

Salt response in pepper (*Capsicum annuum* L.): Components of photosynthesis inhibition, proline accumulation, and K^+/Na^+ selectivity

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ABSTRACT

Three salinity of irrigation water (control, 70, 120, and 170 mM NaCl) were applied to five cultivars of hot pepper (*Capsicum annuum* L.) collected from the South of Tunisia. After 3 weeks, relative water content, photosynthetic pigment levels, gas exchange, proline, soluble sugars, and ions K^+ and Na^+ concentrations were measured. The results showed that the inhibition of photosynthesis was not mainly stomatal, but probably biochemical manifesting by a stability of stomatal conductance up to 120 mM NaCl and accompanied by a continuous increase in intercellular CO_2 concentration under the three concentrations of salt. Proline content increased considerably as a result of stress and reaches almost double those of controls in cultivars Alaya, Skhira, and Sgay and even triple in cultivars Maghraoua and Farch. Solubles sugars do not contribute effectively to the osmotic adjustment. Mineral analysis showed that until 120 mM NaCl, the highest concentration of Na^+ was obtained in stems, probably reflecting the existence of a re-circulation mechanism of Na^+ . Meanwhile, salinity reduced the foliar K^+ content. At stems level, K^+ concentration does not show significantly disruptions compared to control. However, at the roots, there is a continuous increase in potassium content. For K^+/Na^+ shoot ratio and at 70 mM NaCl, all cultivars showed a decrease of selectivity except cv. Maghraoua. At 120 and 170 mM, the same cultivar showed the lowest reduction. In root system, at 70 mM NaCl, cv. Maghraoua showed the greater improvement of selectivity.

KEY WORDS: *Capsicum*, intercellular CO_2 concentration, NaCl, photosynthesis, proline, stomatal conductance

INTRODUCTION

One of the current challenges throughout the world is to promote food production against rapid growth accompanied with increasing demand. Global food production should increase by 38% in 2025 and by 57% in 2050 from current levels of food supply (Wild, 2003). Salinization is one form of soil degradation and is one of the real dangers that may affect crop production, especially in arid and semi-arid which are characterized by scarcity and shortage of hydrological resources (Munns and Tester, 2008). According to the report published by FAO in 2000, saline soils cover 3.1% (397 Mha) of the total land area of the world; 39 Mha of these soils are in Africa, nearly 2% of the total area (Martinez-Beltran and Manzur, 2005). In Tunisia, arable land is estimated by 5 Mha (APIA, 2014), about 1.5 Mha was salt-affected, and this represented over

than 30% of the country's arable land. Saline soils are found throughout the Tunisian territory, but it is mainly in the center and south as the arid climate promotes their extension (Hachicha *et al.*, 1994). Management of salt-affected soils requires a combination of agronomic and management practices and is largely dependent on water availability, climatic conditions, crop standing, and the availability of resources (Hachicha and Abdelgawed, 2003). Several methods of reclamation have been developed; among the 1.5 Mha of saline soils in Tunisia, about 300,000 ha were improved (Hachicha, 2007). We must therefore attempt to increase the yield per unit of land rather than the area cultivated.

In scale of plants, salinity may they affects in several ways: A lower osmotic potential in the soil solution, thus reducing the availability of water to the roots of the

plant; impaired soil physical structure thereby causing poor ventilation and lower permeability of water and an excessive accumulation of ions at a cell causing alteration of cell metabolism (ion toxicity, nutritional disorders, oxidative stress, membrane disorganization, reduction of cell division and expansion, genotoxicity) (Hasegawa et al., 2000; Munns, 2002; Zhu, 2007). Studying plant responses to biotic and abiotic stress and understanding the processes involved in this response from morphological to molecular studies, permit scientists to better improve and stabilizing crops productions.

Vegetable crops are threatened by the problem of salinity. *Capsicum annum* are not exempt from the effect of this constraint. The cultivation, production, and consumption of pepper are greatly extended into the world. Pepper are grown around the world because of their adaptation to different agro-climatic regions and their wide variety of shapes, sizes, colors, and pungencies of the fruit (Qin et al., 2014). In Tunisia, hot pepper is a species in high demand for its flavor and pungency and is commonly used in traditional dishes. This vegetable was grown on 18,700 ha with a production of 270,000 T (GIL, 2012). Internationally, Tunisia is the 11th producer of green chilies and pepper in 2012 and the fifth producer in Africa (FAOSTAT, 2013). Pepper culture is seriously affected by environmental conditions and potential irrigation (Harzallah and Chalbi, 1991). The severity of these problems may explain, in part, the decline in productivity of these species in Tunisia compared to other countries. Therefore, considering this serious problem, the main of current work is to characterize, at various behaviors, five local cultivars of pepper during growth under salt conditions. The studied cultivars were collected from the South of Tunisia which are more exposed and adapted to this environmental stress and which probably have a more interesting genetic potential for subsequent exploitations in plant biotechnology and genetic resources for sustainability and productivity.

MATERIALS AND METHODS

Plant Material and Growth Conditions

Five cultivars of hot pepper (*C. annum* L.) were involved in this assay: cv. Alaya, cv. Skhira, cv. Sgay, cv. Maghraoua, and cv. Farch.

The experience was conducted in a greenhouse in Arid Lands Institute. Seeds, homogeneous in size and without apparent damage, were sown individually in plastic pots of 8 cm deep and 5 cm in diameter and filled with peat and pricked in bases to facilitate drainage. These pots

were put in a greenhouse with a thermohygrometer to measure temperature, fixed at 25°C, the relative humidity at 70% and under natural solar radiation. At the stage of 4 leaves, plants were transferred individually in plastic pots of 30 cm diameter and 50 cm deep, filled at the bottom with thin layer of gravel to facilitate water draining and each containing 14.5 kg as a mixture of soil and organic fertilizer (3V/1V). The soil used was from one of the plots of the institute. It has been screened to remove large particles, and then pots were placed in a plastic greenhouse. The substrate has an electrical conductivity (dS.m⁻¹): 0.9 at 22°C. This salinity was considered during treatment. Three concentrations of NaCl were tested during this experience (70, 120, and 170 mM) compared to control (rainwater). A randomized complete block experimental design was used. 16 plants were prepared for each cultivar, four plants for each treatment. The substrate was maintained at field capacity.

