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Effect of Gibberellins on Sugar, Protein and Malondialdehyde Contents in Savory Plant (*Satureja hortensis* L.) under Salt Stress

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ABSTRACT

Salinity is one of the main obstacles to the development and production of plants. Gibberellin affected physiological and biochemical parameters of plants under salt stress. In present research, the effects of different concentrations of sodium chloride (0, 30, 60, 90 and 120 m M) and gibberellin (0 and 100 µg L^{-1}) on certain of physiological parameters in *Satureja hortensis* were studied. The results showed that soluble sugar and malondialdehyde contents were significantly increased by salt treatments in comparison to control plants, while combined application of GA_3 and NaCl caused reduction of malondialdehyde content. With increasing of NaCl concentration soluble protein content significantly decreased however, adding gibberellin in the medium, soluble protein content increased significantly as compared to control.
These results showed that GA_3 could increase the tolerance of *Satureja hortensis* to salinity stress.
KEY WORDS: Salinity, gibberellin, protein, soluble sugar, malondialdehyde, savory

INTRODUCTION

Plant growth and yield is limited in many regions of the world by various biotic and abiotic stresses. In fact, environmental stresses are the most important factors that reduce the growth and yield of plants in the world (Mahajan and Tuteja, 2005). Overall, survival of plants under salt stress depends on the plants ability to understand and receive stimulus, production and transfer symptoms and beginning of the biochemical, physiological changes (Mahajan and Tuteja, 2005).

Effects of salt stress on changes of primary and secondary metabolic are intricate and unclear. There are some evidences that production of some compounds decline under salt stress but also there are more reasons that these reactions are not permanent and in many cases, increase of metabolics rate have been seen in salt stress conditions (Said Al Ahl and Omer, 2011).

Decrease in water potential and osmotic potential, along with the disappearance of inflammation, stomatal closure and reduction in growth are symptoms of salt stress. Decrease in photosynthesis, reducing the absorption of nutrients, growth and eventually plant death occur with boosting level of salinity (Azevedo Neto et al., 2004).

One of the most important biochemical changes that occur during salinity stress, is accumulation of reactive oxygen species (ROS), which disrupts cellular redox balance and causes oxidative stress, which in turn can causes lipid peroxidation, enzymes inactivation and DNA and proteins structure damaging that finally lead to destroying and reducing of these biological molecules (Moller and Kristensen, 2004).

Phytohormones treatments can effect on responses of plant to salt stress and can reduce damaging effects on plants. One of the most effective hormones is gibberellin, which can play an important role in improving plant growth under salt stress. Gibberellin has an important effect on specific cellular processes that can provide ideal growing conditions for plants (Frantz and Bugbee, 2002).

One of the most important medicinal plant that has long been used by humans, is savory. Savory (*Satureja hortensis* L.) is a herbaceous annual plant of the Lamiaceae (Labiatae) family.

It was obtained that savory can grow in area with salt concentrations of 30 until 50 mM, but savory growth rate and cellular components contents maybe change in these concentrations. So this research for perception of effects of different concentrations of salt and gibberellins on cellular components contents in savory plant was carried out.

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MATERIALS AND METHODS

Growth conditions and stress treatments:

Seeds of savory (*Satureja hortensis* L.) were selected uniformly and sterilized with 5% hypochlorite for 10 min. After germination, uniform seedlings were transferred into plastic pots containing sand. Pots were transferred to a growth chamber (17 h light periods, 200 µmol quanta m^{-2} s^{-1} light intensity, day/night and temperatures of 25±1°C in day and 18±1°C in night). 30-day-old Plants were treated with different concentrations of sodium chloride (0, 30, 60, 90 and 120 mM NaCl) and gibberellin (0 and 100 µg L^{-1}). At last, after 45 days of treatment period, 75-day-old plants were harvested for determination of physiological parameters.

Assay of soluble sugars content:

The contents of soluble sugars were determined according to the method of Somogyi (1952). First soluble sugars of dried leaf (0.1 g) were extracted with 80% ethanol. Then 2 ml of the extract was mixed with 2 ml copper reagent of Somogyi. The mixtures were transferred to boiling water bath for 20 minutes. After cooling 2 ml of Nelson's ars enomolybdate reagent was added. After 10 minutes, the absorbance of the reaction mixtures was measured at 600 nm using Spectrophotometer. The amounts of sugar was calculated with the help of standard curve obtained by using different concentration of standard glucose solution and the contents of soluble sugar was expressed as mg g^{-1} D.W.

