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Full Length Research Paper

The salicylic acid effect on the tomato (*Lycopersicum esculentum* Mill.) sugar, protein and proline contents under salinity stress (NaCl)

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Plants growth is impressed by biotic and abiotic stress inversely. There are many reports about proteins change level in salinity stress. Leaves fill up more soluble sugar of glucose, fructose and proline with treatment of salicylic acid. In this research, tomato seeds planted in pots containing perlite were put in a growth chamber under controlled conditions of 27 ± 2 and 23 ± 2°C temperature, 16 h lightness and 8 h darkness, 15 Klux light intensity and 75% humidity; NaCl concentration of 0, 25, 50, 75 and 100 mM and salicylic acid concentration of 0, 0.5, 1 and 1.5 mM were used in the form of factorial experiment in a complete randomized design (CRD) . Salinity increases the soluble sugar in leaf and root tissues, and salicylic acid decreases it. The leaf protein level decreased because of salinity effect, but salicylic acid could increase it. In the root, salinity increases protein, but salicylic acid with 1.5 mM concentration decreases it. Salinity increases the proline level in leaf and root, and salicylic acid did not significantly change in low salinity levels.

Key words: Salinity, stress, salicylic acid, tomato, proline, protein and sugar.

INTRODUCTION

Plants' growth and production are affected by natural stresses in the form of biotic and abiotic stresses, inversely. The abiotic stress causes loss of hundred million dollars annually, because of reduction and loss of products (Mahajan and Tuteja, 2005). Salinity is the most important limiting factor for crop production and it is becoming an increasingly severe problem in many regions of the world.

Plant's behaviorial response to salinity is complex, and different mechanisms are adopted by plants when they encounter salinity. The soil and water engineering methods increase farm production in the damaged soil by salinity, but achievement of higher purposes by these methods seems to be very difficult (Yokoi et al., 2002). The high salinity of the soil affected the soil penetration, decreased the soil water potential and finally caused physiological drought (Yusuf et al., 2007).The plants under salinity condition change their metabolism to overcome the changed environmental condition. One mechanisms utilized by the plants for overcoming the salt stress effects might be via accumulation of compatible osmolytes, such as proline and soluble sugar. Production and accumulation of free amino acids, especially proline by plant tissue during drought, salt and water stress is an adaptive response. Proline has been proposed to act as a compatible solute that adjusts the osmotic potential in the cytoplasm. Thus, proline can be used as a metabolic marker in relation to stress. The tomato is a major vegetable crop that has achieved tremendous popularity over the last century. It is grown in practically every country of the world - in outdoor fields, greenhouses and net houses.

Tomatoes, aside from being tasty, are very healthy as they are a good source of vitamins A and C. Compared with other plant, much less is known about the mechanism behind tomato plant response to salt stress.

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Salicylic acid is a plant phenol, and today it is in use as internal regulator hormone, because its role in the defensive mechanism against biotic and abiotic stresses has been confirmed. This research studies the salinity and salicylic acid effects on sugar, protein and proline contents of tomato which is sensitive to salinity.

MATERIALS AND METHODS

Planting

At first, the seeds were disinfected with hypocholorid sodium, and then 5 seeds were planted in each pot containing perlite and kept in a growth chamber under controlled conditions of 27 ± 2°C, 23 ± 2°C temperatures, 16 h lightness, 8 h darkness, respectively, 15 Klux light intensity and 75% humidity. Then the pots were irrigated with deionized water and nutrient solution, with 6.5 pH every two day for one month. NaCl factor at 5 levels including 0, 25, 50, 75, 100 mM and salicylic acid treatment at 4 levels including 0, 0.5, 1 and 1.5 mM were used. The experiment was performed as factorial in the form of completely random plan (CRD Design) with 3 repetitions (60 pots). Salicylic acid treatments were sprayed on the leaves every two days for two weeks, and then different levels of salinity factor were used every two days with nutrient solution for 15 days.

The measurement of sugar content based on somogy 1952 method

0.05 g of fresh tissue of leaf and root was weighted by laboratory subtle scale (satrius) BP211D model with 0.0001 g accuracy. Each sample was grinded with 10 ml deionized water in a china mortar, then the mortar content was transferred to small container and located on a heater to boil. After that, the container contents were filtered by watman filter paper (number 1), for plant extraction. 2 ml of each extractionwas transferred to a test tube and 2 ml copper sulfate solution was added to each of the tube. Then, the tube caps were closed with cotton. Each of these tubes was kept in warm water bath with 100°C temperature. In this term, CU^{2+} was reduced to CU_2O by monosacarido aldehid; here a brick red color was observed in the bottom of the test tube.