Measurements

Relative water content (RWC)

The RWC was measured as described by Barrs and Weatherly (1968). RWC was determined on fully expanded leaves of a similar age. Fresh leaves were weighed and placed in distilled water in the dark for 24 h to rehydrate. Leaf turgid weight was recorded. Then, leaves were dried in an oven at 80°C for 48 h, and the dry weight (DW) was determined. RWC was derived by the following equation:

$$\text{RWC (\%)} = [(\text{FW} - \text{DW}) / (\text{FTW} - \text{DW})] * 100$$

Where, FW: Sample fresh weight (g); FTW: Sample turgid weight (g); DW: Sample dry weight (g).

Content of photosynthetic pigments

The extraction of chlorophyll and carotenoids was made according to Arnon's method (1949), while concentration was calculated by McKinney formulas (1941). Fresh leaves (100 mg) were grinded with 80% acetone. After filtration, absorbance was measured using a spectrophotometer at 480, 645, and 663 nm.

Gas exchange

Gas exchange was measured by a Portable Photosynthesis System (ADC, LCi). The following parameters were evaluated: Photosynthetic activity (A) (μmolCO₂.m⁻².s⁻¹), transpiration (E) (mmolH₂O.m⁻².s⁻¹), stomatal conductance (gs) (mmol.m⁻².s⁻¹), and the intercellular CO₂ concentration (μmol.mol⁻¹). The measurements were taken in completely expanded leaves located in the middle part of the plant. A total of three readings were recorded

per plant. Measurements were performed from 9.00 to 11.00 a.m.

Proline content

The extraction was made by the method of Troll and Lindsley (1955) simplified depending Wittmer (1987). 100 mg of fresh material was placed with 2 ml of methanol 40%. The reaction mixture was heated at 85°C in a water bath for 1 h. After cooling, 1 ml was removed from the extract to which 1 ml of acetic acid and 1 ml of the mixture containing (120 ml distilled water + 300 ml of ortho-phosphoric acid) was added. The resulting solution was boiled for 30 min. After cooled, 5 ml of toluene was added. Two phases were separated. The upper phase (organic phase) was recovered. Absorbance measurements were determined using the spectrophotometer at 528 nm. Proline concentration was determined from a standard curve.

Soluble sugar content

The soluble sugars extraction was performed according to the method of Dubois *et al.* (1956). 3 ml of ethanol 80% was added to 100 mg of fresh material. The tubes were incubated at room temperature for 48 h. They were placed in an oven at 80°C to evaporate the ethanol. Then, 20 ml of distilled water was added to each tube. 1 ml of the test solution was placed in a new tube, to which was added 1 ml of phenol solution 5%. The tubes were carefully agitated, and 5 ml of concentrated sulfuric acid was added. The temperature then reaches about 110°C. After rapid shaking, the tubes were maintained at 5°C for 45 min. After spending 30 min in the dark, absorbance measurements were determined using the spectrophotometer at 485 nm. The results were reported on a standard curve of soluble sugars (expressed as glucose).

Mineral Analysis

After washing with distilled water, the plants were divided into roots, stems, and leaves. Samples were dried in an oven at 80°C for 48 h. 100 mg was ground, placed in porcelain capsules and incinerated at 550°C for 5 h in a muffle furnace. After cooling, the ash obtained was dissolved in 4 ml of distilled water and 1 ml of HCl 1%. The capsules were placed on a hot plate until boiling, following by two successive filtrations; the extracts obtained were completed to 100 ml. From this volume, the Na⁺ and K⁺ analysis were performed using standard solutions by Flame Photometer (Sherwood Model-410).

Statistical Analysis

Statistical analysis was performed using SPSS version 16.0. Data were evaluated by analysis of variance (ANOVA) and

differences between means were compared by Duncan test at 5%.

RESULTS

Effects on Physiological Aspect

RWC

In the absence of salt stress, cultivars showed values between 75.39% and 85.45%. When NaCl was supplied, the average RWC showed an increase compared to control ($P < 0.05$) (Table 1). For 70 mM NaCl, the highest stimulation was noted in cv. Alaya (12.89%), followed by cv. Farch (10.18%), while the other cultivars tend to react with a slight decrease in their content of water. For 120 mM NaCl, the largest stimulation of RWC was observed in cv. Skhira (18.23% increase), while at 170 mM NaCl, except the cv. Maghraoua, which showed a slight decrease (0.15%), all other cultivars showed significant improvements which are 23.14% in cv. Alaya, 6.57% in cv. Skhira, 10.25% in cv. Sgay, and 10.21% in cv. Farch.

Chlorophyll A content

Variability of responses was observed ($P < 0.05$). In control plants, the highest and the lowest concentrations were measured in pepper of Sgay and Skhira (6.16 and 4.37 mg/g FW, respectively) (Table 1). At 70 mM NaCl, the leaf tissue of cultivars Sgay, Skhira, and Maghraoua increased their contents with improvements by 10.71%, 12.81%, and 27.53%, respectively. Whereas, pepper of Alaya and Farch are more sensitive thus demonstrating a similar reduction by 12%. At moderate and high salinity levels (120 and 170 mM NaCl), only cv. Maghraoua showed an increase in concentration by 29.48% in moderate dose and 4.61% in the high dose of salt. Other cultivars suffer a decrease in their contents. Cv. Alaya showed the most significant decrease, compared to control, with reduction values by 46.84% and 63.28% in 120 and 170 mM, respectively. The comparing means showed that chlorophyll "A" content increased by 5.57% for salinity 70 mM and decreased by 14.31% and 25.09%, respectively for treatments 120 and 170 mM NaCl.

Chlorophyll B content

The results were presented in Table 1. Chlorophyll "B" content decreased under three irrigation treatments in cultivars Alaya, Sgay, and Farch. While the increase in concentration was recorded in two other cultivars, the most important are in pepper Skhira at 70 mM NaCl (47.59%) and in Maghraoua at 120 and 170 mM NaCl (20.33% and 9%, respectively).