Assay of proteins content:

Frozen leaf samples (0.5 g) were used for protein extraction according to the method of Bradford (1976). Samples were ground in 5 ml of 50 mM phosphate buffer (pH 7.5) using pre-chilled mortar and pestle. The phosphate buffer contained 1 µm EDTA, 1 µm PMSF and 1% PVP-40. Then the extract was centrifuged at 4°C at 15,000 × g for 30 min. The supernatant (protein extract) was used for measurement of protein content. The absorbance of the irradiated solution was read at 595 nm using a spectrophotometer.

Assay of Lipid peroxidation:

Lipid peroxidation was determined by measuring malondialdehyde (MDA) content (Heath and Packer, 1968). The plant materials (0.5 g) were homogenized in 5 mL of 0.1% (W/V) trichloro acetic acid (TCA) and centrifuged at 10,000×g for 20 min. To 1 mL aliquot of the supernatant, 4 mL of 0.5% thiobarbituric acid (TBA) in 20% TCA was added. The mixture was heated at 95ºC for 30 min and quickly cooled in an ice bath. After centrifugation at 10,000×g for 15 min the absorbance of the supernatant was recorded at 532 and 600 nm. The value for nonspecific absorption at 600 nm was subtracted. The concentration of MDA was calculated using extinction coefficient of 155 mM^{-1}.

Statistical analysis: Data were analyzed by using SPSS software and statistical significant was set at $P < 0.05$. Mean comparisons were done through Duncan's Multiple Range Test. All graphs were drown using Excel software.

RESULTS

Results showed that individual effects of sodium chloride and gibberellin and interaction effects of both of them on sugar content were dramatically significant and sugar content increased in all of treatments as compared to control (Figure1).

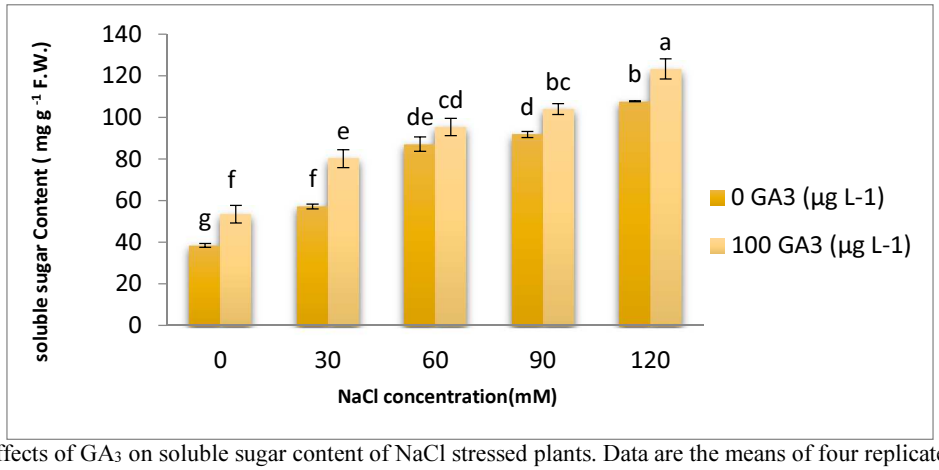

Figure1: The effects of GA_3 on soluble sugar content of NaCl stressed plants. Data are the means of four replicates (Mean ± SE) and different letters indicated significant differences at $P < 0.05$ level.

Soluble protein:

Results showed that individual effects of sodium chloride and gibberellin and NaCl × GA_3 interaction had different effects on soluble protein content. Hence different concentrations of sodium chloride decreased protein content compared to control but by adding 100 µg L^{-1} gibberellin to experimental pots, protein content considerably increased in comparison with other treatments.

Its known that salt is an inhibitor to synthesis of protein but it seems that gibberellin reduces inhibitor effects of salt. Also gibberellin has protection effect on protein molecules in salt stress conditions. The highest and lowest protein content were observed in 120 mM sodium chloride and 100 µg L^{-1} gibberellin with 23.547 mg g^{-1} F.W and 120 mM sodium chloride without gibberellin with 12.549 mg g $^{-1}$ F.W respectively (Figure 2).

