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Effect of salinity on biochimical and anatomical characteristics of sweet pepper (*Capsicum annuum* L.)

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Abstract

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 morpho- physiological and anatomical parameters in two sweet pepper (*Capsicum annuum* L.) varieties: *Super marconi* and *Marconi*. So, an experiment of eight months was carried out underStandard room at and stress is induced by NaCl at 4 concentrations (0, 25, 50, and 150mMol/l). Results showed that increasing salinity stress, for all cultivars, decreased stems (length, fresh and dry weights) and leaves (number and area).As the salinity increased, proline concentration and leaf total soluble sugars also increased significantly compared with the control. The results showed that the accumulation of proline and soluble sugars are good indicators of salinity tolerance. Results also suggest that the plant resists against salinity through osmotic adjustment and ion absorption and sharing within its cells. This process is essential for the survival of plants in salineMicroscopic study demonstrated that .salinity stress significantly decreased cortex thickness because of salinity stress while xylem grown under salinity stress especially high level of salinity .Additionally. There were changed in xylem creation and construction in stressed plants. It is concluded that the variety *Super Marconi* (Sp) is more tolerant to salinity compared to the variety *Marconi* (M). Hence, they have a significant role to play in agriculture, food, and economy.

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Introduction

Soil salinization is one of the major factors of soil worsening. It has reached 19.5 % of the watered land and 2.1 % of the dry-land farming existing on the globe (FAO, 2000). Salinity effects are more easily seen in arid and semiarid areas where 25 % of the watered land is affected by salts. Considering that 52 % of northeast Brazil is within semiarid tropics (Lira *et al.,* 1982).

Irrigation has played an extremely important role in terms of food production worldwide by increasing crop produce and quality. However, to many crops-watering can cause soil worsening mostly by increasing the soil salinity (Trout, 2000). In fact, high levels of salinity have been reported as causing of the loss of 250,000 to 500,000 ha of irrigated land every year. The problem happens mostly in arid and semiarid zones where a total of 100 to 110 million ha are reported as having problems related to salinization which could make them unusable for farming- based purposes (FAO, 2002). The problem is greatest in these areas due to the high level of evapotranspiration which increases salts, introduced via the rising with water (secondary salinization) or as part of the original chemical composition of the soil. 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, 2002). Salinity inhibition of plant growth is the result of osmotic and ionic effects and the different plant species have developed different mechanisms to cope with these effects (Munns, 2002). The osmotic adjustment, reduction of cellular osmotic potential by net solute accumulation, has been believed an important method to salt and drought tolerance in plants. This reduction in osmotic potential in salt stressed plants can be a result of inorganic ion (Na^+, Cl^-, and K^+) and compatible organic solute (soluble carbohydrates, amino acids, proline, roots and leaves contribute to the maintenance of water uptake and cell turgor, allowing physiological processes, such as stomatal opening, photosynthesis, and cell expansion (Serraj and Sinclair, 2002). In addition to their role in cell water relations, organic solute accumulation may also help towards the maintenance of ionic homeostasis and of the C/N ratio, removal of free radicals,and stabilization of macromolecules and organelles, such as proteins, protein complexes and membranes (Bohnert and Shen, 1999, Bray *et al.*, 2000). These solutes may also help towards the control of pH in the cytosol and detoxification of excess NH_4^+ (Gilbert *et al.*, 1998) Although the relationship between osmoregulation and salt tolerance is not clear, there is evidence that the osmotic adjustment appears, at least partially, to be involved in the salt tolerance of certain plant genotypes (Richardson and Mccree, 1985). Therefore, the objective of this investigation was to evaluate the effects of salt stress on growth, water relations and anatomical of different sweet pepper genotypes, commonly grown in northeast Algeria, and at the same time try to correlate these effects with changes in ionic and organic solute accumulation, with a view to a better understanding of the mechanisms of salt tolerance in these genotypes.

Materials and methods

Study area

This study is carried out on two varieties of sweet pepper (*Capsicum annuum* L.) varieties: *Marconi* (M) / the origin: china) and *Super marconi* (Sp) / the origin: china). The experiment was conducted in a culture room at the Abdelhafid Boussouf University Center (Wilaya de Mila, North-East Algeria.), During the year 2016/2017.

The experimental design

The test is conducted according to an experimental device in completely randomized blocks (BAC), comprising 4 blocks (repetitions) comprising 4 blocks (repetitions) of each variety of sweet pepper (*Marconi* and *Super marconi*). Fo each repetition two plants.

Saline solutions

The plants of the two varieties are subjected to the different treatments of NaCl:

So :NaCl (0 m Mol / L (control).

S1: NaCl(25 m Mol / L.)

S2 :NaCl (50 m Mol / L.)
S3 :NaCl (150 m Mol / L.)

Measured parameters

Number of leaves NF**:**

We calculated the number of leaves from the beginnin g of the growth ofthe first two papers until the rotting of the plants for each of the two varieties separately, a nd the averages were calculated.