Table 1: RWC, chlorophyll A, chlorophyll B, and carotenoids concentrations of five *Capsicum* cultivars were estimated in the plants treated with sodium chloride (control, 70 mM, 120 mM, and 170 mM)

| Parameter | Cultivar | NaCl (mM) | | | | Means |
|-------------------------|-----------|--------------------------|--------------------------|--------------------------|---------------------------|--------------------|
| | | 0 | 70 | 120 | 170 | |
| RWC (%) | Alaya | 75.39±11.70 ^a | 85.11±4.17 ^a | 76.11±12.14 ^a | 92.84±7.89 ^a | 81.38 ^a |
| | Skhira | 78.92±3.10 ^{ab} | 78.89±6.89 ^{ab} | 92.31±16.00 ^a | 84.11±3.16 ^{ab} | 84.60 ^a |
| | Sgay | 82.14±8.55 ^{ab} | 82.11±5.44 ^b | 82.12±6.69 ^b | 90.56±2.93 ^a | 83.61 ^a |
| | Maghraoua | 85.45±6.69 ^a | 79.87±7.08 ^a | 90.36±7.95 ^a | 85.32±11.98 ^a | 84.72 ^a |
| | Farch | 80.64±13.81 ^a | 88.85±4.93 ^a | 90.60±6.04 ^a | 88.88±7.05 ^a | 87.13 ^a |
| | Means | 80.33 ^b | 82.89 ^{ab} | 86.34 ^a | 87.59 ^a | |
| Chlorophyll A (mg/g FW) | Alaya | 5.23±0.39 ^a | 4.60±1.19 ^a | 2.78±0.11 ^b | 1.92±0.33 ^b | 3.63 ^c |
| | Skhira | 4.37±1.07 ^a | 4.93±2.17 ^a | 4.16±2.09 ^a | 4.16±0.61 ^a | 4.40 ^{bc} |
| | Sgay | 6.16±1.76 ^a | 6.82±3.07 ^a | 4.28±1.10 ^a | 4.23±0.98 ^a | 5.37 ^{ab} |
| | Maghraoua | 5.63±2.54 ^a | 7.18±0.63 ^a | 7.29±2.95 ^a | 5.89±0.97 ^a | 6.49 ^a |
| | Farch | 5.54±1.91 ^a | 4.87±2.08 ^a | 4.54±2.45 ^a | 3.95±2.22 ^a | 4.72 ^{bc} |
| | Means | 5.38 ^a | 5.68 ^a | 4.61 ^{ab} | 4.03 ^b | |
| Chlorophyll B (mg/g FW) | Alaya | 4.94±1.02 ^a | 3.48±1.05 ^b | 3.05±0.54 ^b | 2.37±0.93 ^b | 3.46 ^a |
| | Skhira | 2.29±0.54 ^a | 3.38±1.54 ^a | 2.31±0.87 ^a | 2.39±0.35 ^a | 2.59 ^b |
| | Sgay | 4.32±1.65 ^a | 4.04±1.27 ^a | 3.00±0.77 ^a | 3.23±1.11 ^a | 3.65 ^a |
| | Maghraoua | 3.00±1.31 ^a | 3.71±1.22 ^a | 3.61±1.93 ^a | 3.27±0.67 ^a | 3.39 ^a |
| | Farch | 3.42±1.04 ^a | 2.54±0.28 ^{ab} | 2.35±0.70 ^{ab} | 1.82±0.67 ^b | 2.53 ^b |
| | Means | 3.59 ^a | 3.43 ^{ab} | 2.86 ^{bc} | 2.61 ^c | |
| Carotenoids (mg/g FW) | Alaya | 1.460±0.09 ^a | 1.253±0.04 ^b | 1.273±0.48 ^a | 1.058±0.06 ^c | 1.26 ^c |
| | Skhira | 1.411±0.21 ^a | 1.577±0.04 ^a | 1.669±0.47 ^a | 1.794±0.26 ^{ab} | 1.61 ^{ab} |
| | Sgay | 1.742±0.17 ^a | 1.815±0.22 ^a | 1.798±0.49 ^a | 1.638±0.32 ^{abc} | 1.74 ^{ab} |
| | Maghraoua | 1.580±0.40 ^a | 1.754±0.05 ^a | 2.069±0.67 ^a | 2.196±0.26 ^a | 1.89 ^a |
| | Farch | 1.387±0.79 ^a | 1.655±0.38 ^a | 1.519±0.33 ^a | 1.345±0.83 ^{bc} | 1.47 ^{bc} |
| | Means | 1.51 ^a | 1.61 ^a | 1.66 ^a | 1.60 ^a | |

Values followed by the superscript same letter are homogeneous at 5% by Duncan's test. FW: Fresh weight, RWC: Relative water content

The comparing means showed the following percentage reductions 4.45%, 20.33%, and 27.29% at 70, 120, and 170 mM NaCl, respectively.

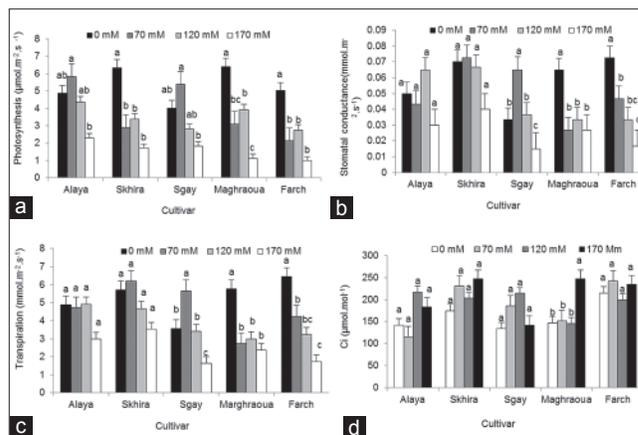
Carotenoids content

ANOVA showed a highly significant effect for the variable cultivar and no significant for the variable treatment. This was confirmed by the comparison of means (Table 1). The increase in salt concentration was accompanied by a small no significant increase in carotenoids content by 6.62%, 9.93%, and 5.96%, respectively, at 70, 120, and 170 mM NaCl. The largest increases in carotenoids content were observed at 70 mM in cv. Farch (19.32%) and at 120 and 170 mM, in cv. Maghraoua (30.94% and 38.98%, respectively). When NaCl was supplemented, cv. Alaya manifested the most significant decreases (14.17%, 12.80%, and 27.53% under the three irrigation water, respectively).

Leaf Gas Exchange Parameters

Photosynthetic activity (A)

Photosynthesis was affected with the accentuation of salt stress ($P < 0.05$) (Graph 2a). After 3 weeks of treatment, results showed that for control plant peppers (Graph 1a), the best activity was recorded in cv. Maghraoua ($6.41 \mu\text{mol}\cdot\text{m}^{-2}\cdot\text{s}^{-1}$) and the lowest in cv.



