all cultivars increase significantly ($p<0.001$) with the increase of salt concentration in irrigation water. This increase was more at 12 g/l NaCl treatment when the five cultivars displayed very high values: 0.094 (Tb cv), 0.096 (Sj cv), 0.206 (Aw cv), 0.214 (Sm cv) and 0.415 mg/gFW (Kb cv) corresponding respectively to 3.6, 2.1, 6, 4 and 7.15 times to the level found in controlled plants.

Table 1. Root length (cm) 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	25.00^{a}	21.70^{ab}	19.30^{bc}	13.40c	7.00^{d}	5.00^{de}	3.10^{f}
Somaa	28.10^{a}	24.00^{b}	20.10^{bc}	17.50^{c}	10.25^{d}	6.00^{e}	4.20^{f}
Korba	27.30^{a}	25.40^{b}	22.80^{c}	19.30^{cd}	15.20^{e}	10.50^{fg}	7.70^{g}
Awlad haffouzz	26.20^{a}	23.10^{b}	13.00^{c}	18.70^{d}	12.20^{e}	9.30^{f}	5.80^{g}
Souk jedid	30.30^{a}	28.10^{ab}	21.20^{c}	15.30^{d}	10.80^{e}	7.00^{f}	2.50^{g}

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

Table 2. Leaves number 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	24.66[a]	20.33[b]	18.66[c]	15.55[cd]	12.87[de]	8.78[e]	3.50[f]
Somaa	33.16[a]	30.26[b]	23.16[c]	17.11[d]	14.13[d]	9.16[e]	5.30[f]
Korba	29.66[a]	21.66[b]	18.16[c]	16.33[cd]	14.83[d]	11.01[e]	9.16[f]
Awlad haffouzz	31.66[a]	25. 01[b]	20.13[c]	14.82[de]	11.66[ef]	9.13[f]	4.11[g]
Souk jedid	40.10[a]	34.33[b]	25.50[c]	17.33[d]	13.16[e]	7.25[f]	5.13[g]

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

Table 3. Leaf area (cm^2) 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	21.50[a]	19. 14[b]	14.24[c]	12.87[c]	9.78[d]	7.78[e]	2.50[f]
Somaa	15.83[a]	13.02[ab]	11.89[b]	9.39[b]	7.85[c]	4.49[d]	2.50[e]
Korba	18.30[a]	15.60[b]	12. 67[c]	10.58[d]	9.50[d]	5.96[e]	5.01[f]
Awlad haffouzz	20.26[a]	17.60[b]	15.20[bc]	8.53[c]	7.93[cd]	6.34[d]	5.39[e]
Souk jedid	10.92[a]	9.55[ab]	7.18[b]	5.79[c]	4.46[d]	3.11[de]	1.01[e]

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

Table 4. Chlorophyll a content (mg/gFW) 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	3.34[a]	2.68[b]	2.18[bc]	1.83[c]	1.59[c]	0.25[d]	0.14[e]
Somaa	3.37[a]	3.14[b]	2.65[c]	1.26[d]	0.79[e]	0.45[f]	0.23[g]
Korba	2.86[a]	1.31[b]	1.05[c]	0.87[d]	0.61[ef]	0.52[f]	0.42[f]
Awlad haffouzz	1.44[a]	1.35[b]	1.23[bc]	1.18[c]	0.52[d]	0.33[e]	0.22[f]
Souk jedid	3.65[a]	3.50[bc]	3.12[c]	2.74[d]	1.64[e]	0.58[f]	0.11[g]

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

Table 5. Chlorophyll b content (mg/gFW) 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	1.76[a]	1.64[ab]	1.40[b]	0.98[c]	0.72[d]	0.41[e]	0.21[f]
Somaa	1.03[a]	0.86[b]	0.61[c]	0.54[c]	0.35[d]	0.21[e]	0.17[f]
Korba	1.77[a]	1.57[b]	1.25[c]	0.80[d]	0.53[e]	0.32[f]	0.21[g]
Awlad haffouzz	0.89[a]	0.81[ab]	0.72[b]	0.43[c]	0.22[d]	0.13[e]	0.07[f]
Souk jedid	1.84[a]	1.61[b]	1.31[c]	0.85[d]	0.61[e]	0.31[f]	0.13[g]

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

Table 6. Effect of NaCl (0, 2, 4, 6, 8, 10 and 12 g/l) on PS-II efficiency (Fv/Fm) 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	0.775	0.712	0.689	0.671	0.656	0.641	0.573
Somaa	0.785	0.687	0.674	0.669	0.653	0.631	0.591
Korba	0.792	0.751	0.723	0.711	0.671	0.657	0.635
Awlad haffouzz	0.807	0.747	0.714	0.698	0.681	0.652	0.621
Souk jedid	0.760	0.746	0.708	0.684	0.665	0.621	0.603