Measuring leaf area SF (cm2): The leaves area was cal culated during the seedling growth stage of both culti vars during theremoval of seedlings for study and the averages were calculated.

Fresh weight (PF / g): We calculated wet weight durin g the seedling growth stage and afterremoval of the se edlings by a sensitive balance.

Dry Weight Measurement (PS / g):

After gaining swelling weight, we wrap the balanced seedlings individually in the aluminum foil and then put them in the incubator for drying under 80 ° C for48 hours to dry completely.

Dry seedlings and we make the color we get dry weight.

Leaf proline estimation

Extraction and estimation of proline were conducted according to the procedures described by Bates *etal.* (1973).

Leaf total soluble sugars estimation

The total soluble sugars were measured using the phenol-sulfuric acid. To measure leaves' total soluble sugars from the solution of5% $ZnSO_4$, 0.3 N Ba $(OH)_2$, 5% (v/v) phenol solution and sulfuric acid was used based on Stewart method. Finally, absorption was read at 485 nm by spectrophotometry (Stewart, 1989).

Anatomical study

According to Saadoun (2005) a realization of the cross sections of the stems of the two varieties of pepper (*Capsicum annuum* L.) with a razor blade.

This method is based on the use of certain dyes: methyl green, Congo red or carmino-green Mirande, or methyl green and carmine alum. It makes it possible to exactly color the cell walls according to their chemical composition. The staining principle of the raised sections is based on the following steps:

First, Making cuts by hand using a simple razor, the organ is delivered in slices of a few micrometers thick so that the light rays can cross it.

Second, The sections are immersed, before staining, in a 12% sodium hypochlorite bath for a quarter of an hour to evacuate the contents of the cells.

Third, Rinsing with distilled water.

Fourth, Immersion in 2% acetic acid for five minutes to eventually fix the dyes on the cells and remove all traces of sodium hypochlorite.

Fifth, Rinse briefly with distilled water.

Sixth, Immersion in the double dye (methyl green / Congo red) for 10 to 15 minutes.

Finally, Rinsing with distilled water, then mounting the finest cuts between blade and coverslip. Observation under a microscope equipped with a digital camera (Motic).

Statistical analysis

The Excel Stat (2017) software was used to perform all variance analyzes as well as the Newman keuils test ($\alpha = 0.05$) to compare the averages between the control and processed samples for each parameter analyzed.

Results and discussion

Leaf number

The different saline concentrations resulted in a significant decrease in the number of leaves for both cultivars. There was also a difference in the average number of leaves between the two cultivars, especially in the concentrations (25-50 mM / L).

Table 1. Analysis of variance on sweet pepper leaf number affected by salt stress.

Source	DDL	Sum of Squares	Averag Squares	F	Pr> F
Model	7	151,889	21,698	6,873	0,000
Error	24	75,766	3,157		
Total corrected	31	227,654			

The highest mean number of leaves (12.25) 11.5) in Super marconi in the control plants. In the concentration (50 mM), Super marconi surpasses the average number of leaves (9.43) on Marconi, which has an average of 6.5 sheets.(50-150 mmol / L). The average number of leaves ranged between(7.25-7.81)inthe concentration (50 mM / L) and between(6.06-6.26) in the concentration Saline (150 mM / L).

Table 2. Analysis of variance on sweet pepper leaf area affected by salt stress.

Source	DDL	Sum of Squares	Averag Squares	F	Pr> F
Model	7	148,969	21,281	17,462	< 0,0001
Error	24	29,250	1,219		
Total corrected	31	178,219			

The analysis of variance showed a significant effect (p <0.01) on all traits due to salinity stress.

The results are shown in Table 1.The New man Keuilstest confirms these differences between cultured plants in the different saline media and between the two cultivars. *Super marconi* recorded the highest values of mean number of leaves in all saline = concentrations compared to *Marconi*.

Which confirms the results of Unlükara *et al.* (2008). Inhibition of the formation of leaf primordia under salinity stress could be the probable reason for low leaf number. Iyenger *et al.* (1977) reported that saline irrigation water reduced the number of leaves.

Table 3. Analysis of variance on sweet pepper dry weight affected by salt stress.

Source	DDL	Sum of Squares	Averag Squares	F	Pr> F
Model	7	0,180	0,026	657,223	<0,0001
Error	24	0,001	0,000		
Total corrected	31	0,181			

Leafs area

According to the results shown in Fig. 2 the effect of salt stress negatively affects the paper area of the studied species. The highest concentration of paper area in the control plants of Super marconi (9.75 cm2) and average values (6.75-8 cm2) in the concentrations (25-50 mmol / L) The lowest value of paper area (4 cm2) was recorded in the high salt concentration (150 mM / L).(S = 0) and saline concentration (1S = 25 mM / L) and the paper area (6.5 cm2) and the mean values in the concentration (S2 = 50 mM / l)) And the lowest (2.25 cm2) in the high salt concentration (S3 = 150 mmol / L).