After cooling the pipes, 2 ml phosphomolibdic acid solution was added to them; after a moment, blue color appeared, and the test pipe was well shaken to spread the color within the test pipe. The solution absorption was in 600 nm, determined by spectrophotometer system, and then the sugar concentration was measured by using of standard curve. For spectrophotometer setting, a solution instead of plant extraction, which includes deionized water and the rest solution with sugar values, was measured and presented by using of relevant standard curve based on mg/g fw.

The measurement of protein concentration based on Bradford 1976 method

For protein extraction of root and leaf , one gram of each fresh tissue (leaf and root) was grinded in a chain mortar; it included 5 ml buffer Tris - HCl 0.05 M with pH=7.5. The obtained computable solution was transferred to centrifuge pipe and then, the samples were centrifuged by a refrigerator centrifuge for 25 min in 10000 g and 4°C. The obtained extraction was used for the measurement of protein solution concentration. Also, 0.1 ml protein extraction and 5 ml biore reagent were added to the test pipe, and vortexes quickly. After 25 min, their absorption was read by spectrophotometer system in 595 nm. The protein value was measured and presented by using of relevant standards curve based on mg/g fw.

Proline measurement method

0.02 g of root and fresh leaf tissue was grinded with 10 ml, 3% sulfosalicylic acid solution; the obtained extraction was centrifuged by using centrifuge napco 2028R model for 5 min in 10000 g. Then 2 ml of upper liquid was mixed with 2 mg ninhydrin reagent and 2 ml pure acetic acid; they were kept in hot water bath at 100°C for 1 h. After that for stopping all reactions, the pipes were cooled in ice bath, and then 4 ml Tollen's reagent was added, with the pipes well shaken. Separated layers were formed by fixing the pipes for 15 - 20 s. For measurement of proline concentration, the upper color layer of Tollens' reagent and proline were used. The absorption of some specific color material was determined through 520 nm, and the proline of each sample was obtained by using standard curve, based on mg/g fw.

Statistical analysis

In this study, the total number of experiments was done in different stages in completely randomized design with 3 repetitions; and the htest considers the reciprocal effect of Salicylic acid and salinity on different parameters as factorial. The levels of 0, 25, 50, 75, 100 mM of salinity were used and the levels of Salicylic acid were 0, 0.5, 1, 1.5 mM. The comparison of means was done with Duncan test to SPSS 12.0 software in probability level of 1%. For drawing graph, we used Excel 2003 software.

RESULTS

Leaf sugar

By high salinity concentrations, the level of sugar in leaf increases. Salicylic acid decreases the sugar at 0 and 25 mM salinity, which is related to 1 mM salicylic acid concentration. The highest reduction in the level of sugar in leaf in 50, 75, 100 mM salinity is related to 0.5 mM salicylic acid concentration. Salicylic acid balances in leaf sugar level by salinity control that decreases the sugar level due to salinity stress indirectly. The highest increasing sugar level at salinity is 100 mM and 0 mM salicylic acid concentration. The least sugar level is observed at 0 mM salinity and 1 mM salicylic acid (Figure 1).

Root sugar

According to Figure 2, at 0, 25 and 50 mM salinity level, salicylic acid decreases the sugar and at 75 and 100 mM NaCl concentrations, the sugar level increases at different salicylic acid concentrations. The balanced role of salicylic acid through salinity control is also observed at root sugar level, and the highest root sugar level is in 100 mM salinity and 0.5 mM salicylic acid concentration.