Graph 1: Effect of saline irrigation in photosynthetic activity (a), stomatal conductance (b), transpiration (c) and intercellular CO₂ concentrations (d) of five cultivars of pepper after 21 days of treatment at three levels of salinity (70, 120, and 170 mM NaCl)

Sgay ($4.02 \mu\text{mol}\cdot\text{m}^{-2}\cdot\text{s}^{-1}$). At 70 mM, the two cultivars Alaya and Sgay improved their activities (19.88% and 33.95%, respectively). The others cultivars presented a reduction where the more pronounced was measured in cv. Farch (57.61%). Moderate and high salinity inhibited more photosynthesis machinery; the lowest reduction was recorded in pepper Alaya (10.24% and 52.39% at 120 and 170 mM, respectively) and the most pronounced in pepper Skhira (46.82%) at 120 mM and in plants of Maghraoua (82.48%) at 170 mM.

Stomatal conductance (gs)

The treatment effect is highly significant ($P < 0.05$) for this parameter. In the presence of salt, plants pepper exhibit remarkable response. Comparison of means shows that stomatal conductance remained statistically stable up to 120 mM NaCl compared to control (Graph 2b). At 170 mM NaCl, the reduction is important (56.9%). A wide cultivar (Graph 1b), an increase was recorded in the leaves of the cv. Sgay by 96.96% and in cv. Skhira by 3.57% at 70 mM NaCl. At 120 mM, gs are also statistically stable with an average $0.046 \text{ mmol.m}^{-2}.\text{s}^{-1}$. Improvements are also observed among cultivars Alaya (30%) and Sgay (9.09%). At 170 mM, the conductance was reduced in five cultivars by an average 56.89%. The lowest limitation was measured on leaves Alaya (40%). The largest reductions were recorded in cv. Maghraoua (60%) with 70 mM and in cv. Farch (54.16% and 77.77%) with 120 and 170 mM NaCl, respectively.

Transpiration (E)

The statistical data show that after 3 weeks of treatment, the effect of the cultivar is not significant, while the treatment effect is highly significant (Graph 2c). The result (Graph 1c) showed that pepper Sgay increased transpiration by 58.27% at 70 mM. At 120 mM, a very slight increase is recorded only in pepper of Alaya by 0.49%. While at 170 mM, the lightest limitation characterized Skhira plants (37.92%). The largest reductions were noted at 70 mM in cv. Maghraoua (52.87%) and at 120 and 170 mM in cv. Farch (49.95% and 72.98%, respectively).

Intercellular CO_2 concentration (Ci)

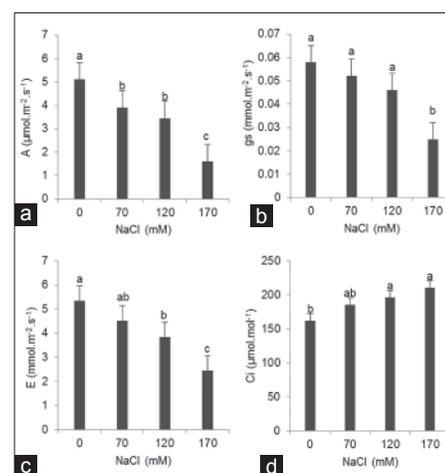
The ANOVA conducted for intercellular CO_2 concentration showed a highly significant effect for the variable cultivar and significant for the treatment ($P < 0.05$). This concentration shows an increase with the severity of stress (Graph 2d). It grew by 14.3%, 20.91%, and 29.8%,

respectively at 70, 120, and 170 mM NaCl. A wide cultivar (Graph 1d), pepper Sgay showed the most important increases of Ci at 70 and 120 mM (39.15% and 60.35%, respectively). At 170 mM, the maximum elevation was recorded in cv. Maghraoua (68.36%).

Effects on Biochemical Aspect

Proline content

Increased accumulation of proline was measured in leaves with accentuation of salinity ($P < 0.05$, Table 2). In the absence of salt, plants presented relatively low concentrations; the lowest concentration was recorded in cultivars Sgay, Maghraoua, and Farch. High salinity level significantly stimulated the accumulation of this osmotic compound, reaching almost double those of controls in cultivars Alaya, Skhira, and Sgay and even triple in cultivars Maghraoua and Farch. The most build-up of this



Graph 2: Effect of salinity on means of gas exchanges: Photosynthetic activity (a), stomatal conductance (b), transpiration rate (c) and intercellular CO_2 concentrations (d) of five cultivars of pepper after 21 days of treatment with a control and three concentrations of salt (70, 120, and 170 mM NaCl)

Table 2: Effect of salinity on the levels of proline and soluble sugars in five cultivars of *Capsicum*

| Parameter | Cultivar | NaCl (mM) | | | | Means |
|--------------------------|-----------|---------------------------|--------------------------|--------------------------|--------------------------|---------------------|
| | | 0 | 70 | 120 | 170 | |
| Proline (mg/g FW) | Alaya | 0.048±0.023 ^b | 0.031±0.002 ^b | 0.082±0.007 ^a | 0.088±0.022 ^a | 0.062 ^a |
| | Skhira | 0.046±0.005 ^b | 0.065±0.018 ^b | 0.061±0.005 ^b | 0.086±0.013 ^a | 0.064 ^a |
| | Sgay | 0.021±0.004 ^d | 0.039±0.003 ^c | 0.048±0.005 ^b | 0.056±0.002 ^a | 0.041 ^c |
| | Maghraoua | 0.023±0.004 ^b | 0.047±0.004 ^a | 0.060±0.010 ^a | 0.072±0.027 ^a | 0.050 ^b |
| | Farch | 0.029±0.009 ^c | 0.029±0.007 ^c | 0.059±0.005 ^b | 0.075±0.006 ^a | 0.048 ^{bc} |
| | Means | 0.033 ^d | 0.042 ^c | 0.062 ^b | 0.075 ^a | |
| Soluble sugars (μg/g FW) | Alaya | 0.603±0.056 ^a | 0.615±0.108 ^a | 0.523±0.021 ^a | 0.621±0.043 ^a | 0.590 ^a |
| | Skhira | 0.596±0.050 ^a | 0.678±0.050 ^a | 0.575±0.091 ^a | 0.833±0.321 ^a | 0.670 ^a |
| | Sgay | 0.548±0.034 ^a | 0.535±0.028 ^a | 0.561±0.053 ^a | 0.693±0.280 ^a | 0.584 ^a |
| | Maghraoua | 0.589±0.098 ^a | 0.596±0.079 ^a | 0.744±0.417 ^a | 0.718±0.226 ^a | 0.662 ^a |
| | Farch | 0.605±0.106 ^{ab} | 0.559±0.048 ^b | 0.681±0.021 ^a | 0.550±0.028 ^b | 0.599 ^a |
| | Means | 0.588 ^a | 0.597 ^a | 0.617 ^a | 0.683 ^a | |

Values followed by the superscript same letter are homogeneous at 5% by Duncan's test. FW: Fresh weight

compound was noted on leaves of Maghraoua; it increases by 104.34%, 160.86%, and 213.04%, respectively at 70, 120, and 170 mM. Comparison of means shows a significant stimulation on the biosynthesis of proline under saline conditions. Improvements are 27.27%, 87.87%, and 127.27%, respectively, at three tested salinity.