The two varieties generally showed a different response in the paper area in all saline treatments, where the *Super marconi* exceeded *Marconi*. The ANOVA study showed that the paper area recorded very significant differences as shown in Table 2.

The New man Keuils test confirms these differences between plants grown in different saline cultures and between the two cultivars. *Super marconi* recorded the highest values of the leaf evolution index in all saline concentrations compared to *Marconi*. Sagi *et al.* (1997) also found the adverse effects ofsalinity stress on leaf area. It has been reported that, decline in plant biomass may be due to excessive accumulation of NaCl in chloroplasts of sweet pepper, which affects growth rate, and is often associated with a decrease in the electron transport activities of photosynthesis (Kirst, 1989) and inhibition of PSII activity (Kao *et al.*, 2003).

Table 3. Analysis of variance on sweet pepper dry weight affected by salt stress.

Source	DDL	Sum of Squares	Averag Squares	F	Pr> F
Model	7	0,180	0,026	657,223	<0,0001
Error	24	0,001	0,000		
Total corrected	31	0,181			

In general, salinity reduces leaf number leaf area, shoot and root dry weight, leading to low yields (Essa, 2002; Hamdy *et al.*, 1993; Li *et al.*, 2006; Sharifi *et al.*, 2007).Our results are inconsistence with Gosset and Lucas (1996) who reported that NaCl highly reduced total leaf area; it seems that plant height was more sensitive to salinity than leaf number or leaf area expansion.

Dry weigth and fresh weigths

Results shown in Fig. 3 show salinity has a negative effect that resulted in a clear decrease in average dry weight by increasing saline concentrations. The highest mean dry weight (0.28 g) was recorded in saline concentration (S0 = 0) in control plants and (0.14 g) in saline concentration (S1 = 25 mM / L) for *Marconi* species, while lowest values were recorded for the same cultivar Saline (50-150 mM / L) ranged between 0.05 g and -0.08 g.

Table 4. Analysis of variance on sweet pepper fresh weigth affected by salt stress.

Source	DDL	Sum of Squares	Averag Squares	F	Pr> F
Model	7	7,653	1,093	7,556	< 0,0001
Error	24	3,473	0,145		
Total corrected	31	11,126			

Super marconi had the highest mean dry weight (0.23 g) in the control plants, and in the concentrations (25-50 mM / L), the mean wet weight values ranged from 0.11 g to 0.12 g while the lowest mean Wet weight (0.07 g) in high salt concentration (150 mM / L).

Salinity stress was diminished plant growth and significantly decreased total dry weight. There was downward decrease in shoot weight because of deterrent effect of salinity on plant height (Table 3). Salt stress adversely affects the growth and development of crops, and the results of our study confirm that all growth variables of sweet pepper drastically decreased with NaCl treatment.

Fresh weigth

The results shown in Fig. 4 showed that the negative salinity effect resulted in a clear decrease in average wet weight by increasing saline concentrations. The highest mean wet weight (2.16 g) was recorded in saline concentration (S0 = 0) in the control plants and (1.37 g) in the saline concentration (S1 = 25 mM / L) of the Marconi species. Saline (50-150 mM / L) ranged between (0.38 g - 0.94 g). *Super marconi* had the highest mean wet weight (1.54 g) in the control plants and in the concentrations (25-50 mM / L). The mean wet weight values ranged between (1.19 g - 1.22 g) while the lowest mean Wet weight (0.88 g) in high salt concentration (150 mM / L).

Table 5. Analysis of variance on sweet pepper proline affected by salt stress.

Analysis of the variance (Variable Pro L)					
Source	DDL	Sum of Squares	Averag Squares	F	Pr> F
Model	8	2101,371	262,671	336,591	< 0,0001
Error	24	18,729	0,780		
Total corrected	32	2120,101			

Table 6. Analysis of variance on sweet pepper soluble sugars affected by salt stress.

Analysis of the variance (Variable Sug L) :					
Source	DDL	Sum of Squares	Averag Squares	F	Pr> F
Model	8	2990,653	373,832	6219,405	< 0,0001
Error	24	1,443	0,060		
Total corrected	32	2992,096			

The ANOVA study showed that the average wet weight recorded very significant differences. As shown in Table 4 The New man Keuils test confirms these differences between cultured plants in the different saline media and between the two cultivars. Super marconi recorded the lowest values for mean wet weight in saline concentrations (0-25 mM / L) and highest values In saline concentrations (50-150 mM / L) compared to Marconi. At the lower salinities, cell expansion may have been sufficient to diluted the ion concentration within the plant; thus avoiding toxic accumulation (Munns, 2002). Plants irrigated with salinities of 3.5 dS·m−1 and greater may have had a limited water uptake due to high osmotic potential in the root zone. Salt-stressed plants tend to intrinsically save water; thus restricting growth as an adaptation to low water availability (Binzel and Reuveni, 1994).

Table 7. Pearson correlation between growth parameters affected by salt stress.

