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Effects of salinity stress on certain morphological traits and antioxidant enzymes of two *Carica papaya* cultivars in hydroponic culture

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Key words: antioxidant enzymes, *Carica papaya*, hydroponic, morphological factors, salinity stress.

Abstract: *Carica papaya* L. is the only species belonging to the *Carica* genus. Salinity stress in soil or water, especially in hot, arid regions, could limit plant growth and reduce its yield. This research studied six-month old seedlings of two cultivars of papaya ('Sinta' and 'Solo'), in solid, disease-free form for two weeks inside a half-dose of Hoagland solution. Results obtained from the effects of salinity stress indicated that the longest root and shoot were observed in the control treatment in 'Sinta'. Moreover, there was no significant difference between the two cultivars in terms of root length, shoot length, fresh weight of roots and fresh weight of shoots in different salinity levels. The highest dry weights of roots and shoots were found in the 'Sinta' control treatment, while the lowest was observed in 'Solo' 6 dS/m treatment. There was no significant difference between the two cultivars in terms of dry weights of roots and shoots. Finally, the interaction of salinity levels showed that increasing salinity in both cultivars led to higher peroxidase, catalase, superoxide dismutase and ascorbic peroxides activity. By increasing the salinity level, the total protein and proline greatly increased in both cultivars, where the maximum value was found in the 'Sinta' 6 dS/m salinity treatment, and this was significantly different from other treatments. A comparison of the different salinity levels showed that there was a significant difference between the 6 dS/m salinity treatment and other treatments.

1. Introduction

The ability of plants to tolerate salinity stress is facilitated by a series of biochemical pathways which maintain or absorb water, protect plants chloroplast function and sustain an ionic balance. Some of these pathways include the synthesis of active osmotic metabolites (Zhifang and Loescher, 2003). Some proteins and enzymes destroy free radicals (Mittova *et al*., 2003). *Carica papaya* L. is the only species belonging to the *Carica* genus. It can be found in tropical region of America. The cultivation of this crop is common in southern Mexico, Central America and South America, as well as most countries in the tropics. Papaya is a fast-growing tree (it produces fruit in the third year after planting), extremely sensitive to cold, and planted only in the tropics. Papaya fruit is rich in carotenoids, vitamins B, C, lycopene and mineral fibers. The skin, flesh and seeds of this product contain a number of phenolic compounds. Salinity stress in soil or water, especially in hot, arid regions, could limit plant growth and reduce its yield (Koca *et al*., 2007). Plants growing in areas with extreme salinity are divided into halophytes and glycophytes. Most glycophyte plants do not have the ability to tolerate salinity stress (Sairam and Tyagi, 2004). During salinity stress, all the main processes, including photosynthesis, lipid metabolism and energy, are affected (Sairam and Tyagi, 2004). The first response is to reduce the development rate of leaf area and then complete cease. However, growth process resumes as soon as the problem is fixed (Parida and Das, 2005). The plant either tolerates the stress, or avoids it. The former generally occurs at the cell level while the latter occurs at the plant level. During salinity stress, a plant can undergo dormancy (avoidance) or make certain cellular adjustments in order to resist drought stress (Yokoi *et al*., 2002). The

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aim of this study is to survey the effect of salinity stress on certain morphological traits and also on antioxidant enzymes of two *Carica* cultivars in hydroponic culture condition.

2. Materials and Methods

Six-month old seedlings of two cultivars of papaya ('Sinta' and 'Solo'), solid and disease-free, were placed for two weeks in a glass containing 700 ml of a half-dose Hoagland solution, and were then transferred to the hydroponic system. Afterwards, 500 ml of the half-dose Hoagland solution per seedling was added to the hydroponic system. After two weeks, the salinity treatments were initiated at pH=6 as salinity stress continued for 8 weeks. Then, the leaf samples were placed in aluminium foil and frozen at -20°C for further measurements of protein and antioxidant enzymes. Plant height was measured with a ruler. At the end of the experiment, the fresh weight of plant organs was measured and then rinsed through distilled water and finally kept in the oven at 70°C until the dry weight was stabilized. Then, shoots and roots dry weights were measured. A factorial experiment was conducted based on completely randomized design with 5 replications in the greenhouse of the Horticultural Science Department, University of Shiraz. The factors included salinity treatments: 0 (Control), 2, 4, 6, 8 and 10 dS/m NaCl which were added to half dose Hoagland solution and 2 cultivars of papaya ('Sinta' and 'Solo'). Data analysis was done using SAS (version 9.2; SAS Institute, Cary, NC, USA), mean comparisons were carried out using LSD test at 5% of probability.