Table 7. Effect of NaCl (0, 2, 4, 6, 8, 10 and 12 g/l) on leaf proline content (mg/gFW) in 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	0.026^{f}	0.031^{e}	0.051^{d}	0.067^{cd}	0.073^{bc}	0.082^{b}	0.094^{a}
Somaa	0.053^{g}	0.103^{f}	0.127^{e}	0.169^{de}	0.193^{c}	0.207^{b}	0.214^{a}
Korba	$0.\ 058^{g}$	0.121^{f}	0.178^{e}	0.205^{d}	0.351^{cd}	0.385^{b}	0.415^{a}
Awlad haffouzz	0.034^{g}	0.057^{ef}	0.081^{e}	0.115^{d}	0.134^{c}	0.187^{b}	0.206^{a}
Souk jedid	0.046^{e}	0.053^{d}	0.068^{c}	0.073^{c}	0.081^{b}	0.091^{b}	0.096^{a}

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

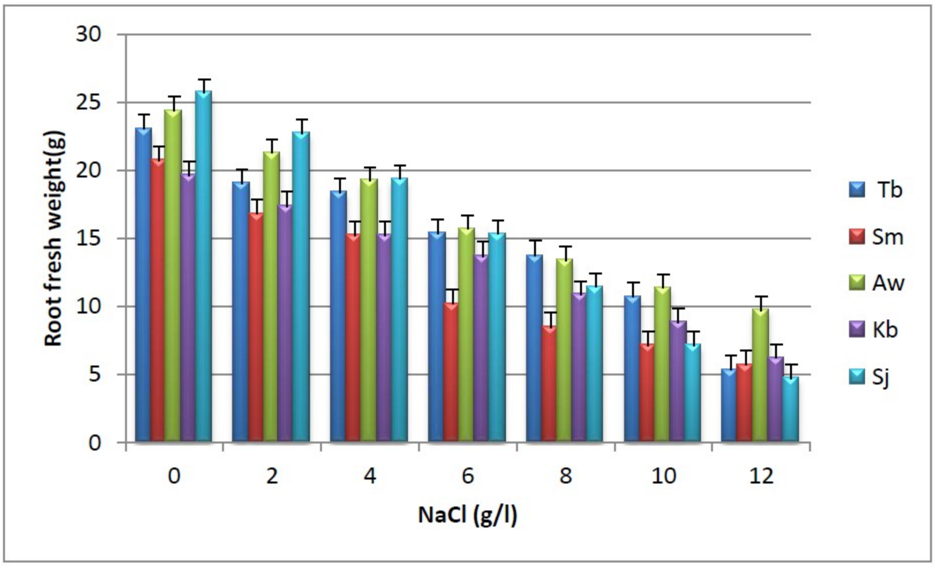

Figure 1. Roots fresh weight (g) of five Tunisian chili pepper cultivars watered during 60 days with NaCl (0, 2, 4, 6, 8, 10 and 12 g/l)

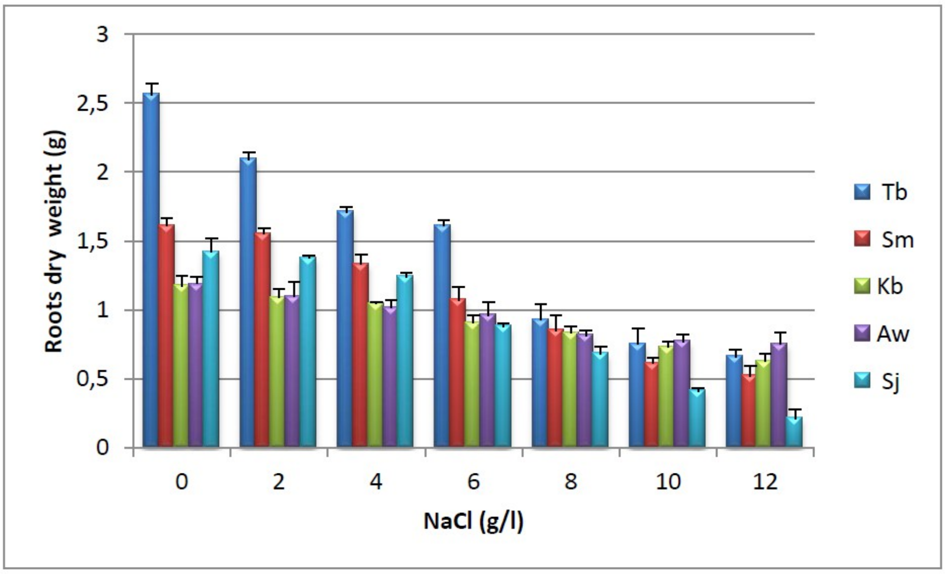

Figure 2. Roots dry weight (g) of five Tunisian chili pepper cultivars watered during 60 days with NaCl (0, 2, 4, 6, 8, 10 and 12 g/l)