Variable	Leaf number	Leaf dry weightt	Leaf fresh weigth	Leaf area	Leaf proline	Leaf suger
Leaf number	1					
Leaf dry weigth	0,740**	1				
Leaf fresh weigth	0,585	0,766**	1			
Leaf area	0,570	0,591	0,546	1		
Leaf proline	-0,622*	-0,765**	-0,577	-0,687*	1	
Leaf suger	-0,421	-0,655*	-0,462	-0,318	0,576	1

*** Significant at the 0.05 and 0.01probbility levels, respectively.

Plants may have higher concentrations of Na^+ and Cl^- in the leaves. Both Bethke and Drew (1992) and Chartzoulakis and Klapaki (2000) reported higher Cl^- content than Na^+ in the leaves of peppers at high salinities, whereas Blom-Zandstra *et al.*,(1998) reported the reverse. Both processes which ultimately limit nutrient uptake, causing nutrient imbalances (Grattan and Grieve, 1999). Based on our results, it could be speculated that Cl^- concentration in the leaves might have been more harmful than Na+ based on the fact that peppers are incapable of Cl^- exclusion (Chartzoulakis and Klapaki, 2000) and that chloride was the most abundant ion in our saline irrigation water.

Kirnak *et al.*, (2003) found that mulched plants under water stress had significantly greater water content in leaves, shoot dry weight and stem diameters than plants grown in bare soil. Under non-saline conditions mulches have been found to significantly increase plant height and stem diameter (Locher *et al.*, 2005), shoot fresh weight (Aziz, 1994) and number of leaves (Siwek *et al.*, 1994).

Proline

Results of the measurement of the amino acid proline content, as shown in Figure (5), is the most studied amino acid among the other amino acids involved in the formation of proteins as a measure of the physiological condition of the plant (salt stress).

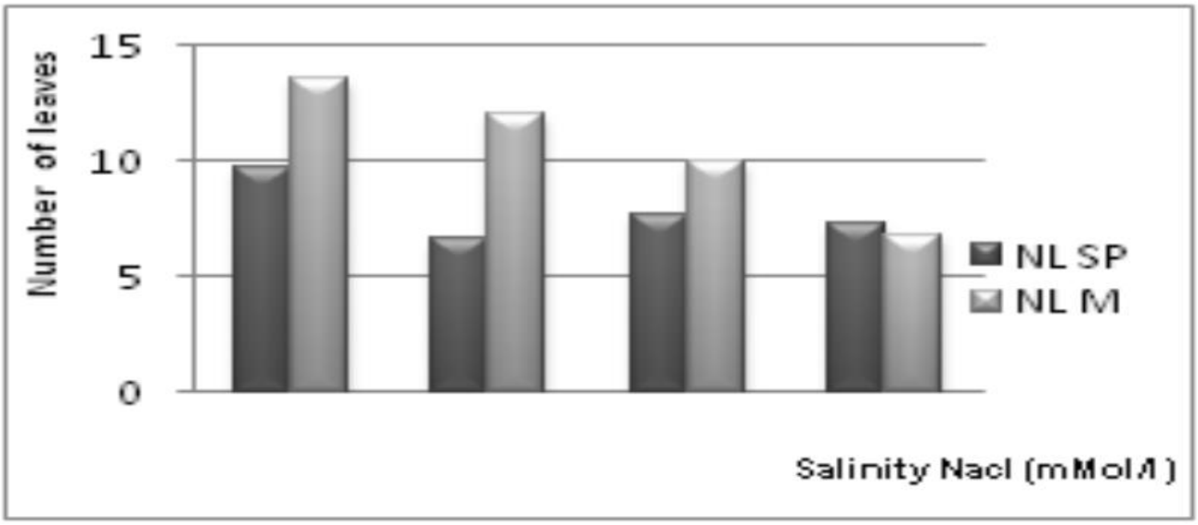

Fig. 1. Effect of NaCl on leaves numbers of two sweet pepper cultivars during 60 days.

In the normal case (S0 = 0), the ratio of the amino acid proline in the leaves of the pepper varieties was weak, ranging from (3.05-4.76 µg / 100mg / MF). While the amount of amino acid proline in the leaves increased by saline concentrations ranged between (4.89-6.06 µg / 100mg / MF) in the concentrations (25-50 mM / L) of sodium chloride salt and the maximum values were recorded in the high salt concentration Mol / l) for both studied cultivars (9.39-11.10 µg / 100mg / MF).