Extraction for measuring the amount of protein and antioxidant enzymes

For extraction, 0.5 g of root or leaf sample was first ground in liquid nitrogen and then 2 ml of extraction buffer was added and homogenized in a porcelain mortar. Then this mixture tube was centrifuged at 13,000 rpm for 15 min at 4°C. The upper phase was isolated for the purpose of reading the protein content and enzyme activity. For the preparation of the extraction buffer (50 ml), 0.607 g of tris(hydroxylmethyl)aminomethane and 0.05 g of Polyvinylpyrrolidone (PVP) were dissolved into 45 ml of distilled water (pH 8.0).

Total protein

The Bradford assay (1976) was used to determine the protein concentration. To measure the protein concentration, 20 ml extract was diluted in 80 µl of extraction buffer; 5 ml of fresh reagent Coomassie was added, stirred for 2 min, and finally, after 5 min, the optical density was read at a wavelength of 595 nm. Besides, the extraction buffer was used as control. The concentration of protein in the sample was obtained according to the absorption, using the standard curve. Bovine serum albumin was used as the standard and total soluble protein concentrations were expressed in mg g^{-1} fresh weight.

Proline

The method of Bates *et al*. (1973) was employed to measure the concentration of proline. According to this method, 0.5 g of leaves from each sample was placed in 10 ml of an aqueous solution of sulfosalicylic acid (3%) and the mixture was completely homogenized in a porcelain mortar. Then, the homogenized mixture was filtered through paper no. 2. In the next stage, 2 ml of solution was mixed with 2 ml of a reagent, creatininedimenhydrinate and 2 ml of acetic acid was added to each tube. Then, the samples were placed in bain-marie bath for 1 h at a temperature of 100°C and were immediately transferred into an ice bath for a few minutes. Afterwards, 4 ml of toluene was added to each tube and the samples were stirred through Vortex for 15 to 20 s until they were completely homogeneous. The supernatant phase was used to determine proline concentration based on the proline standard curve in the spectrometer at a wavelength of 520 nm. Proline concentration was calculated using L-proline for the standard curve.

Guaiacol peroxidase (POD) activity

To measure the quantitative concentrations of this enzyme, the method of Chance and Mahly (1955) was used with minor modifications. Measurements were done according to the oxidation of guaiacol by that enzyme. In this method, 33 mol of extract was dissolved into 1 ml of a peroxidase solution containing 13 Mm guaiacol, 5 mM hydrogen peroxide (H_2O_2) and 50 mM potassium phosphate buffer (pH=7), and the absorbance values were read for one minute at 10-s intervals and at a wavelength of 470 nm. To prepare 1000 ml of potassium phosphate buffer, 39 ml potassium phosphate and 50 mM monohydrate were mixed with 61 ml potassium dihydrogen and 50 mM phosphate.

Ascorbic peroxidase (APX) activity

To measure the quantitative concentrations of this enzyme, the method of Nakano and Asada

(1981) was used. According to this method, 50 ml of the extract was mixed with 1 ml of ascorbic peroxidase containing 50 mM potassium phosphate buffer (pH=7), 0.1 mM EDTA, 0.5 mM ascorbic acid (ASA), and 0.15 mM peroxide hydrogen (H_2O_2). Then, the absorption at a wavelength of 290 nm was read within one minute through a spectrophotometer. An enzymatic unit of ascorbic peroxidase is equivalent to a dissolution of 1 mM ascorbic acid in a minute.