Leaf protein

According to Figure 3, salinity decreases the leaf protein

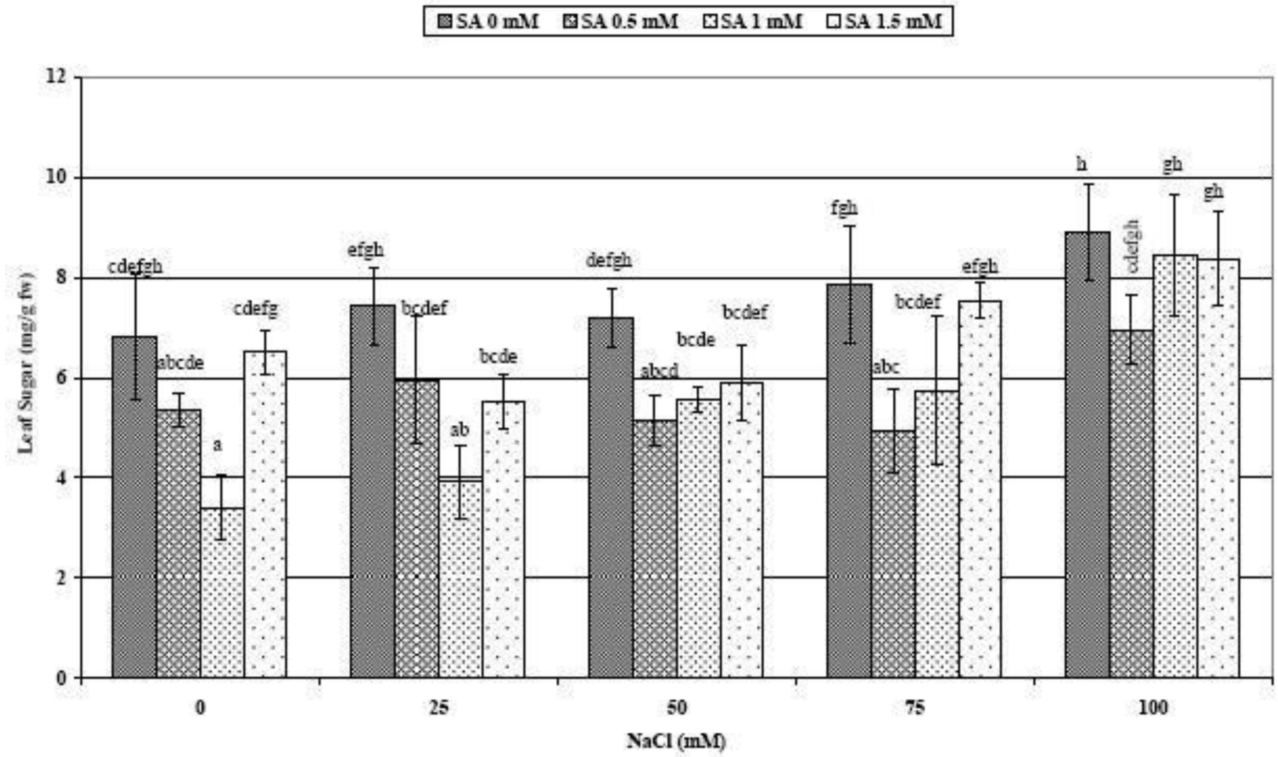

Figure 1. The effect of NaCl salinity concentrations and salicylic acid on the leaf sugar content.