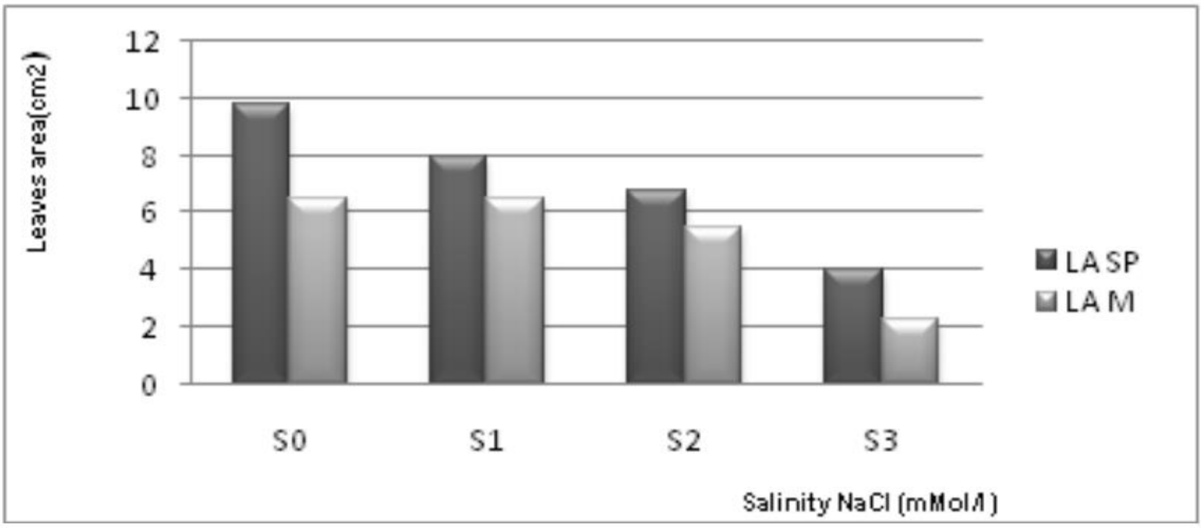

Fig. 2. Effect of NaCl on leaves area of two sweet pepper cultivars during 60 days.

The ANOVA study showed that the amino acid content of proline in the leaves of the two varieties of paprika has recorded very significant differences. As shown in Table (5). The New man Keuils test confirms these differences between plants grown in different saline cultures and between the two cultivars. Super marconi recorded the highest values of the leaf evolution index in all saline concentrations compared to Marconi.

Solubles sugars

Figure 6 shows that the content of sugars decreased significantly in non- salted plants. While the effect of salinity varied on sugar content between the two studied cultivars with the highest values of (9.82-10.15 µg / 100mg / MF) were recorded in the Super marconi cultivars (25-50-150 mM / L), while the lowest values were recorded in the Marconi cultivars in the control plants and the low salinity concentration (25 mM / L) ranged between (9.16-9.37 µg / 100mg / MF) compared with high salinity concentrations with the highest values ranging from (9.50-9.94 µg / 100mg / MF).

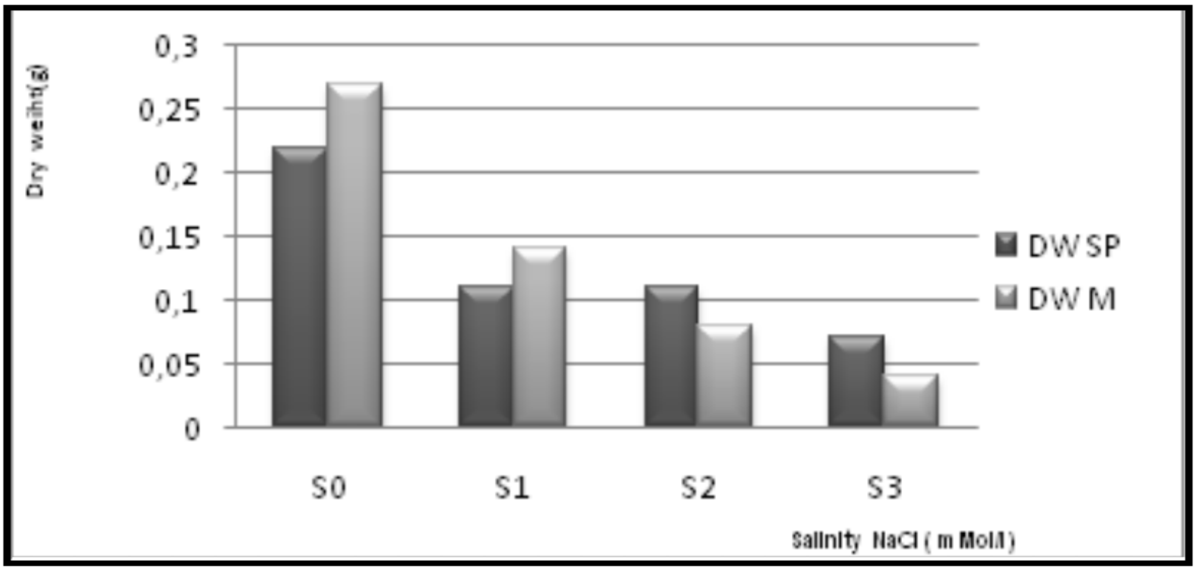

Fig. 3. Effect of NaCl on dry weigth of two sweet pepper cultivars during 60 days.