Catalase (CAT) activity

To measure the quantitative concentrations of this enzyme, the method of Nakano and Asada (1981) was used. According to this method, 50 ml of the extract was mixed with 1 ml of catalase containing 50 mM potassium phosphate buffer (pH=7) and 15 mM hydrogen peroxide (H_2O_2). Then, the absorption at a wavelength of 240 nm was read within one minute through a spectrophotometer. An enzymatic unit of catalase is equivalent to a dissolution of 1 mM hydrogen peroxide (H_2O_2) in a minute.

Superoxide dismutase (SOD) activity

To measure the quantitative concentrations of this enzyme, the method of Beauchamp and Fridovich (1971) was used. The measurement was based on the ability of SOD enzyme to stop the photochemical reduction of NBT by superoxide radicals in the presence of riboflavin in light. According to this method, 50 ml of the extract was mixed with 1 ml of superoxide dismutase containing 50 mM potassium phosphate buffer (pH 7.8), 75 mM of NBT, 13 mM of L-methionine, 0.1 mM of EDAT and 2 mM riboflavin. It should be noted that the solution was stored separately in a dark container and after the addition of soluble extract and measurement solution, superoxide dismutase was added to Qt. The mixture reacted when placed in the light chamber for 15 min. The solution was then placed in a spectrophotometer and the absorbance was measured and read at a wavelength of 560 nm.

3. Results

The interaction between salinity levels and cultivars on root length showed that the highest root length was found in the control treatment in Sinta, while the minimum value was found in the 6 dS/m treatment of both 'Sinta' and 'Solo'. Seedlings of both cultivars in the 8 and 10 dS/m treatments exhibited no salinity resistance and were dried, therefore, these two treatments were excluded from the results; results also showed no significant differences between the two cultivars, in terms of root length, at various salinity levels (Fig. 1).

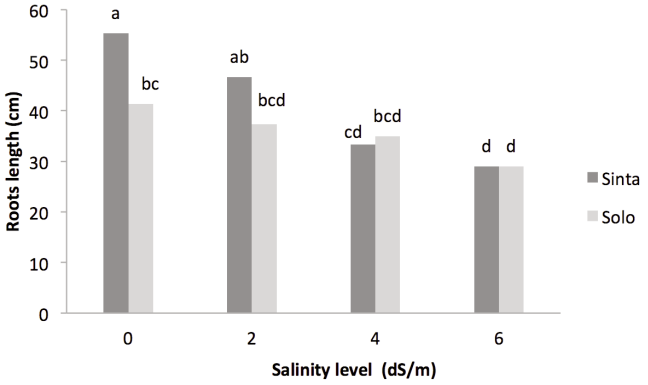

Fig. 1 - Interaction of cultivar and salinity levels on roots length. The means followed by the same letters were not significantly different at p≤0.05.

As shown in figure 2, the highest shoot length was observed in the 'Sinta' control treatment, which was significantly different from other treatments. Moreover, there was no significant difference between the two cultivars in terms of salinity levels.

The control group of both cultivars generally had a significant difference with other treatments, while there existed no significant difference between concentrations of 2, 4 and 6 dS/m.

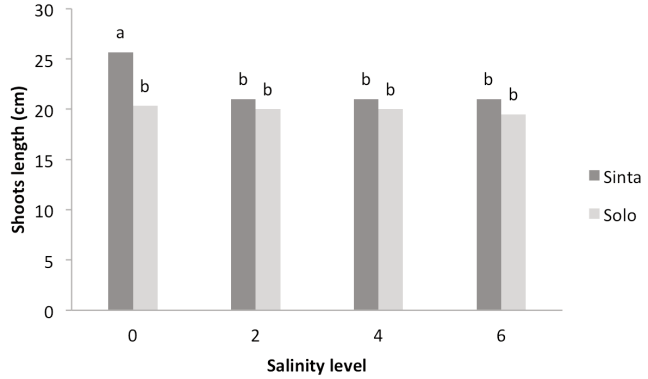

Fig. 2 - Interaction of cultivar and salinity levels on shoots length. The means followed by the same letters were not significantly different at p≤0.05.

Evaluation of salinity effect on roots fresh weight between the two cultivars showed that the highest and lowest values were observed in the control treatment and the Sinta 6 dS/m treatment, respectively. There was no significant differences between the two cultivars in terms of root fresh weight. The comparison of the various salinity levels, showed a significant difference between the control and other salinity treatments, while there was no significant difference

between treatments 2, 4 and 6 (dS/m) (Fig. 3).