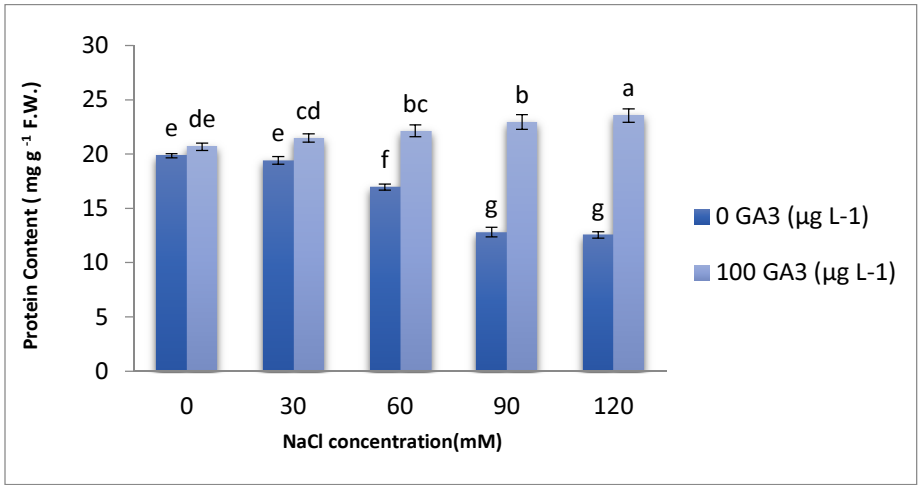

Figure2: The effects of GA_3 on protein content of NaCl stressed plants. Data are the means of four replicates (Mean ± SE) and different letters indicated significant differences at $P < 0.05$ level.

Lipid peroxidation:

Decrease of membrane stability index and increase of malondialdehyde (MDA) concentration are one of lipids peroxidation reasons that rise during oxidative stress.

Malondialdehyde (MDA) increased in individual treatment of sodium chloride and gibberellin and also interaction NaCl and GA_3 compared to control (Figure3), however, increasing levels of MDA were lower in gibberellin treatments. So it can be concluded that gibberellin can partly reduce lipids peroxidation and protect cells from salinity damages.

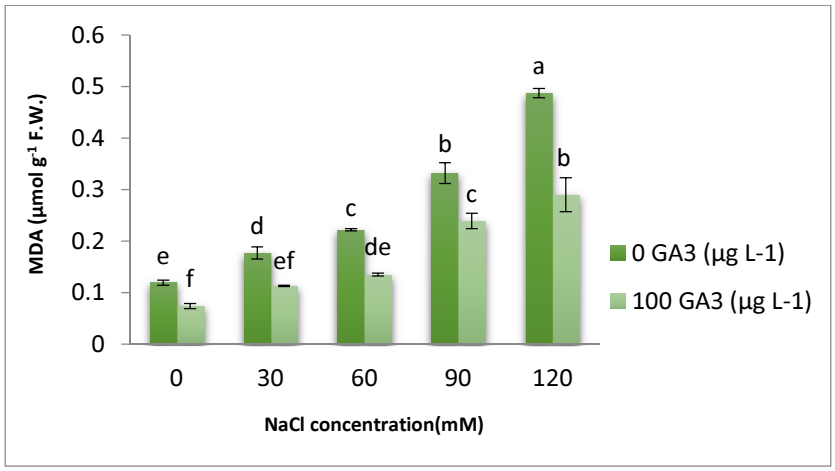

Figure3: The effects of GA_3 on the MDA of NaCl stressed plants. Data are the means of four replicates (Mean ± SE) and different letters indicated significant differences at $P < 0.05$ level.

DISCUSSION

Effect of NaCl and gibberellin on soluble sugar content:

Soluble sugars are the group of osmotic regulators that are increased within of plant cell under stress condition. They are compatible solutes, and do not interfere with enzymatic reactions within cells during osmotic stress, and act as osmotic protectors (Bohnert et al., 1995). Inflammation and water uptake are happened by accumulation of osmotically active solutes in salt stress. Carbon compounds for osmotic adjustment can be provided by both photosynthesis and sources of reserved carbon.