The ANOVA study showed that the sugar content of the leaves showed very significant differences. As shown in Table (6). The New man Keuils test confirms these differences between plants grown in different saline media and between the two cultivars. Super marconi recorded the highest sugar content in all saline concentrations compared to Marconi.

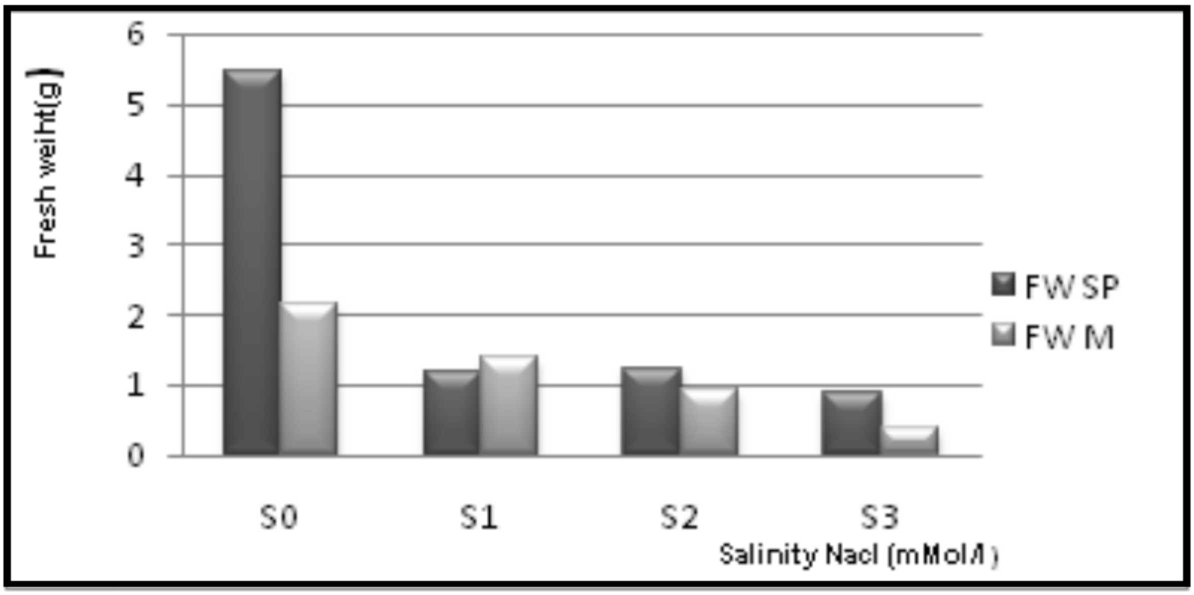

Fig. 4. Effect of NaCl on fresh weight of two sweet pepper cultivars during 60 day.

In this study, proline concentration in the leaf of *Capsicum annuum L.* was increased with increasing salinity, particularly at the highest external salt level, thus, showing the positive role of proline in the salt tolerance of this crop. Proline, as a signaling/regulatory molecule, can activate multiple responses, which are component to the process of adaptation to abiotic stresses including salt stress (Ashraf and Orooj, 2006).

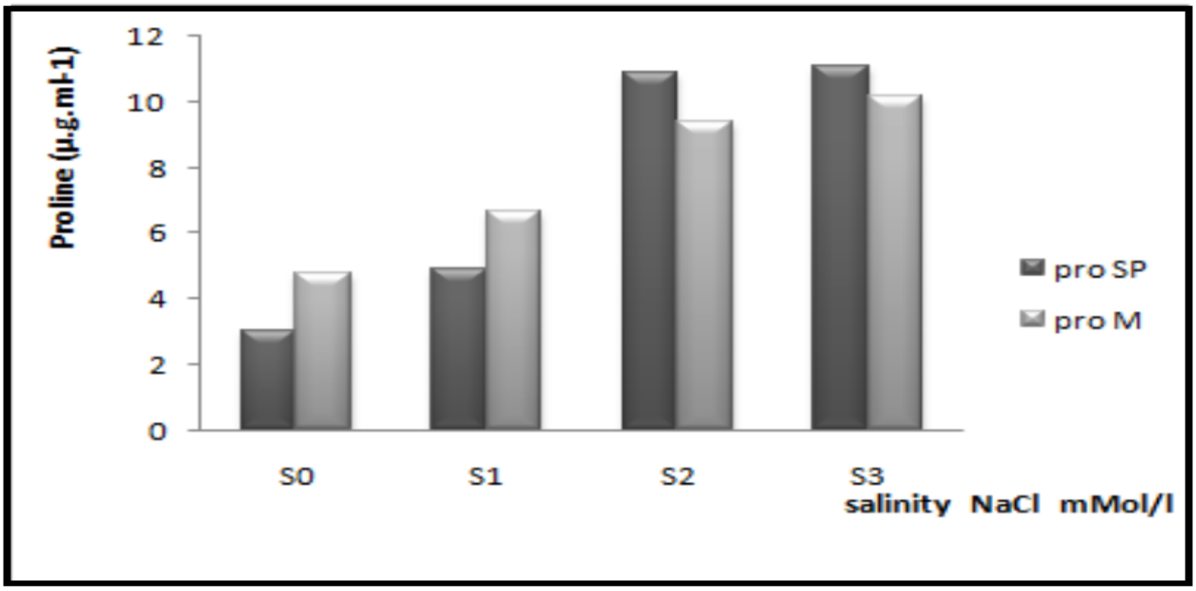

Fig. 5. Effect of NaCl on proline of two sweet pepper cultivars during 60 day.