In this study maximum root dry weight was observed in the 'Sinta' control (Fig. 4) while the minimum was observed in the 'Solo' 6 dS/m treatment. The comparison between the two cultivars showed a significant difference between them in terms of roots dry weight. The mean salinity concentrations indicated that the control had a significant difference with other concentrations, while there was no significant difference between concentrations of 2 and 4 dS/m.

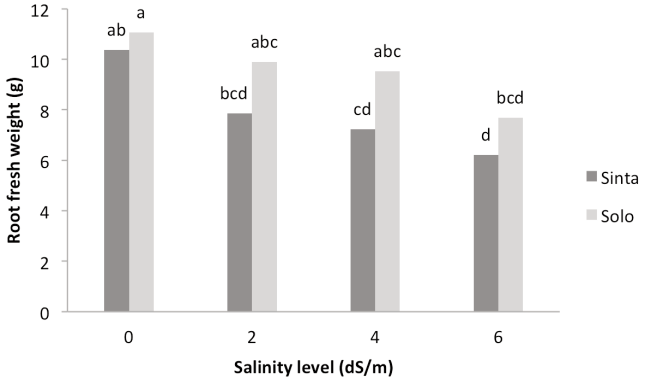

Fig. 3 - Interaction of cultivar and salinity levels on root fresh weight. The means followed by the same letters were not significantly different at p≤0.05.

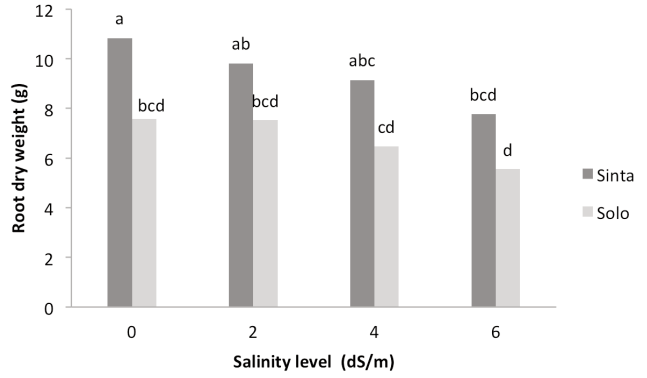

Fig. 4 - Interaction of cultivar and salinity levels on root dry weight. The means followed by the same letters were not significantly different at p≤0.05.

Evaluation of treatments applied in this study on shoots fresh weight indicated that control treatment in both 'Sinta' and 'Solo' had the highest values, while treatments 4 and 6 dS/m had the lowest values. A comparison of the two cultivars showed no significant differences in term of shoot fresh weight. In the comparison of salinity concentrations, no statistically significant difference was found between the control and 2 dS/m (Fig. 5).

According to figure 6, the findings of this study regarding the shoots dry weight showed that the highest dry weight was found in the control 'Sinta', where there was no significant difference between

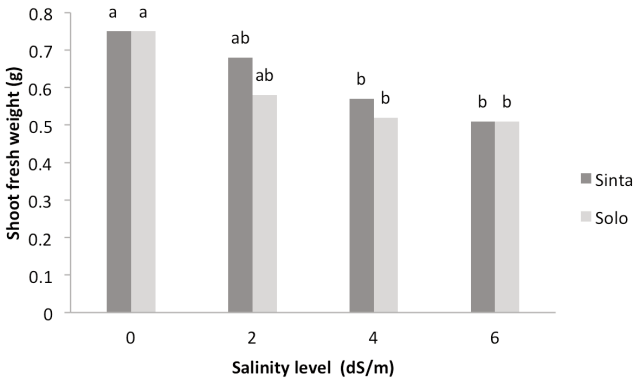

Fig. 5 - Interaction of cultivar and salinity levels on shoot fresh weight. The means followed by the same letters were not significantly different at p≤0.05.