Soluble sugars content

No significant accumulation of soluble sugars was detected in stressed plants. Cultivars and salinity means showed no significant differences (Table 2). At 70 and 170 mM, the largest accumulations were measured in cv. Skhira (13.75% and 39.76%). At 120 mM, pepper of Maghraoua was the richest in soluble sugars (26.31%).

Effect on Mineral Nutrition

Na⁺ supply

In absence of salt stress, means of Na⁺ content in the leaves, stems, and roots of five cultivars were minimal with minimum average amount 0.837 mg.g⁻¹ DW in roots. With treatment, these levels were increased in all parts of the plant. A wide organ and up to 120 mM NaCl, the stems has maximum Na⁺ accumulation, followed by leaves and then roots (Table 3). While at

170 mM, the most pronounced accumulation was noted in leaves Alaya, Sgay, and Maghraoua. These cultivars showed symptoms of dryness. In leaves, at low and high concentration of NaCl, the lower accumulation was observed in pepper Farch (82.22% and 723.97% increase, respectively), at moderate concentration, in cv. Skhira (154.45%).

K⁺ supply

The ANOVA showed highly significant effects for the variables cultivar, treatment and their interaction (*P* < 0.01) for the levels of K⁺ at the leaves, stems, and roots.

According to the results shown in Table 3, in the absence of salt, the leaves contain the most potassium content, followed by stems and finally the roots. With salt water, K⁺ content was decreased at leaves, increased in the roots and remained statistically stable in the stems. When 170 mM NaCl was applied, K⁺ content did not significantly differ between the three parts of plants. Regarding the cultivars, in three levels of salinity, cv. Farch improved its accumulation of K⁺ by 1887.69%, 697.22%, and 1865.07%, respectively.

Table 3: Sodium and potassium content (mg/g DW) in leaves, stems, and roots of five cultivars of *C. annuum* under salt stress conditions

| Cultivars | Leaves Na ⁺ | | | | | Stems Na ⁺ | | | | | Roots Na ⁺ | | | | |
|-----------|------------------------|--------------------|--------------------|---------------------|--------------------|-----------------------|--------------------|--------------------|--------------------|--------------------|-----------------------|--------------------|--------------------|--------------------|--------------------|
| | NaCl (mM) | | | | | NaCl (mM) | | | | | NaCl (mM) | | | | |
| | 0 | 70 | 120 | 170 | Means | 0 | 70 | 120 | 170 | Means | 0 | 70 | 120 | 170 | Means |
| Alaya | 1.111 | 2.117 | 6.117 | 37.726 | 11.76 ^a | 0.955 | 3.058 | 9.117 | 7.058 | 5.048 ^d | 0.855 | 0.955 | 8.045 | 6.545 | 4.101 ^b |
| Skhira | 2.011 | 3.117 | 5.117 | 10.117 | 5.089 ^d | 1.117 | 10.117 | 14.117 | 8.058 | 8.353 ^b | 0.455 | 6.045 | 5.545 | 4.045 | 4.023 ^d |
| Sgay | 1.411 | 4.117 | 6.117 | 30.226 | 10.46 ^c | 1.611 | 1.511 | 2.058 | 10.117 | 3.825 ^e | 0.805 | 0.755 | 4.045 | 5.545 | 2.788 ^e |
| Maghraoua | 0.058 | 4.117 | 4.779 | 37.726 | 11.66 ^b | 9.558 | 7.117 | 12.117 | 9.117 | 9.478 ^a | 0.955 | 0.705 | 5.545 | 9.045 | 4.063 ^c |
| Farch | 0.855 | 1.558 | 6.117 | 7.045 | 3.894 ^e | 0.255 | 7.058 | 4.558 | 11.117 | 5.747 ^c | 1.111 | 4.045 | 6.545 | 7.045 | 4.687 ^a |
| Means | 1.090 ^d | 3.006 ^c | 5.650 ^b | 24.563 ^a | | 2.700 ^d | 5.773 ^c | 8.394 ^b | 9.094 ^a | | 0.837 ^d | 2.502 ^c | 5.945 ^b | 6.446 ^a | |

| Cultivars | Leaves K ⁺ | | | | | Stems K ⁺ | | | | | Roots K ⁺ | | | | |
|-----------|-----------------------|--------------------|--------------------|--------------------|---------------------|----------------------|--------------------|--------------------|--------------------|---------------------|----------------------|--------------------|--------------------|--------------------|--------------------|
| | NaCl (mM) | | | | | NaCl (mM) | | | | | NaCl (mM) | | | | |
| | 0 | 70 | 120 | 170 | Means | 0 | 70 | 120 | 170 | Means | 0 | 70 | 120 | 170 | Means |
| Alaya | 8.252 | 2.509 | 3.009 | 3.452 | 4.305 ^{bc} | 3.6 | 3.009 | 8.509 | 2.6 | 4.429 ^a | 0.05 | 0.252 | 5.452 | 4.452 | 2.551 ^a |
| Skhira | 11.505 | 5.509 | 6.009 | 4.009 | 6.757 ^a | 6.509 | 5.2 | 3.4 | 3.6 | 4.695 ^a | 0.252 | 4.952 | 4.452 | 3.452 | 3.277 ^a |
| Sgay | 8.752 | 8.009 | 2.509 | 4.452 | 5.930 ^a | 4.8 | 4 | 3.509 | 2.4 | 3.683 ^{ab} | 0.752 | 0.252 | 5.952 | 3.452 | 2.602 ^a |
| Maghraoua | 7.009 | 10.009 | 1.252 | 2.952 | 5.305 ^{ab} | 2.8 | 4.509 | 2.8 | 6.009 | 4.029 ^a | 0.752 | 1.752 | 3.952 | 3.952 | 2.602 ^a |
| Farch | 0.252 | 5.009 | 2.009 | 4.952 | 3.055 ^c | 4.4 | 2.4 | 2.009 | 2.6 | 2.859 ^b | 1.252 | 3.452 | 3.952 | 3.952 | 3.152 ^a |
| Means | 7.154 ^a | 6.208 ^a | 2.957 ^b | 3.963 ^b | | 4.431 ^a | 3.833 ^a | 4.050 ^a | 3.442 ^a | | 0.611 ^d | 2.132 ^c | 4.752 ^a | 3.852 ^b | |