Osmotic regulators in plants are adjusted by conversion of polysaccharides like as starch to monosaccharides, because osmotic potential depends on number of insoluble molecules (Hendry,1993).

Guard cell of chloroplasts also contain large starch grains and when stomata is opened starch content of chloroplast will be decreased and when stomata is closed starch content will be increased.

Starch is an insoluble polymer of glucose with heavy weight that has no reducing effect on osmotic potential of the cells but hydrolysis of starch to soluble sugars, reduces the osmotic potential and osmotic pressure in stomata guard cells under stress condition (Taiz and Zeiger, 2012).

Cell development in plants is reduced during salinity stress and decreases of the conversion of soluble sugars to polysaccharides like as cellulose is happened and thus it causes the accumulation of soluble sugars in plant. Consequently increase of soluble sugar in stressful condition seems to be reason of cessation of growth and destruction of unsoluble sugar (Kerepesi and Galiba, 2000).

Koch (1996) revealed that the soluble sugars, during salt stress, can reduce inhibitory effects on the transcription of certain genes. For instance, decrease in transcription of small and large subunits of rubisco during salt stress, is a controlling mechanism that can be one of the reasons of soluble carbohydrates accumulation in leaves.

As mentioned above, the amount of soluble carbohydrates during salinity change and its amount has a direct correlation with photosynthesis, transfer of photo assimilates and respiration. In response to salinity, synthesis and accumulation of osmotic regulators within the plant are increased until to reduce osmotic potential and consequently can resist to salt stress. Increase amount of soluble carbohydrate in salinity stress has been reported in wheat seedling and is coincident with findings of this present study. The plant is more resistant to salt, the internal sugar synthesis capability is more (Kerepesi and Galiba, 2000).

Plaut and coworkers (2004) showed that sodium chloride cause increasing of soluble carbohydrates in tomato, this increased amount of glucose, fructose and sucrose in tomato fruit, create an osmotic balance and are followed by increasing activity of sucrose phosphate synthase enzyme (SPS) in fruits and leaves of tomato.

Gibberellin can increase leaf area and also can increase absorption of water and nutrient conductivity so this benefits of gibberellin in boosting of photosynthesis rate and sugar synthesis can be affected and used in plant under salinity stress (Colebrook et al., 2014).

Effect of NaCl and gibberellin on protein content:

Plants for reconstruction, tolerance, resistance and response to stressful conditions change the protein content (Swindell et al., 2007).

Retention of proteins in the form of their performance and prevention of unnecessary accumulation of protein for growth and maintenance of cells is important under stress conditions. Different families of proteins have been known that are synthesized and accumulated or that denatured and reduced in plant in the response to stress. These proteins are related to signal transduction, translation, immune mechanisms, metabolism of carbohydrates and amino acids that in the genomic level in the face of stress factors play direct or indirect roles. So to clarify the mechanisms of plant response to stress and its role in resistance to stress are important (Timperio et al., 2008). For example in plants, there is a set of 20 kDa proteins that are named as cbp-20.

The role of abscisic acid under stress conditions is very important for stomatal closure. Lack of function of the protein cbp-20 causes extreme sensitivity to abscisic acid, severely reduction of stomata conductivity and increases the tolerance to stress (Bacso et al., 2008).

The results of experiments on *Arabidopsis thaliana* showed that mutant plant of these proteins, have a better responses to stress, and protective role of mutation against water deficiency stress has showed. Photosynthesis in mutants decreased in response to stress and essential substances uptake in mutant plant was higher at a significant level and also peroxidation of lipids in response to water deficiency was lower (Bacso et al., 2008).

In addition, a series of proteins in nuclei of plant cell were identified and were named Della which perform the role of inhibitory mechanisms in plant. Mutation that effect on the genes of these proteins cause a double production level against wild type under fourteen day stress condition (Neumann, 2008).

Mentioned examples are cases that refers to the reduction of protein content in plants under stress. The results of this research also showed that increasing salinity decreased the amount of total protein content in savory leaves. This decreasing maybe the result of increasing of protein degradation by ROS in salinity stress condition. Reactive oxygen species (ROS), is made in NaCl stress, can cause oxidation of amino acid chains, formation of protein-protein links and finally fragmentation of proteins. The protein content depends on the difference between synthesis and degradation (Bartels and Sunkar, 2005).