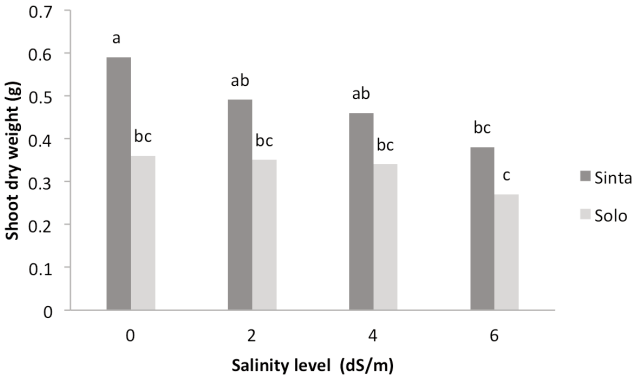

Fig. 6 - Interaction of cultivar and salinity levels on shoot dry weight. The means followed by the same letters were not significantly different at p≤0.05.

this treatment and the 2 and 4 dS/m salinity treatments. The lowest shoot dry weight was found in the 'Solo' 6 dS/m salinity treatment. A comparison of the two cultivars represented a significant difference in the shoots dry weight. Moreover, the comparison of various salinity levels showed no significant difference between the control and 2 dS/m salinity treatments for both cultivars.

The interaction effect of cultivar and salinity levels in this study showed that an increase in the salinity levels enhanced peroxidase activity in both cultivars. The highest enzyme activity was observed in 'Sinta' 6 dS/m salinity treatment while the lowest was in 'Solo' control treatment. A comparison of values between the cultivars showed a significant difference in terms of peroxidase activity. Moreover, the comparison of various salinity levels showed a significant difference between the 6 dS/m salinity treatment and other treatments for both cultivars (Fig. 7).

According to figure 8, the increased salinity levels in both cultivars enhanced catalase activity. The maximum level of this enzyme was found in the 'Sinta' 6

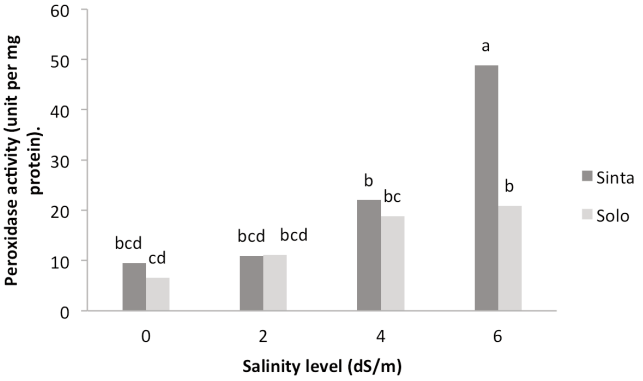

Fig. 7 - Interaction of cultivar and salinity levels on peroxidase activity. The means followed by the same letters were not significantly different at p≤0.05.

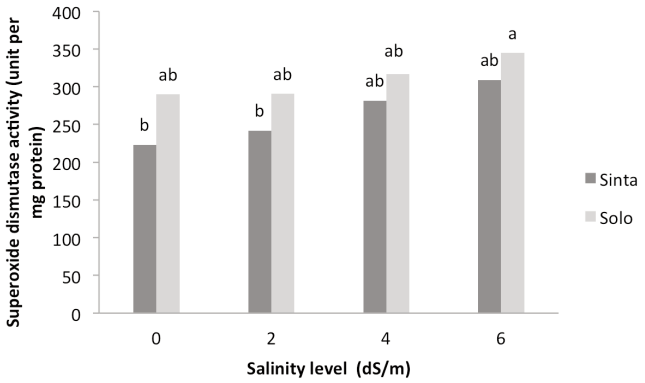

Fig. 9 - Interaction of cultivar and salinity levels on superoxide dismutase activity. The means followed by the same letters were not significantly different at p≤0.05.

dS/m salinity treatment, which showed a significant difference with other treatments, while the minimum values were observed in the control treatment of both cultivars. A comparison of the two cultivars showed no significant differences. In the showed results, there was a significant difference between the different levels of salinity in the 6 dS/m treatment and the control 2 dS/m, while there was no significant difference when compared to the 4 dS/m treatment.