Increase in proline under salinity has also been reported in some medicinal plants (Hajar *et al.*, 1996; Munns., 2002; Ashraf and Orooj, 2006; Abdul *et al.*, 2007). Leaf soluble sugars considerably increased in response to the increase in salinity. Accumulation of soluble carbohydrates in response to environmental stress has been widely reported despite specific reduction in net CO2 assimilation levels (Chaves *et al.*, 2003; Meloni *et al.*, 2008). Sugars, in addition to the role of regulating osmotic balance, also act as the metabolic signals in the stress conditions (Chaves *et al.*, 2003).

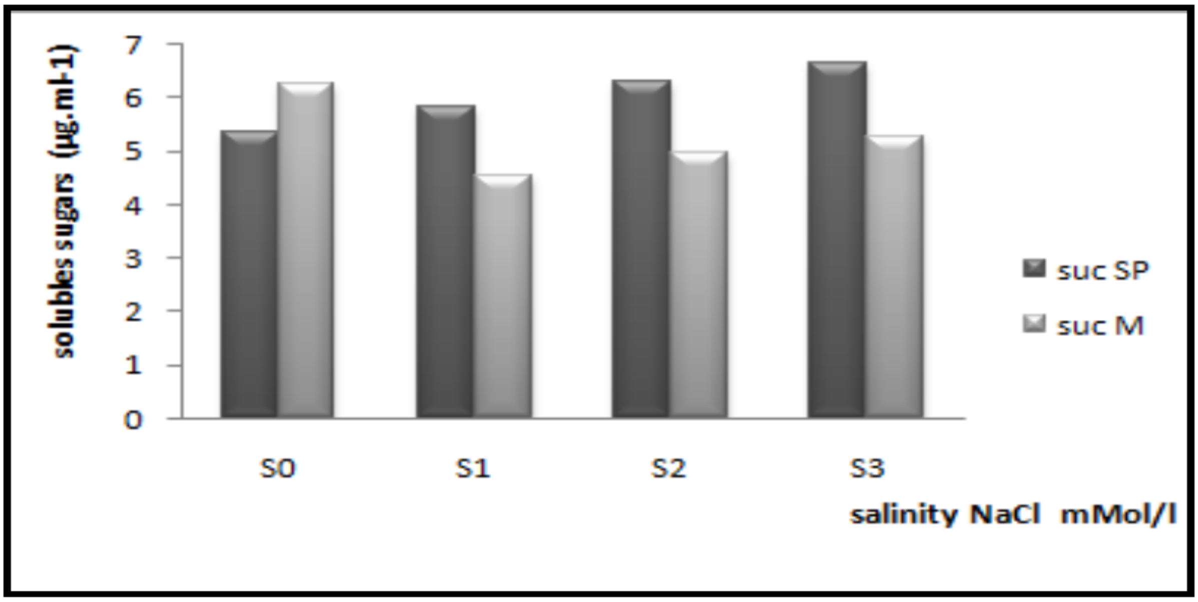

Fig. 6. Effect of NaCl on soluble sugars of two sweet pepper cultivars during 60 day.

Anatomical results

A comparison of the anatomical cuts made on the pepper stems (*Capsicum annuum* L.) of the control and stressed plants shows that the stem of the control plants consists of two zones: the bark and the central cylinder (Fig. 5). In stressed pepper plants, an increase in parenchymal cell size is observed under saline treatment of 25 mMol/l, followed by an increase in parenchyma thickness. However, under treatment of 50-150 mMol/l, cell thickness decreases. Furthermore, we observed that, in salt stressed plants, number of trichomes was increased from epidermal stem cells.

