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ORIGINAL ARTICLE

The salicylic acid effect on the *Salvia officianlis* L. sugar, protein and proline contents under salinity(NaCl) stress

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Plant 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, *Salivia officialis* seeds planted in pots containing perlite were put in a growth chamber under controlled conditions of 27 ±2 ^{0}C and 23 ±2 ^{0}C temperature, 14h lightness and 10h darkness; NaCl concentration of 0,4,8,12 ds/m and salicylic acid concentration of 0,1,2,4 mM were used in the form of factorial experiment in a complete randomized design (CRD). The results demonstrated that increasing of proline and sugars due to osmotic slope in plants lead to increasing of tolerance against dehydrations of leave content and acceleration of plant developments in stress conditions.

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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 behavioural 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 purpose 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.* 2008). The plants under salinity condition change their metabolisms 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 draught, 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. *Salvia officinalis* (garden sage, common sage) is a small, perennial, evergreen sub shrub, with woody stems, grayish leaves, and blue to purplish flowers. It is a member of the family Lamiaceae and is native to the Mediterranean region, though it has naturalized in many places throughout the world. It has a long history of medicinal and culinary use, and in modern times as an ornamental garden plant. The common name "sage" is also used for a number of related and unrelated species. 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 *Salvia officials*.

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 ±2ºc, 23±2ºc temperature, 14 h lightness, 10 h darkness. Then the pots were irrigated with deionized water and nutrient solution, every two day for one month. NaCl factor at 4 levels including 0,4,8,12 ds/m and salicylic acid treatment at 4 levels including 0,1,2 and 4 mM were used. The experiment was performed as factorial in the form of completely random plan (CRD design) with 3 repetitions (48 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 14 days.

The measurement of sugar content based on somogy1952 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 extraction was transferred to a test tube and 2 ml copper sulphate 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 100ºC temperature. In this term, Cu^{Z+} was reduced to Cu_20 by monosaccharide 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 - HCI 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 1000 g and 4ºC. 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. (Bradford, 1976)

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 1000 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 100ºC. 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.

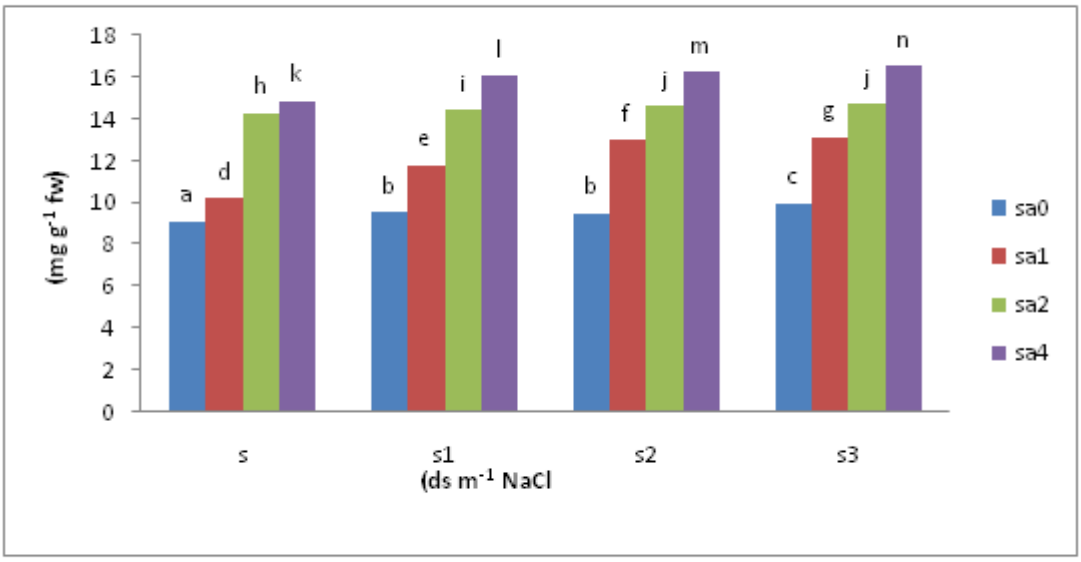

Figure 1.The effect of NaCl salinity concentrations and salicylic acid on the leaf sugar content

Statistical analysis

In this study, the total number of experiments was done in different stages in completely randomized design with 3 repetitions and the test considers the reciprocal effect of salicylic acid and salinity on different parameters as factorial. The levels of 0, 4, 8, 12 ds/m of salinity were used and the levels of salicylic acid were 0, 1, 2, 4 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. Also salicylic acid increases the sugar. The highest increasing sugar. level at salinity is 4 mm and 12 ds/m salicylic acid concentration. The least sugar level is observed at 0 mM salinity and 0 ds/m salicylic acid (Figure 1).

Leaf protein

According to Figure 2, salinity decreases the leaf protein level. Salicylic acid increases protein concentration. The least protein level is observed at 0 mM salinity level with 0 ds/m salicylic acid concentration, and also the highest level of it could be observed at 4 mm salinity level with 12 ds/m salicylic acid concentration.