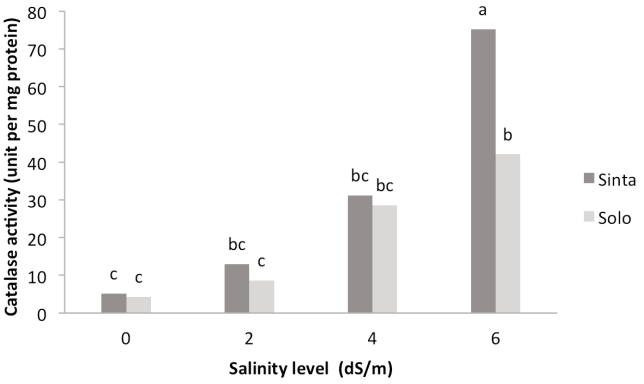

Fig. 8 - Interaction of cultivar and salinity levels on catalase activity. The means followed by the same letters were not significantly different at p≤0.05.

In this study, the interaction effect of salinity stress on superoxide dismutase activity showed an increase at higher salinity levels in both cultivars. The maximum activity of this enzyme was found in the Solo 6 dS/m salinity treatment which was not significantly different when compared with other treatments and the 'Sinta' 4 and 6 dS/m salinity treatments. The comparisons between the cultivars were significantly different. The comparison of different salinity levels showed no significant difference between the 4 and 6 dS/m treatments (Fig. 9).

Results reported in figure 10 showed that as salinity levels increased, the ascorbic peroxidase activity also increased. An evaluation of the interaction of salinity levels on the enzyme activity showed that the highest value was found in Sinta 6 dS/m treatment while there was no significant difference with the same treatment in 'Solo'. The comparison between the cultivars showed no significant difference. Moreover, a comparison of various salinity levels showed a significant difference between 6 dS/m salinity treatment and other treatments for both cultivars.

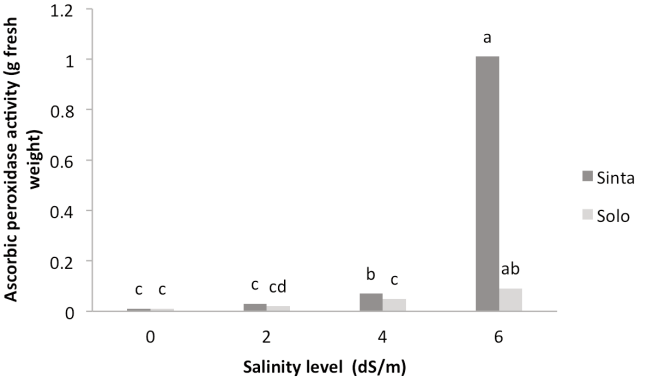

Fig. 10 - Interaction of cultivar and salinity levels on ascorbic peroxidase activity. The means followed by the same letters were not significantly different at p≤0.05.

From figure 11, the interaction effect of salinity levels on activity of total protein in this study showed that as salinity levels increased there was higher protein content in both cultivars. The maximum value was observed in 'Sinta' of 6 dS/m salinity treatment, which was significantly different from other treatments, while the lowest was in the control for the two cultivars which were not significantly different. No significant difference was found when the culti-

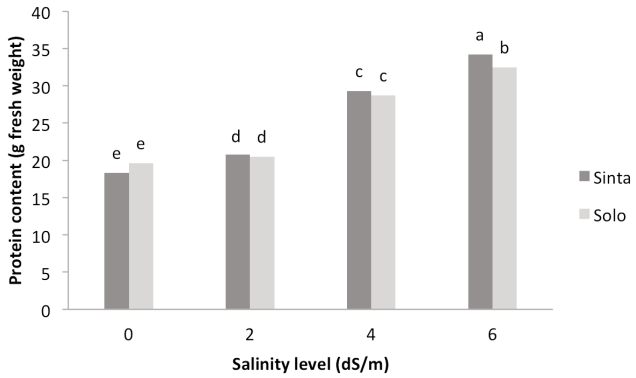

Fig. 11 - Interaction of cultivar and salinity levels on protein content. The means followed by the same letters were not significantly different at p≤0.05.

vars were compared. As for the various salinity levels, the results showed that the 6 dS/m salinity treatment was significantly different from other treatments.