Figure 3. Effect of NaCl on roots of Tunisian chili pepper, Kb cv: reduction in length and biomass

Figure 4. Effect of NaCl on leaves of Tunisian chili pepper, Aw cv: reduction in leaf area and appearance of chlorosis

DISCUSSION

Salinity treatment during two months using NaCl (0, 2, 4, 6, 8, 10 and 12 g/l) in five Tunisian cultivars of chili pepper (Tb, Kb, Sm, Aw and Sj) had significant effect on the vegetative growth where the highest concentration of NaCl resulted in severe reduce on the corresponding parameters in study. Similar results were reported in potato (Kerkeni, 2002) for root length, in canola (Byubordi, 2010) for leaf area and in groundnut (Mensah et *al.* 2006) for number of leaves. Al Thabet et *al.* (2004) working on sprout, Singh et al. (2007) on peanut, Farhoudi and Tafti (2011) in soybean and Keshavarzi (2011) on savory found also that under salinity stress plant growth is inhibited due to the low water potential, ion toxicity and imbalance excreted by salinity (Greenway and Munns, 1980).

As consequence, a decrease in photosynthetic pigment content was observed with increasing salt concentration in all cultivars. At 12 g/l, we noted a reduce of 93% and 97% respectively in chlorophyll b and chlorophyll a content compared to control (Sj cv). This result is in agreement with those of Tewari and Singh (1991) in lentil, Beinsan et *al.* (2003) in bean, Iqbal et *al.* (2006) in wheat, Chen and Yu (2007) in *Glycine max* seedlings, Moussa Helal (2006) in maize, Molazem et *al.* (2010) in corn, Malik et *al.* (2010) in cucumber, El Iklik et *al.* (2011) in tomato and Rahdari (2012) in Purslane,

Parida and Das (2005) suggested that decrease in chlorophyll content in response to salt stress is a general phenomenon which led to disordering synthesizing chlorophyll and appearing chlorosis in plant. According to Rao and Rao (1981), NaCl stress decreased total chlorophyll content of the plant by increasing the activity of the chlorophyll degrading enzyme: cholorophyllase, inducing the destruction of the chloroplast structure (Blumenthal-Goldschmidt and Poljakoff-Mayber, 1968) and the instability of pigment protein complexes (Dubey, 1997). In another study, Ali et *al.* (2004) attributed this reduction in chlorophyll concentration by NaCl to the inhibitory effect of the accumulated ions of various salts on the biosynthesis of the different chlorophyll fractions.

Working on potato and gladiolus leaves, Mehouachi (1993) and Bettaieb *et al*. (2008) respectively affirmed that the decrease of chlorophyll content is due to a decrease of photosynthetic activity. Based on this last hypothesis, we choose to study photosynthetic activity through the measurement of chlorophyll fluorescence estimated by the ratio (Fv/Fm) which is considered as an indicator of the efficiency of Photosystem II (Maxwell and Jonhson*,* 2000). Baker (1991) confirmed that photosystem II (PSII) plays a key role in the response of leaf photosynthesis to environmental perturbation.

The maximum photochemical efficiency (Fv/Fm) indicates the capacity of absorption of excitation energy by leaves and it is usually decreasing thereafter as a consequence of leaf senescence and decrease of photosynthetic assimilation (Barbagallo *et al.* 2003). In our study, the ratio (Fv/Fm) showed parallel trend with chlorophyll a and chlorophyll b content. Increasing salinity level is accompanied by a significant reduce in Fv/Fm ratio below 0.8.: a normal value reported for most the plants and showing the health and vigor of the plant (Bjorkman and Demmig-Adams, 1995) while value below 0.8 indicates that plants are experiencing stress conditions (Schreiber *et al*. 1995). DeEll et *al.* (1999) confirmed this and reported that the ratio for a normally functioning leaf varies between 0.75 and 0.85 and a decline in this ratio is indicative of photoinhibitory damage. Our results can be related to some earlier findings in which it has been observed that salt stress has significant effect on PSII photochemical activity, e.g., in strawberry (Rahimi and Biglarifard, 2011) and maize (Suriyan and Chalermpol, 2009). However, there are some reports that suggest that salt stress may not causes changes in Fv/Fm ratio in wheat (Akram et *al.* 2006) and pepper (Ibn Maaouia Houimli et *al.* 2008). The reduced quantum yield as obtained in our study may result from a structural impact on PS II (Everard et *al*.1994; El-Shintinawy, 2000;) induced by salinity which affects reaction centers of PSII either directly (Masojidek and Hall, 1992) or via an accelerated senescence (Hasson and Paljokoff-Mayber, 1981; Kaya et *al.* 2002;).