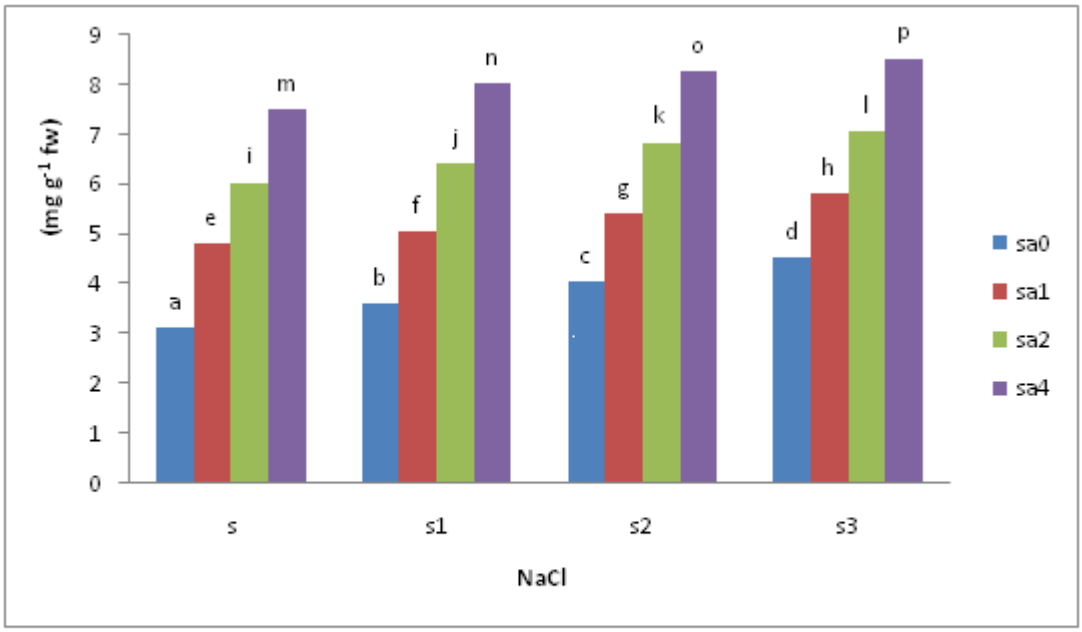

Figure 2.The effect of NaCl salinity concentrations and salicylic acid on the leaf protein content

Leaf proline

Figure 3 shows that with increasing salinity level, proline increase, also increases proline level by high salicylic acid level.

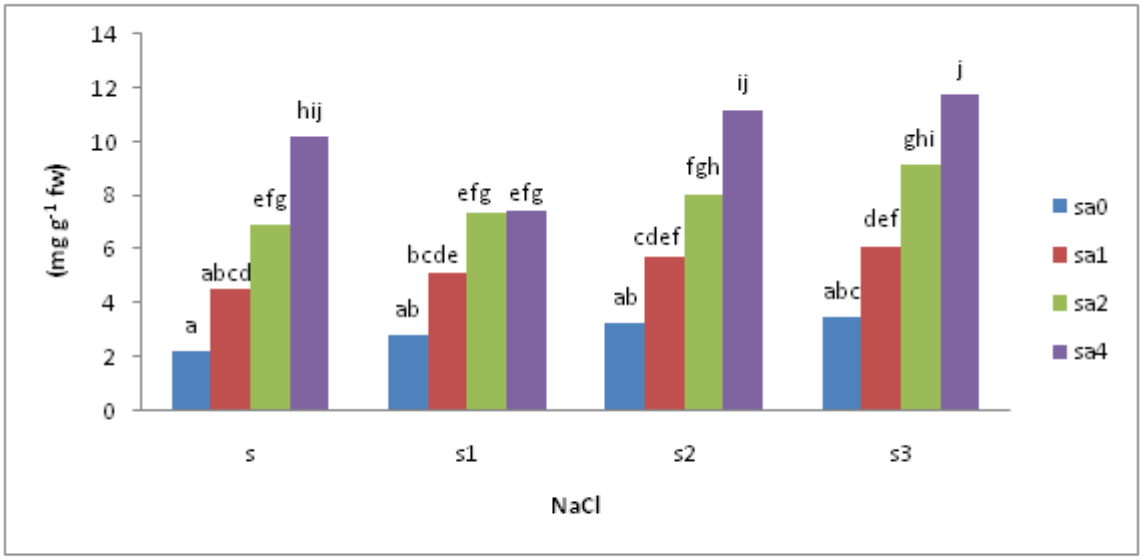

Figure 3.The effect of NaCl salinity concentrations and salicylic acid on the leaf proline content

DISSUSSION

Sugar

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 struction 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 this research salt stress and salicylic acid increases leaf sugar. Salicylic acid cause 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, 1996). 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 (El Midaoui *et al*, 1999). Marian and colleagues (2000) reported increase in soluble sugar content of the root of tomato under salt (NaCl) stress. 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 polysaccharide hydrolysis (Khodary, 2004).

Protein

There are many reports about increasing and decreasing of protein level in salicylic stress. The soluble protein and free amino acid in barley organs (root and bud) increases with NaCl increasing. The study of maize plant and also all amino acid 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 root 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). In this research, the salt stress decreases the protein level in leaf, but salicylic acid increases the protein levels. The proteins at salt stress condition accumulate and act as osmotic regulator (Fairduddin *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-Bassiony 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. Increasing proline leads to increase in resistance to salt stress the amount of this increase is different between different varieties. (Sairam *et al,* 1998). Source of proline is total protein and total amino acid.

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 leaf. In high level of salt , the proline level increases. There is a direct relation between accumulation of osmolates (proline and sugars) and increasing resistance in plants in abiotic stress condition (Ramanjulum *et al.* 1998) . High protection of abscisic acid in treated plants with salicylic acid and under salt stress increases proline and defensive proteins (Shakirova, Sakhabutdinova, 2003).

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