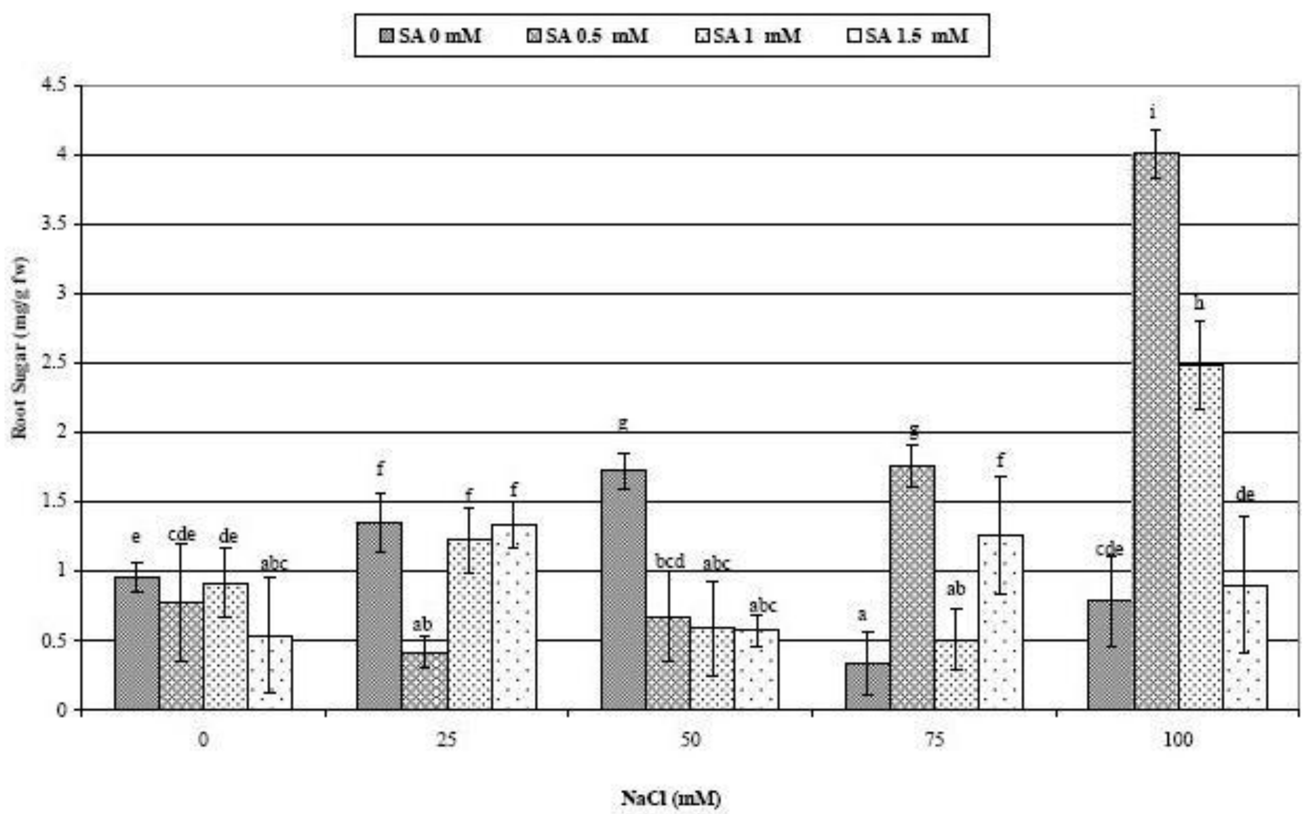

Figure 2. The effect of NaCl salinity concentrations and salicylic acid (SA) on root sugar content.