In fact, chlorophyll fluorescence is related to only Chlorophyll a, but in our research, the decrease in Fv/Fm ratio throughout the experiment in all cultivars in study coincided also with a decrease in Chlorophyll b content under salt-stress conditions. The result agrees with those of Lutts et *al.* (1996) in rice and Rahimi and Ali (2011) in strawberry. Kocheva et *al.* (2004) suggested that decrease in Chlrophyll b content in leaves might lead to structural/conformational changes in the PS-II antennae as chlorophyll b is mainly associated with PS-II antenna. Hall and Rao (1999) reported that analysis of fluorescence characteristics such as quantum yield reflects the properties of the chlorophyll molecules and their interaction with the external environment and also with associated physiological processes. It has been used since long by crop physiologists to evaluate response of various crop species to determine the influences of abiotic stresses at various stages of plant growth to have a quantitative assessment to be used in ranking plant species for their tolerance and/or sensitivity towards environmental stresses (Maxwell and Johnson, 2000). Thus, the varieties studied can be ranked in order of decreasing tolerance as follows: Kb, Aw, Sm, Tb and Sj.

As a response to salt stress, leaves of pepper plants accumulate proline, a common phenomena observed in all organisms ranging from bacteria to higher plants to survive both water deficit and high salinity (Ahmad and Jhon, 2005). Thus, proline content increased significantly in the leaves of all the genotypes of the chili pepper in study as the salt concentration increased. Our result is supporting findings in *Triglochin bulbosa* (Naidoo and Naidoo, 2001), barley (Sadeghi, 2009), mulberry (Harinasut et *al.* 2000 ; Kumar *et al*., 2003), mangrove (Parida *et al*. 2002), maize (Cicek and Cakirlar, 2002), sorghum (De Lacerda *et al*. 2003), *Phaseolus aureus* (Misra and Gupta, 2005), *Morus alba* (Ahmad et *al.* 2007), *Sesamum indicum* (Koca et *al.* 2007), cotton (Desingh et *al.* 2007), wheat (Khan et *al.* 2009; Shafi et *al.* 2011), *Paulownia imperialis* (Astorga and Melendez, 2010), *Atriplex* (Ouiza et *al.* 2010), rapessed (Farhoudi, 2011) and Chookhampaeng (2011) in pepper, where salt stress resulted in extensive proline accumulation.

Proline is an organic solute known to be involved in osmoregulation which reduces the cell osmotic potential to a level to provide high turgor potential for maintaining growth (De Lacerda *et al*. 2005; Ashraf and Harris, 2004; Chaum *et al*. 2004). Also, apart protection of macromolecules from denaturation and carbon and nitrogen reserve for stress relief, proline has several other functions during stress: e.g. osmoprotection (Kavi Kishor et *al.* 2005), free radical scavenger and antioxidant activity (Sharma and Dietz, 2006). It is also compatible with other cytoplasmic components and can be easily converted to glutamate that takes part in the synthesis of other essential amino acids (Rains, 1981). Recently, Matysik *et al*. (2002) reported that proline accumulation protects plants against free radical induced damage by quenching of singlet oxygen. In many species like Alfalafa (Fougere *et al*. 1991; Petrusa and Winicov, 1997), rice (Lutts et al. 1996) and mulberry (Ramanjula and Sudhakar, 2001), a positive correlation between magnitude of free proline accumulation and salt tolerance has been suggested as an index for determining salt tolerance potentials between cultivars. In our study, a similar behavior in the seedling of chili pepper was also observed: proline accumulation in the salt tolerant pepper cv. Korba was significantly higher than that in the salt sensitive one (cv. Souk jedid).

CONCLUSION

In conclusion, our study showed that salt stress at higher concentration, especially 12g/l NaCl is harmful to vegetative growth of the five cultivars of pepper since the root length, fresh and dry weight of roots, number of leaves and leaf area decreased significantly. In addition to this morphological features, photosynthetic parameters (quantum yield and chlorophyll content) were adversely affected while proline amount in leaves was activated and increased by increasing salinity level. Awlad haffouzz and Korba cultivars of chili pepper are classified as salt tolerant whereas cv Souk Jedid as susceptible based on various parameters studied. It's thus apparent from the present investigation that salinity stress tolerance in pepper is attributed to the biosynthesis of proline which makes plants able to continue growth even under higher salinity level. However, literature affirmed that there is no single parameter could be suggested as sole factor responsible for salinity stress tolerance; it is the combination of many characters. Thus, in future, a mineral analysis (Na^+, Cl^-, K^+ and Ca^{2+}) and measurement of soluble protein content and percent soluble sugar are worthy to be studied.

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