| Cultivar | Shoots selectivity K ⁺ /Na ⁺ | | | | | Roots selectivity K ⁺ /Na ⁺ | | | | |
|-----------|--|--------------------|--------------------|--------------------|--------------------|---|--------------------|--------------------|--------------------|--------------------|
| | NaCl (mM) | | | | | NaCl (mM) | | | | |
| | 0 | 70 | 120 | 170 | Means | 0 | 70 | 120 | 170 | Means |
| Alaya | 5.732 ^a | 1.066 ^c | 0.756 ^a | 0.136 ^e | 1.923 ^a | 0.059 ^e | 0.264 ^e | 0.678 ^d | 0.681 ^b | 0.421 ^e |
| Skhira | 5.756 ^a | 0.810 ^e | 0.490 ^c | 0.419 ^a | 1.869 ^c | 0.554 ⁺ | 0.819 ^c | 0.803 ^b | 0.854 ^a | 0.757 ^d |
| Sgay | 4.482 ^b | 2.134 ^a | 0.736 ^b | 0.170 ^d | 1.881 ^b | 0.933 ^b | 0.334 ^d | 0.472 ^a | 0.623 ^c | 0.840 ^b |
| Maghraoua | 1.020 ^d | 1.293 ^b | 0.240 ^e | 0.192 ^c | 0.686 ^e | 0.787 ^c | 2.482 ^a | 0.713 ^c | 0.437 ^e | 1.105 ^a |
| Farch | 4.184 ^c | 0.860 ^d | 0.377 ^d | 0.416 ^b | 1.459 ^d | 1.126 ^a | 0.854 ^b | 0.604 ^e | 0.561 ^d | 0.786 ^c |
| Means | 4.235 ^a | 1.232 ^b | 0.520 ^c | 0.267 ^d | | 0.692 ^c | 0.951 ^a | 0.854 ^b | 0.631 ^d | |

Values followed by the superscript same letter are homogeneous at 5% by Duncan's test. DW: Dry weight, *C. annuum*: *Capsicum annuum*

Selectivity K^+/Na^+

The ratio K^+/Na^+ were negatively affected in shoot system by reductions of 70.9%, 87.72%, and 93.69%, respectively, at 70, 120, and 170 mM NaCl as a consequence of the decrease in K^+ and increase of Na^+ content. At 70 mM, all cultivars showed a decrease of selectivity except cv. Maghraoua which exhibited an increase by 26.76%. When 120 and 170 mM was supplied, the same cultivar showed the lowest reduction (76.47% and 81.17%, respectively).

In root system, K^+/Na^+ ratio was increased with low and moderate salinity by 37.42% and 23.41%, respectively. At high treatment, the reduction was 8.81% compared to control. With addition of 70 mM NaCl, cv. Maghraoua showed the greater improvement of selectivity (215.37%). Under 120 and 170 mM NaCl, the best values were measured in cv. Alaya (1049.15% and 1054.23%, respectively).

DISCUSSION

Response of plants to salt stress is a complex reaction involving several interconnected mechanisms controlled by multigenic trait and variable depending on the species and stage of stress application (Munns and Tester, 2008). In current work, sodium chloride was used because it is the most soluble and widespread salt (Munns and Tester, 2008).

During the growth phase and after 21 days of application of saline irrigation, the RWC was effectively protected. This content is one of the key indicators of plant water status. It increased for the three tested treatments with maintaining a constant conductance somatic statistically up to 120 mM NaCl. A relative turgidity less affected by stress reflects good efficiency saving water. This improvement can be explained, in part, by the effective accumulation of organic osmolytes. Correlations between RWC and proline content ($r = 0.168^*$) and soluble sugars ($r = 0.105^*$) are significant, which suggests the existence of mechanisms of osmotic adjustment lead to the preservation of the structural and functional integrity of the tissue (Blum, 1988). On the other hand, the improvement of water status of plants may be in part a consequence of the no significant reduction in stomatal conductance for 70 and 120 mM NaCl and significantly at 170 mM, to limit water loss through transpiration. This latter was attenuated by salt stress under the three tested doses. The decrease in transpiration rate indicates that the plant has difficulty drawing water from saline soil and try to minimize its loss. According to Silva *et al.* (2008), salinity significantly reduced root hydraulic conductance,

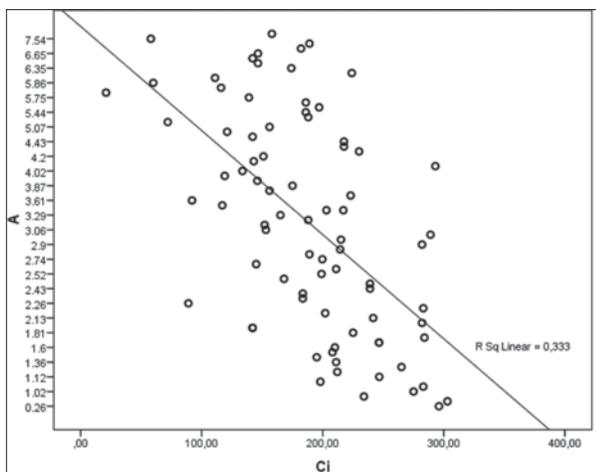
this reduction is closely related to the reduction in the activity of aquaporins at the plasma membrane of roots.

For osmotic adjustment, accumulation of proline under salt stress is cited by various authors, but until now, its role is controversial. Some studies have cited that the buildup of this amino acid is an important regulatory mechanism under salt stress (Huang *et al.*, 2013). However, other studies have reported a negative correlation between his accumulation and salt tolerance (Chen *et al.*, 2007). Matysik *et al.* (2002) showed that proline alleviates salt-stress-induced enhancement in the oxygenase and carboxylase activities of Rubisco and protects plants from free-radical-induced damage by the quenching of singlet oxygen. Considering that early responses to water and salt stress have been considered mostly identical (Munns, 2002), Sziderics *et al.* (2010) found that, in plants grown under drought stress, the elevation of the synthesis of proline in the roots combined with transport of the compound from leaves to roots can be regarded as an adaptation strategy in this species exposed to water deficit.