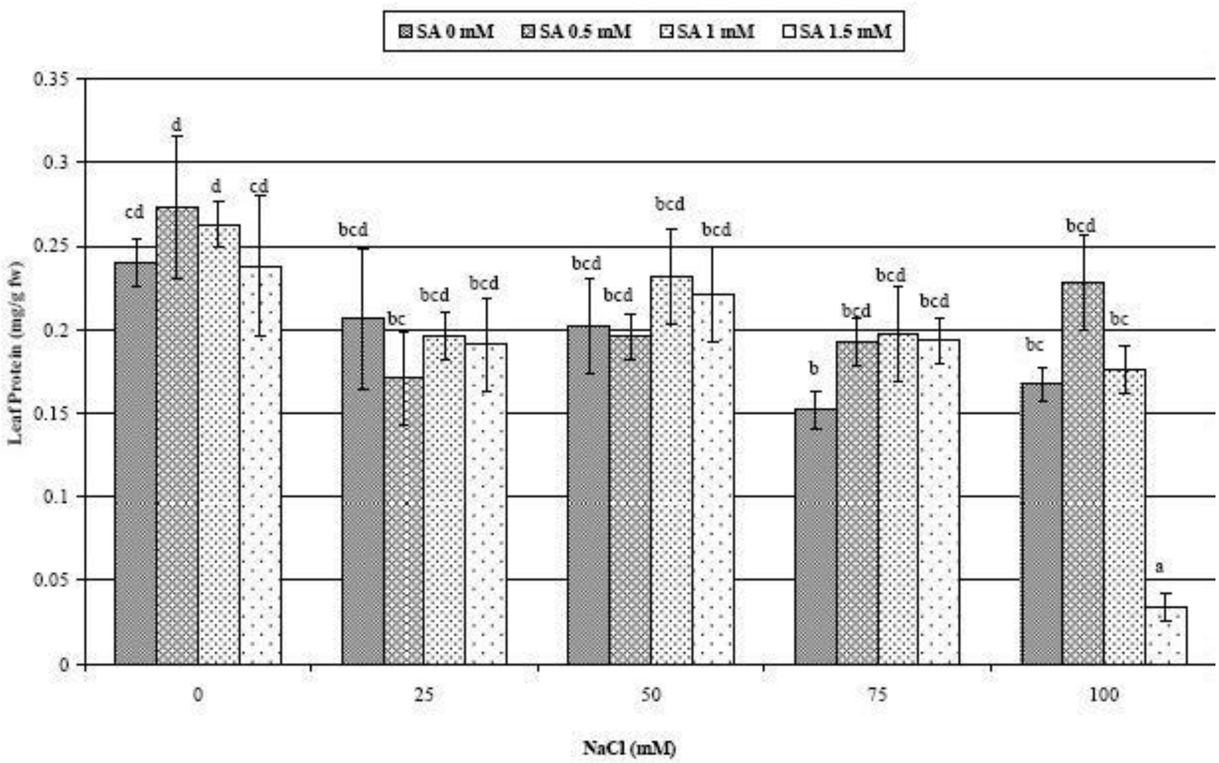

Figure 3. The effect of NaCl salinity concentrations and salicylic acid on leaf protein content.

level, which is more reduction at salinity high concentration (75 and 100 mM). Salicylic acid increases protein concentration by 50, 75 mM salinity levels. The least protein level is observed at 100 mM salinity level with 1.5 mM salicylic acid concentration, and also the highest level of it could be observed at 0 mM salinity level with 0.5 mM salicylic acid concentration.

It seems that there is negative interaction at high salinity concentration (100 mM) and salicylic acid treatment with high concentration (1.5 mM).

Root protein

All salinity levels except 100 mM level increases the protein. It seems that high defensive metabolism salinity makes trouble, but salicylic acid causes balance and decreases the protein level. The salicylic acid role could be observed at the balancing of high salinity concentration (Figure 4).

Leaf proline

Figure 5 shows that with increasing salinity level, proline increases, but decreases proline level by high salinity level at 75 and 100 mM, with use of salicylic acid treatment.

Root proline

Figure 6 shows that with increasing salinity, the root proline increases. But, there was no significant change atlow salinity level (0 - 25 mM), with use of salicylic acid treatment. With increasing salicylic acid concentration at 50, 70 and 100 mM salinity level, the proline decreases.

DISCUSSION

Sugar

The soluble sugar in oat organ plant (root and bud) increased with NaCl increasing (El-Tayeb, 2005). With salicylic acid, the leaves fill up more soluble sugar and proline (Szepesi, 2006). The increasing of photosynthesis carbohydrate is a signal for water deficiency tolerance. The high carbohydrate concentration with its role to reduce water potential helps to prevent oxidative losses and protein structure maintenance during water shortage. Also carbohydrates play a molecule role for sugar responsible genes that give different physiological response like defensive response and cellular expansion (Koch, 1996).

In one study on *prosopis alba*, salt stress increases soluble carbohydrate in the roots (Meloni et al., 2004).