Evaluation of interaction effect between salinity levels on proline showed an increase in salinity level in both cultivars, followed by higher proline. In fact, the maximum amount of proline was observed in the Sinta 6 dS/m salinity treatment which was significantly different from other treatments. There was no significant difference when both cultivars were compared. Moreover, the comparison of various salinity levels showed a significant difference between the 6 dS/m salinity treatment and other treatments for both cultivars (Fig. 12).

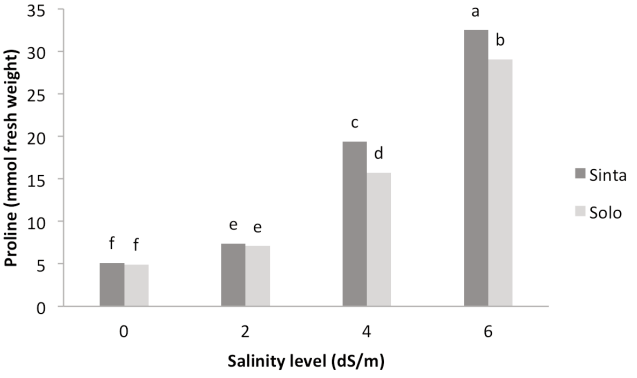

Fig. 12 - Interaction of cultivar and salinity levels on proline. The means followed by the same letters were not significantly different at p≤0.05.

4. Discussion and Conclusions

As mentioned in the results, the vegetative indicators decreased upon initiation of salinity treatments. Literature indicates that plants, especially glycophytes, are highly sensitive during their early vegetative growth, for instance chickpea (Khan *et al.,* 2016), pepper (Penella *et al.,* 2016), grapevine (Ikball *et al.,* 2014), tomato (Manai *et al*., 2014), rice (Horie *et al*., 2012), cantaloupe (Botia *et al*., 2005). Increased salt leads to Na^+ sediment into root growth area, thus reducing the ability to select K^+ versus Na^+ for root cells, which ultimately reduces their growth rate (Zhong and Lauchli, 1994). When exposed to saline conditions, plants show reduced uptake and low tissue retention of $K^{+.}$ (Chakraborty *et al.,* 2012; Gharsallah *et al.,* 2016). Accordingly, K^+ is considered as a key regulatory elements in plant metabolic process by promoting Na^+ exclusion and osmotic adjustment (Chakraborty *et al.,* 2016; Gharsallah *et al.,* 2016). Reduced shoot growth due to salinity usually appears as shoots with low growth and reduced leaf area (Lauchli and Epstein, 1990). Saline conditions reduce root growth and reduce water movement through the root with a decrease in hydraulic conductivity (Acosta-Motos *et al*., 2017). Root hydraulic conductance is expressed in terms of root dry weight. Root dry weight values which determine the root length and surface area, may vary greatly, thus affecting the water absorption (Jonathan *et al.,* 2006; Zobel *et al.,* 2007; Acosta-Motos *et al*., 2017). The results of our study are in accordance with these results. Decreasing in fresh weight or dry weight has been observed in all plant tissues subjected to salt stress especially in the aerial part (Acosta-Motos *et al*., 2017). The stem growth is also reduced by salinity conditions. One of the important reason for decreasing root and shoot growth under saline condition could be the decreasing of nitrogen uptake in response to external NaCl salinity due to antagonism between Na^+ and NH_4^+ or between Cl^- and No_3^- (Parihar *et al.*, 2015; Salachna and Piechocki, 2016). Another reason responsible for the reduction of vegetative index in the shoots, similar to what was mentioned for roots, is ion imbalance and increased ratio of Na to Ca (Neves-Piestun and Bernstein, 2005). In the current experiments, the concentration of proline, which has an important role in eliminating free radicals and enzymes, increased at higher salinity levels. Accumulation of proline under salinity conditions has been indicated to correlate with salt tolerance (Mansour and Ali, 2017).

In fact, to eliminate osmotic stress created by high salinity, plants need to synthesize compatible organic solutes such as proline in the cytosol (Gharsallah *et al*., 2016).