According to Lignowski and Slittstoesser (1971), proline is synthesized from the acid via 1-pyrroline-5-carboxylic acid but also via the arginine and ornithine. The first path explains interconnecting the biosynthesis of chlorophyll and that of proline. This may explain the negative correlation obtained between this two parameter in our essay ($r = -0.327$). This probability should be confirmed by biochemical analyzes such as the evaluation of glutamine synthetase, an enzyme involved in the biosynthesis of glutamate, which is a common precursor to chlorophyll pigments and proline.

Other compatible solutes can be accumulate in tissue plants under salt stress include carbohydrates such as sugars (glucose, fructose, and sucrose), and their major functions have been reported to be an osmotic adjustment, carbon storage, and radical scavenging (Bohnert *et al.*, 1995). In the current study, analyze of soluble sugars in the control and treated plants showed no significant increase in the effect of treatment. Therefore, soluble sugars were not involved effectively in osmotic regulation in pepper plant under salt stress.

At gas exchange level, the effects of salinity on photosynthesis ranging from restriction of the diffusion of CO_2 into the chloroplast, by limiting the stomatal conductance controlled by the root and aerial hormonal signals and CO_2 transport in the mesophyll, to alterations in photochemistry and carbon metabolism in leaf (Chaves *et al.*, 2009). According to Bethke and Drew (1992),



Graph 3: Negative correlation between photosynthesis (A) ($\mu\text{mol.m}^{-2}.\text{s}^{-1}$) and intercellular CO_2 concentration (Ci) ($\mu\text{mol.mol}^{-1}$) after 21 days of application of stress

the inhibition of photosynthesis in plants of pepper under salt stress is due to stomatal effects and/or non-stomatal. Stomatal limitation is manifested by restricting in the supply of CO_2 to photosynthetic tissues following induction of stomatal closure, while the non-stomatal limitation reflects effects on the photochemical process and *in vivo* probably due (and not *in vitro*) to the activity of ribulose biphosphate carboxylase (Cheeseman, 1988). In current work, the reduction of photosynthetic activity is accompanied by a parallel limitation in transpiration and partial limitation of stomatal conductance. However, the internal CO_2 concentration increased significantly (Graph 3).

At 70 mM NaCl, the average of photosynthesis was reduced compared to the control. The total chlorophyll content showed a slight no significant increase. Thus, the hypothesis of a degradation of chloroplasts was relatively removed. Stomatal conductance (gs) was statistically stable, compared to control, with non-significant average reduction. In addition, transpiration (E) is less affected than A. Furthermore, the intercellular CO_2 concentration (Ci) was significantly increased. Therefore, the reduction of photosynthetic activity at 70 mM can be explained probably by a biochemical problem such as carbon fixing due to a reduction of the efficiency of the regenerative capacity of ribulose-1,5-carboxylase biphosphate (Sharkey and Seemann, 1986) or sensitivity of photosystem to NaCl (Ball and Anderson, 1986). At 120 and 170 mM, carbon assimilation restrictions were more pronounced. These changes are accompanied by a continuous increase in Ci. The two parameters (A and Ci) are negatively correlated, and the correlation between them was highly significant ($r = -0.568^{**}$). Chlorophyll contents were decreased, and

the correlation between this contents and A is significant ($r = 0.171^*$). gs was slightly decreased at 120 mM and significantly at 170 mM. It was significantly correlated with A ($r = 0.463^*$). For E, reductions are lower than those of A. Thus, at 120 mM NaCl, the limitation of A is caused by, in addition to the problem of CO_2 fixation, a reduction in chlorophyll content. While at 170 mM NaCl, further to the problem of CO_2 fixation and reduction of the pigment content, A is affected by important limitation of gs. High doses of NaCl generated osmotic effects and ion toxicity. In addition, salinity can cause a decrease in concentrations of essential ions such as Ca^{2+} and Mg^{2+} in mesophyll cells (Netondo *et al.*, 2004). Our results are in agreement with those reported by Bethke and Drew (1992) showed that photosynthetic inhibition in pepper under salt stress is primarily not stomatal but biochemical. Chartzoulakis and Klapaki (2000) suggest that the inhibitory effect of salt on photosynthesis is caused by the partial stomatal closure and increased ion concentration. The results of Lycoskoufis *et al.* (2005) indicate that exposure of half of the root system to the saline solution improves difficulty the physiological responses of plants in comparison with the plants root systems completely salinized, indicating that the response is governed by the effects of ions on roots and not the difficulty to supply the water. Furthermore, Silva *et al.* (2008) have shown, by comparing the effects of salt solution at 60 mM and an iso-osmotic solution characterized by an increase in its concentration of macronutrients, that the first solution is more affected growth pepper plants by reducing the hydraulic conductivity, stomatal conductance, and the percentage of open stomata.

Chlorophyll "A" showed a little no significant increase at 70 mM, while it was noted in one hand, a no significant increase in the surface leaf (data not shown), so the hypothesis of an increase in concentration due to a reduction in the size of cells was eliminated. On the other hand, there was a no significant increase in the RWC would normally lead to a dilution of chlorophyll. This improvement can be explained by an accumulation of Na^+ in leaves at levels below those causing the onset of degradation of chlorophylls (Asch *et al.*, 2000). Aldesuquy and Gaber (1993) have interpreted the increase in chlorophyll concentration by an increase in the number of chloroplasts at the leaves of stressed plants. For 120 and 170 mM NaCl, the means reductions were more marked. This can be due to a toxic accumulation of Na^+ and Cl in the leaf tissue causing the degradation of this biomolecule, or due to decreased gene expression of chlorophyll pathway genes. Rao and Rao (1981) explained this decrease by increasing the activity of the

chlorophyll degrading enzyme: Chlorophyllase, inducing the destruction of the chloroplast structure (Blumenthal-Goldschmidt and Poljakoff-Mayber, 1968). The same depressive effect of salt was observed in three cultivars of *C. annuum* (Aloui *et al.*, 2014).

Carotenoids are essential components required for photosynthesis, photoprotection, and the production of carotenoid-derived phytohormones including abscisic acid and strigolactone (Cazzonelli, 2011). Under salt stress, stimulation of production of carotenoids is one antioxidant strategy developed by the plant and contributes to the elimination of reactive oxygen species (Verma and Mishra, 2005). Semi-quantitative real-time polymerase chain reaction expression data have demonstrated the declined expression of five major genes of the carotenoid pathway as the salt concentration gradually increased in pepper plants (Kumar *et al.*, 2015). By studying the growth under salinity in *Cymbopogon nardus*, Mane *et al.* (2010) showed that the carotenoid content of the leaves at lower levels of salinity (50 mM) was increased and that higher concentration of salt (100-300 mM) did not show much inhibitory effect which might be due to the protective role of carotenoids for chloroplast from photo-oxidative damage by acting as accessory pigments. In current work, we detect a slight no significant increase in the concentration of carotenoids under the three salinity.