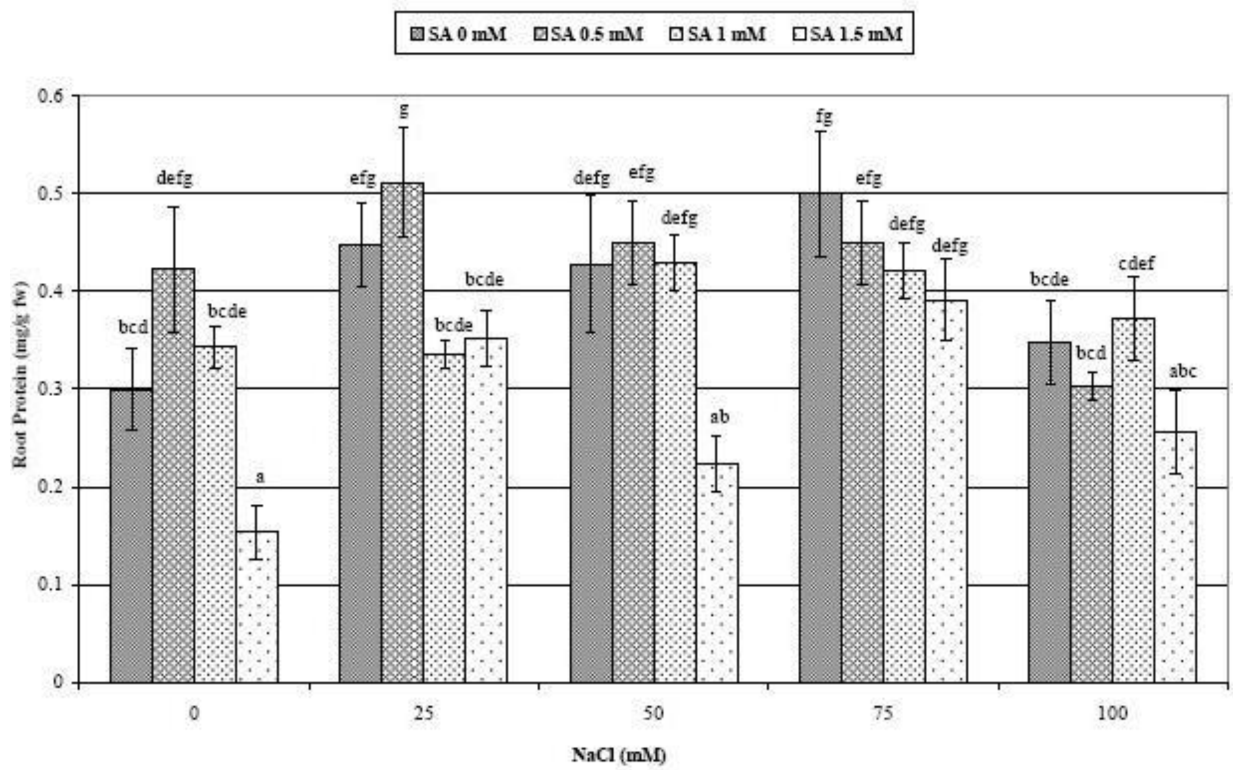

Figure 4. The effect of NaCl salinity concentrations and salicylic acid (SA) on the root protein content.