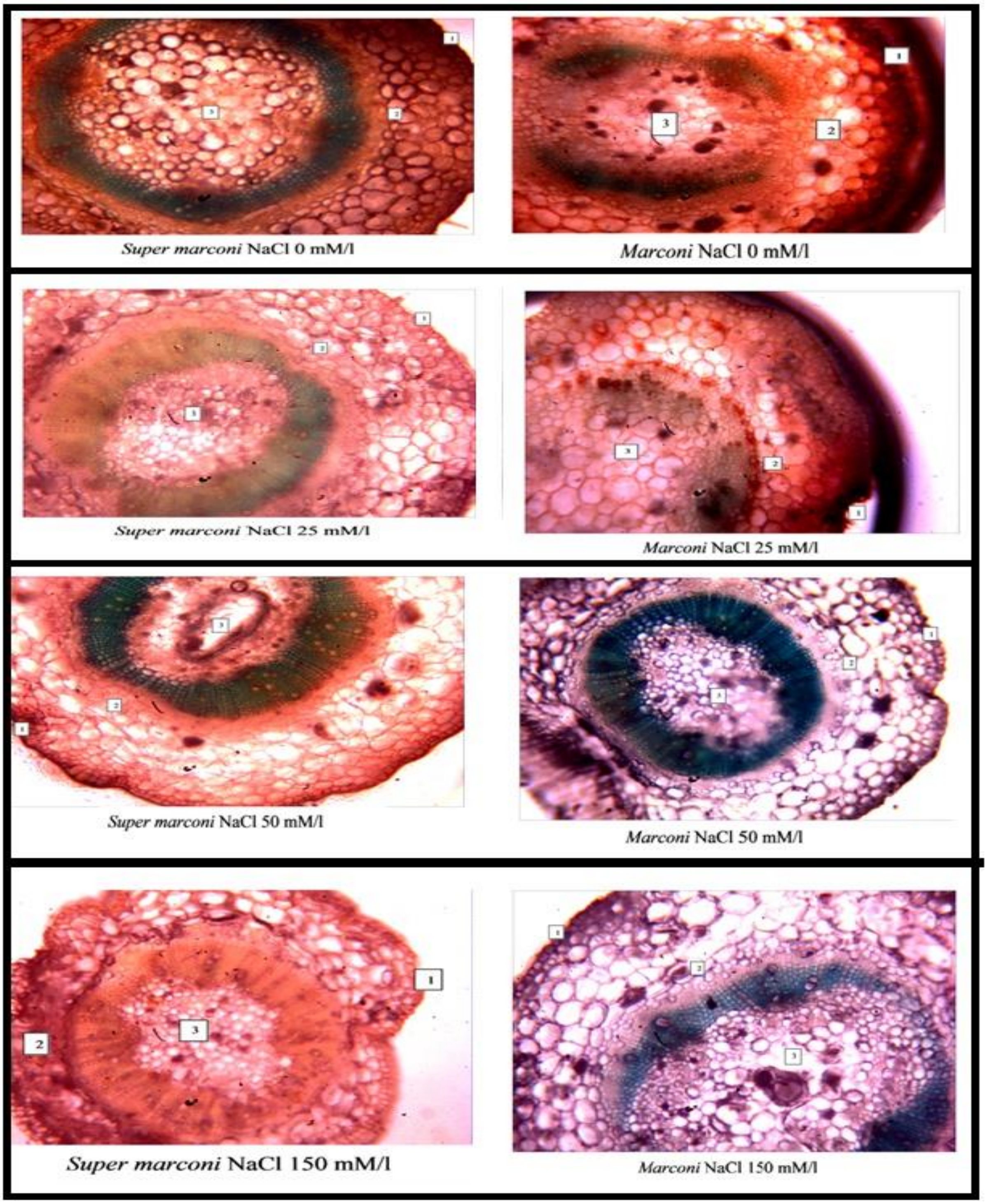

Fig. 7. Anatomical structur of sweet pepper stem under salinity stress.1 :Exodermis,2 : Endodermis,3 : central cylinder.

In the other word, increase of salinity level led to more trichomes on epidermal layer in compare with control plants (Data are not shown) (Fig. 5). There are several reports on increased trichome density under environmental stresses such as drought and salinity (Abernethy *et al.*, 1998; Aguirre-Medina *et al.*, 2002). Increase of trichome density may be a mechanism to increase of tolerance to salt stress.

It was recently suggested that leaf glandular trichomes could contribute to the high salt tolerance by the excretion of ions (Gucci *et al.*, 1997). Salinity induced structural changes in xylems in stems.

In salt stressed plants, stems vascular cell thickness was much larger than control treatment; the salinity effect was concentration dependent. Generally, plants grown in saline solution showed higher thickness in cuticle, vascular tissues and vessel than unstressed plant while cortex zone thickness was decreased (Fig. 5). when the cell is under stress and when itdifferentiates to particular specialized tissues, notably the xylem (Christensen *et al.*, 1998).

Salinity stress has been associated Witha greater deposition of lignin in vascular tissues and/caused earlier and stronger lignifications, which has been or xylem development.

In bean- root vascular tissue, NaCl suggested to be a factor that inhibits root growth and, consequently, represents an adaptation mechanism in resisting salinity-imposed stress (Cachorro *et al.*, 1993).

Conclusion

The results show differences and similarities between the two varieties during saline treatments. *Super marconi* (Sup) is more tolerant to salinity compared to *Marconi* (M).

In conclusion, this study shows that salt stress decreases sweet pepper growth and induces changes in anatomical characteristics such as increment of cut in synthesis on epidermal stem cells and also changes in xylem structure and lignification of them in soybean stems.

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