Besides its role as an osmolyte, proline contributes to scavenging ROS, stabilizing sub cellular structures and functioning as a signal (Szabados and

Savoure, 2010). Generally, plants under salinity conditions need to sustain their water potential (below potential of ground water) because they can continue absorbing water from the soil in order to maintain their turgor (Tester and Davenport, 2003). To maintain osmotic potential as well as ionic balance, plant cells during stress tend to accumulate any substance compatible with metabolism which does not interfere with other biochemical processes and are active in osmotic terms (Zhifang and Loescher, 2003). These substances cover a wide range of compounds such as carbohydrates, proteins and amino acids, including proline. This amino acid accumulates at a higher concentration than other amino acids in plant cells (Abraham *et al.,* 2003). It is possible that proline as a signaling molecule or regulator can activate the response as an adjustment process (Maggio *et al*., 2002). Hence, given the foregoing facts, there is a positive relationship between proline and curtailed damage of salt sensitivity, where the current study was consistent with previous works. In relation to salt damage to the overall growth of plant, the concentration of enzymes eliminating free radicals is very important. In their resistance against the damaging effects of reactive oxygen species, plants possess anti-free radical enzymes such as catalase, peroxidase, superoxide dismutase and other enzymes which eliminate reactive oxygen species and free radicals (Mittova *et al*., 2003). In our study, the concentration of eliminating free radicals enzymes such as peroxidase, catalase, superoxide dismutase and ascorbic peroxidase increased at higher salinity levels. The increases in theses enzymes activities are an adaptive trait to overcome salt damage by reducing toxic levels of H_2O_2 and provide protection against oxidative stress (Chawla *et al*., 2013; Gharsallah *et al*., 2016). Catalase, ascorbic peroxidase and glutation peroxidase have been reported as antioxidant enzymes in different plant tissues (Chawla *et al.,* 2013).

During salinity stress, the balance between production and consumption of reactive oxygen species (ROS) is disrupted, leading to the formation of condensation oxide (Spychalla and Desborough, 1990). ROS has the potential to damage cellular structures, perchloric acids, fats and proteins (Valko *et al*., 2006). In some salt-tolerant plants, increased in catalase activity have been recored after increasing NaCl, such as those described in myrtle, suggesting increased photorespiratory activity (Acosta-Motos *et al*., 2015, 2017). Catalase is often related to an enhanced tolerance to salt stress (Gao *et al.,* 2008; Gharsallah *et al*., 2016). Similarly, ascorbate peroxidase activity under salinity stress increases (Mittova *et al.,* 2004; Gharsallah *et al*., 2016). These responses to salinity were the results of differentially increased activities of ascorbate peroxidase and catalase over that of superoxide dismutase (Mittova *et al.,* 2004; Gharsallah *et al*., 2016). The results of our study were in accordance with these results.

The results of this study indicated also that as salinity levels increased, there was higher protein content in both cultivars. This result was in agreement with that of Abdel-Haleem (2007) who reported as increase in protein band which might be involved in mungbean tolerance. Plants growing in saline environments show distinct changes in the pattern of synthesis and accumulation of proteins. Salinity causes either decreased or increase in the level of soluble proteins or completely disappears in some proteins when compared to the control treatment (Win and Zaw, 2017).

Salinity is an important stress in arid and semi-arid region that reduces crops productivity, including that of the cultivars of *Carica papaya* L. here investigated ('Sinta' and 'Sola'). Shoots and roots growth were decreased under salinity conditions. There was no significant difference between the two cultivars in terms of root length, shoot length, fresh weight of roots and shoots in different salinity levels. Interestingly, on the other hand, in both cultivars increasing salinity led to higher peroxidase, catalase, superoxide dismutase and ascorbic peroxidase activity to provide protection against oxidative stress. Also the increases in those enzymes activities are an adaptative trait to overcome salt damage by reducing toxic levels of H_2O_2. Furthermore, in this experiment the concentration of proline, which has an important role in eliminating free radicals, increased at higher salinity levels in both cultivars; plants need to synthesize compatible organic solutes such as proline. Finally, in this study increasing salinity led to higher protein content in both cultivars; proteins play a major role in salt stress acclimation and plant cellular adjustment. Salinity causes either decrease or increase in the level of soluble proteins.

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