Mineral nutrition of pepper under salt stress is seriously disturbed. By applying NaCl treatment, there was an increase of Na⁺ content in leaves, stems, and roots. Our results are in agreement with previous work in pepper (Chartzoulakis and Klapaki, 2000; Genhua *et al.*, 2010). Until 120 mM, the highest concentrations are obtained in the stems, probably reflecting the presence of a re-circulation mechanism of Na⁺ in pepper to protect photosynthetic tissues from toxic effects of salt (Blom-Zandstra *et al.*, 1998). By studying the flow of Na⁺ in pepper plants under moderate stress 15 mM NaCl, the latter cited author proved the characterization of this species by a strategy of re-circulation of Na⁺ ions, they concluded that sodium recirculation is strictly regulated: When the sodium concentration in the nutrient solution is kept constant, it accumulates in xylem and pith cells and is continuously released into the phloem where it is transferred downward through the stem and pumped into the xylem, either in the roots or at the stem base. As soon as sodium is deprived from the roots, the diffusion resistance for passive sodium efflux will decrease, and sodium is released into the medium in the roots or may be at the stem base. Thus, the external sodium concentration controls regulatory mechanisms for internal sodium

fluxes. This behavior is exclusive (Khurram *et al.*, 2009). A study by Slama (1991) in 10 plants about the role of stem on the ascending path of Na⁺ to the leaves showed that exclusion of Na⁺ from the leaves of susceptible plants is associated with a specific accumulation of this ion in the stems. At 170 mM, cultivars Alaya, Sgay, and Maghraoua showed symptoms of dryness. These cultivars have accumulated large concentrations of Na⁺ in leaves. Therefore, in high dose, the ionic balance does not more balanced and plants export the excess of ions to the leaves where it is stored in the vacuoles of cells, so the plants seem does not control the absorption of sodium in their root systems. The accumulation of sodium in the roots is increasing progressively with the severity of the stress but remains relatively low compared to stems and leaves. Contrary to these results, Chartzoulakis and Klapaki (2000) reported that salinity up to 150 mM in pepper plants result in a significant accumulation of Na⁺ in roots than leaves. Bethke and Drew (1992) found that non-stomatal inhibition of photosynthesis is correlated with the levels of Na⁺, this confirms the significant negative correlation obtained ($r = -0.486^*$) between these two parameters in our work.

Meanwhile, salinity reduced the foliar potassium content; this effect is interpreted by a mechanism of competition between Na⁺ and K⁺ at binding sites because of the great chemical similarity between the two cations (Navarro *et al.*, 2000; Kaya *et al.*, 2003). At stems level, contained K⁺ does not show significantly disruptions compared to control. Whereas, at the roots, there is a continuous increase in potassium content under three salinity. Maintaining K⁺ content sufficient to sustain the growth of the various parts of the plant requires a good selectivity of absorption, storage, and transport of K⁺ from the Na⁺.

In shoot system, the selectivity K⁺/Na⁺ gradually decrease for three salinity levels. In root system, they increase up to 120 mM and then decreased. The reduction ratios K⁺/Na⁺ leads to inhibition of enzyme activities in the cytoplasm (Hawighorst, 2007) and impaired protein synthesis (Blaha *et al.*, 2000). Asch *et al.* (2000) show that high ratios for these two ions under salt stress characterize the plants more tolerant.

Regarding the cultivar effect, the results of photosynthetic activity showed that cv. Sgay is the most tolerant at 70 mM NaCl by improving the activity. This superiority is achieved through the largest increase in stomatal conductance. However, it showed the lowest increase in chlorophyll "A." At 120 and 170 mM, cv. Alaya showed the

lowest reduction in photosynthetic assimilation. While, this cultivar showed the most pronounced reduction in chlorophyll contents. Marked superiority in photosynthesis activity in the latter cultivar can be explained by recording a maximum improvement of stomatal conductance by 30% in 120 mM and a minimum reduction by 40% at 170 mM NaCl. At the three treatments, cv. Maghraoua showed the highest improvements in chlorophyll content. However, photosynthesis was greatly reduced. This reduction is explained by a high limitation of stomatal conductance. For K^+/Na^+ shoot ratio and at 70 mM NaCl, all cultivars showed a decrease of selectivity except cv. Maghraoua which exhibited an increase. When 120 and 170 mM was supplied, the same cultivar showed the lowest reduction. In root system and with the addition of 70 mM NaCl, cv. Maghraoua showed the greater improvement of selectivity. Under 120 and 170 mM NaCl, the best values were recorded in cv. Alaya. This result is in agreement with that of Chartzoulakis and Klapaki (2000) confirming that the salinity tolerance in plants is dependent on the cultivar. Bojórquez-Quintal *et al.*, (2012) confirmed that NaCl doses between 0 and 150 mM affect the growth of pepper plants, depending on the species, genotype, and condition of growth. Furthermore, Thouray *et al.* (2013), by studying the effect of NaCl on the growth of three pepper varieties, proved the genotypic dependence *viz.* salinity.

CONCLUSION

Results show that cv. Maghraoua is the most tolerant *viz.* to salinity. The reduction of its photosynthetic activity, accompanied by physiological superiority at high salinity with significant accumulation of proline, improvements in chlorophyll pigments content and a better selectivity of K^+ compared to Na^+ , appears as an adaptation strategy to protect its tissues. It is the only cultivar that could produce fruit at 120 mM NaCl (data not represented). Regarding the effect of treatment, a low concentration (70 mM NaCl) has, relatively, no deleterious effect on the physiological state of the plant. A higher concentration (120 mM NaCl) growth was reduced significantly in terms of contents of chlorophyll pigments, photosynthesis, and transpiration. However, the high dose (170 mM NaCl) caused a strong growth disorder. Significant accumulation of proline was registered under all the treatments. Whereas, soluble sugars and carotenoids contents appear to have not been affected by the negative effect of NaCl. Inhibition of photosynthesis in *C. annuum* is mainly not stomatal but probably biochemical. Further work is needed to understand this non-stomatal inhibition in pepper plants under saline conditions.

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