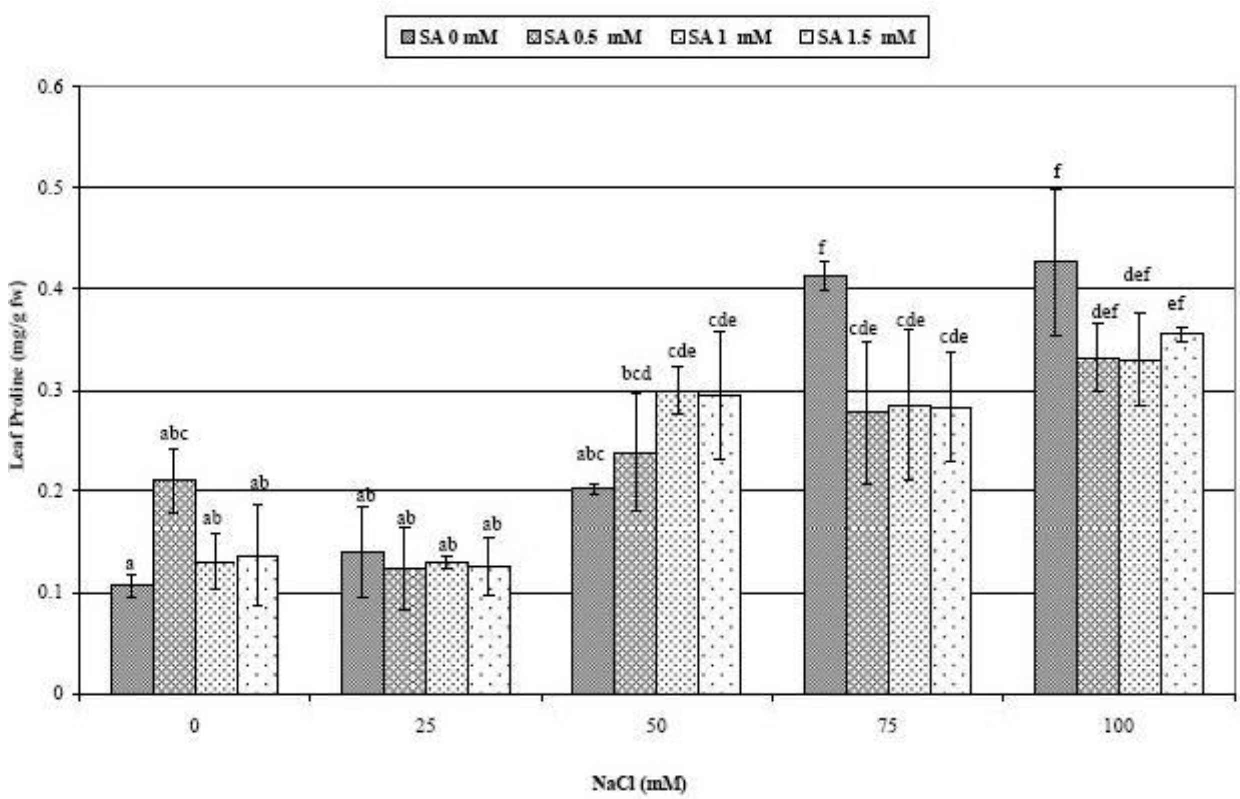

Figure 5. The effect of NaCl salinity and salicylic acid concentrations on leaf proline content.

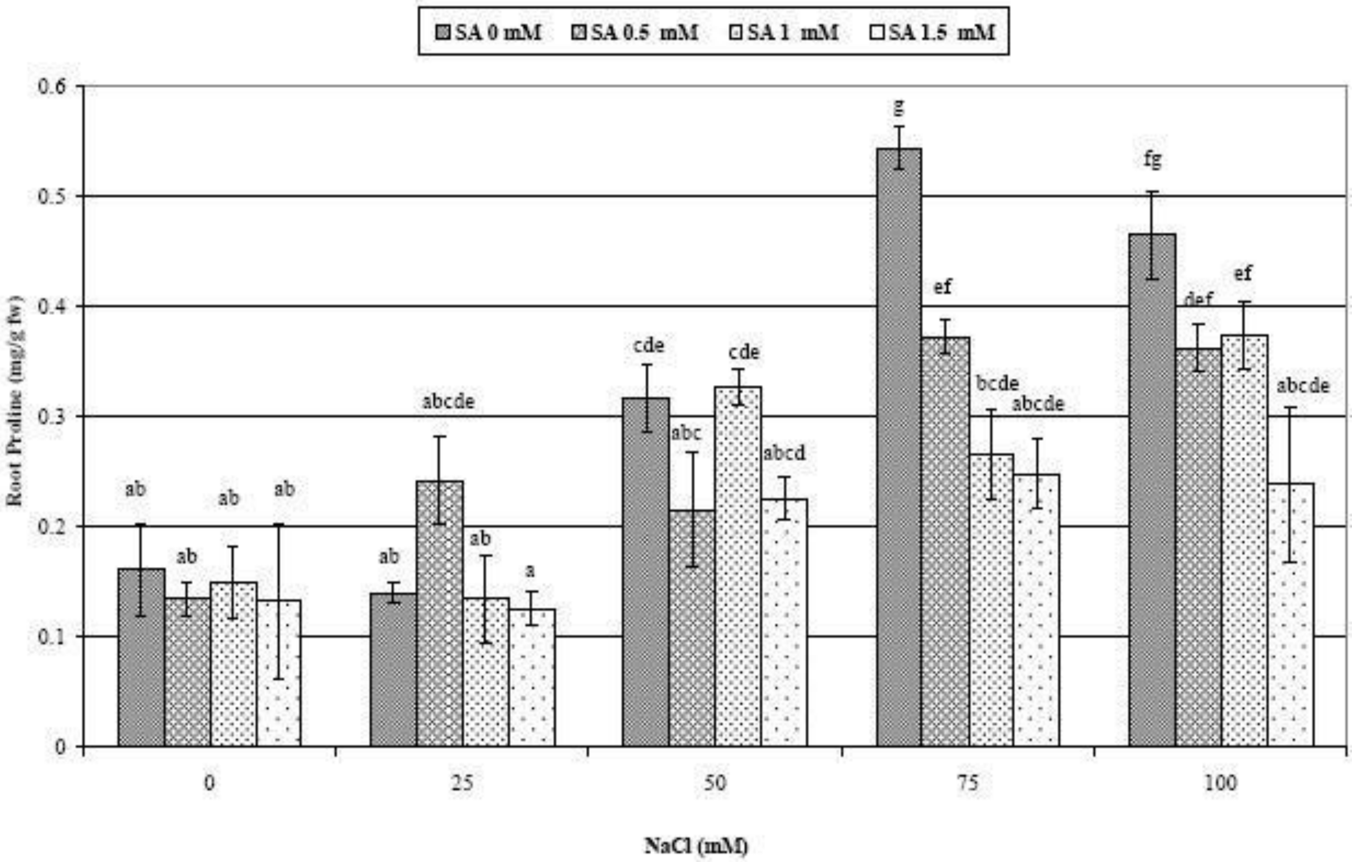

Figure 6. The effect of NaCl salinity and salicylic concentrations on root proline content.