Many researchers have reported decrease in protein content and increase in contents of nitrate, ammonium and amino acids in plant cell under salinity. Decrease in protein content can be for of decrease the activity of nitrate reductase, glutamine synthetase and glutamine oxoglutarate aminotransferase under salt stress (Younis et al., 1993).

There are different reports on the increase of protease enzyme activity in salinity (Parida and Das, 2005). Thus increase the amount of protein degradation by protease enzymes may decrease the amount of total protein in savory plants. Roy and coworkers (2005) reported that there was a reduction in total protein in radish (*Raphanus sativus*) under salt stress.

In addition, many results showed that GA_3 application can alleviate adverse effects of NaCl on proteins content and in this way augments content of proteins in plants under salt stress (Wen et al., 2010).

In present research, also the leaves of savory under salt stress that were sprayed with gibberellin solution showed one increase in total protein content. Gibberellin maybe increase the amount of heat shock protein (HSP) and these proteins are identified as resistance proteins, that are one of the symptoms of tolerance to salinity in plant and plant survival will be provided in salinity conditions. It has been found that the accumulation of toxic compounds including reactive oxygen species in the cells during salt stress, stimulate the expression of heat shock protein (Timperio et al., 2008).

Generally heat shock proteins (HSPs) accumulate in response to various environmental stresses, including heat, drought, salt and oxidative stress (Timperio et al., 2008).

For example study on heat shock proteins in transgenic tobacco showed an important role of these proteins in stress tolerance under water deficit and also there is a direct correlation between expression of heat shock proteins and drought tolerance (Cho and Hong, 2006).

On the other hand, gibberellin has a destructive role on structure of reactive oxygen species and protects macromolecules like as DNA and proteins from these compounds and so increase the protein contents. Gibberellin also increase the biosynthetic activity of proteins (Wen et al., 2010).

So, in this study, gibberellin certainly with destruction of ROS in decrease of protein degradation, and also increase of enzymes activities that function in protein biosynthesis has showed its role.

Effect of NaCl and gibberellin on lipids peroxidation:

Salinity stress has a destructive effect on membrane lipids metabolism. Because membrane lipids is the first cell structure that face with salt stress, so one of the salinity resistance mechanism is related to two-layer lipids and unsaturated fatty which ensure stability of cell membrane under salt stress (Sairam et al., 2002).

ROS that are made in salinity stress, are caused lipids peroxidation and increase of MDA content so it is related to damage of cell membrane (Borsani et al., 2001; Sairam et al., 2005). Lipid peroxidation, which can damage the membrane performance can be measured (Sairam et al., 2002). Evidence suggests that MDA is a breakdown product of unsaturated fatty acids used as a biomarker to measure lipid peroxidation (Mittler, 2002). In the present study, a significant increase in MDA level in savory herb plants that were treated with sodium chloride showed that this reflects the effect of salinity on membrane destruction.

Increase in MDA content in salt stress in rice (Demiral and Turkan, 2005) and lentil (Bandeoglu et al., 2004) have also been reported.

The intensity of the response to the production of malondialdehyde can be vary due to soil salinity and water or plant species, for example, there was the same salinity levels, different membrane lipid responses in different cultivars of rapeseed (*Brassica napus* L.) with different degrees of resistance to salt, followed (Apse and Blumwald, 2002).

On the other hand, it has been reported that application of gibberellin under conditions of salinity reduced MDA contents in the plant (Sairam et al., 2005), which corresponded with the results of this research. Studies have shown that, gibberellin plays its role by prevention of damage to fatty acids, decrease in membrane permeability and protection of thylakoid membrane during salinity stress and likely doing this effect by decreasing the amount of H_2O_2 (Borsani et al., 2001).

On the other hand, the reduction of lipid peroxidation in plants can be the result of gibberellin in the elimination of reactive oxygen radicals. Probability effect of these hormones on the activity of free radicals scavenger enzymes

decrease superoxide radicals and hydrogen peroxide content, and also prevent from activity of lipoxygenase and decomposition of membrane fatty acids (Colebrook et al., 2014).

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