In this research, salt stress increases leaf sugar, but salicylic acid decreases it. The same result was obtained in the root. Salicylic acid also causes balance in the sugar level at salinity stress condition. The increasing of induced glucose storage by salt stress is possible, that is for storage demand reduction of carbon or starch decomposition (Tattini, 1990). The increasing of all soluble carbohydrate in the root during salinity stress is effective on the balance against osmotic pressure. The plant cell for escaping from plasmolysis performance and creation during salt stress conditions should be changed and analyzed from macro molecule to micro molecule. Sucrose breaks down to glucose and fructose, and starch decomposition to glucose increases its osmotic pressure cell (Benbella, 1999). The use of salicylic acid could activate the consumption of soluble sugar metabolism by increasing osmotic pressure. It is supposed that salicylic acid treatment deranges the enzymatic system of polysacarid hydrolysis (Khodary, 2004). The salt stress in soybean varieties decreased sugar level (EL-Samad and Shaddad, 1997). In the accumulation of sugar at stress conditions, a protective mechanism enters the cell via sodium entry. So, some more of this kind of carbohydrate in cell area increases their membrane tolerance and selectivity versus ion entry like sodium and chloride (Fernando et al., 2000).

Protein

There are many reports about increasing and decreasing of protein level in salinity stress. The soluble protein and free amino acid in barley organs (root and bud) increased with NaCl increasing. The study of maize plant and also all amino acids increased with salicylic acid (El Tayeb 2005). The increasing of amino acid in the plant tissue under stress is related to protein fraction (Hussein et al., 2007). In this research, it is written that the leaf protein level decreased by salt stress but salicylic acid could increase it. The cause of protein reduction at salinity condition is the prevention of nitrate reductase activity (Undovenko, 1971). The salt stress induced some changes on the protein of rice leaf shoots and roots, but not effective on leaf blade. The level of some protein decreases because of protein synthesis reduction (Kong-Ngern et al., 2005) . Under high water stress, some plants produce materials with low molecular weight such as amino acid and polyamines, which reduce water potential

(Dantas et al., 2005). The plants produce some proteins in response to biotic and abiotic stresses, that some of these proteins deduct by phytohormones such as salicylic acid (Hussien et al., 2007). The increasing of nitrate reductase activity by salicylic acid depends on the material action with special inhibitors of nitrate (Ahmad et al., 2003). In this research, the salt stress increases the protein level in root, but salicylic acid decreases the protein levels.

The proteins at salt stress condition accumulate and act as osmotic regulator (Ahmad et al., 2003). The salinity stress deducts special protein in root and leaves of barley. Salinity stress increases amino acid content in wheat varieties (EL- Bassiouny and Bakheta, 2005). There are many reports about protein changes along with compatible stages that adapt the plants with changed environment (Kong-Ngern et al., 2005). The salinity stress interferes with nitrogen consumption and absorption. The salt stress condition could have effect on different stages of nitrogen metabolism, such as absorption, ionic reduction and protein synthesis (Meloni et al., 2004).

Proline

Proline is one of the most important compounds of plants defensive mixed action to salt stress. There are many reports about increasing proline under salt stress conditions in pretreatment wheat seedlings by salicylic acid and under salt conditions; the high level of ABA protected improves antistress activities (Sakhabutdinova et al., 2003; Inal, 2002).

According to the other studies, the proline accumulation by salicylic acid treatment increases in wheat, oat, bean and tomato, under oxidative stresses, (Tasgin et al., 2006). The more tolerant plants store more proline (Desnigh and Kanagaraj, 2007). In the present research, increasing salt increases proline in root and leaf, but salicylic acid at 0 and 25 mM of salt did not have a significant change on proline level. But, in high level of salt, the proline level decreases. Salinity and water shortage deduct the proline accumulation in seedlings which regulate osmotic pressure (Inal, 2002). High protection of abcissic acid in treated plants with salicylic acid and under salt stress increases proline and defensive proteins (Shakirova and Sakhabutdinova, 2003).

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