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Full Length Research Paper

Effects of salinity stress on growth, chlorophyll content and osmotic components of two basil (*Ocimum basilicum* L.) genotypes

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Salinity stress is a severe environmental factor limiting crop yield worldwide. To better understand salt stress responses in crop plants, we compared effects of salinity stress on growth, chlorophyll content and osmotic components in two basil genotypes. Seedling of two basil genotypes (B_1 = *Ociumum basilicm* L and B_2= *Ociumum minimum* L.) were grown in controlled environment in Hoagland nutrient solution containing 0, 3 and 6 ds/m NaCl, respectively. Proline, soluble carbohydrates, chlorophyll 'a, b' and carotenes of leaves were determined 20 days after initiation of salinity stress. The results reveal that salinity caused significant decreases in growth of basil plants as measured by fresh weight. By increasing NaCl levels from 0 to 6 ds/m, the content of chlorophyll a and b, and carotenes reduced. Maximum reduction was observed at 6 ds/m of NaCl. Mean values of data showed that B_2 = *O. minimum* had the maximum reduction of chlorophyll a and b, and carotenes under salinity stress. This indicates that the B_2 genotype was more susceptible to salinity stress than B_1 genotype. In this study, salinity had no significant effect on soluble carbohydrate but the proline content varied among the basil genotypes whether the plants were grown with or without salinity stress. With 6 ds/m NaCl applied, proline content was enhanced in two genotypes. B_2 = *O. minimum* had the maximum proline content in leaves when plants were exposed to high salinity level.

Key words: Salinity, basil, chlorophyll content, osmotic components.

INTRODUCTION

Environmental stresses including salinity and temperature affect nearly every aspect of the physiology and biochemistry of plants and significantly diminish the yield. At present, about 20% of the world's cultivated land and approximately half of all irrigated land are affected by salinity (Zhu, 2001). Therefore, salinity is one of the most significant abiotic factors limiting crop productivity (Munnas, 1993). The most important process that is affected in plants, growing under saline conditions is photosynthesis. Reduced photosynthesis under salinity is not only attributed to stomata closure leading to a reduction of intercellular CO_2 concentration, but also to non-stomata factors. There is strong evidence that salt affects photosynthetic enzymes, chlorophyll and carotenoids (Stepien and Klobus, 2006). High salt concentrations, usually sodium chloride cause osmotic stress by decreasing water potential within the cells, and ionic stress due to specific inhibition of metabolic processes.

Plants respond to salinity by sequestering toxic ions in the vacuoles and accumulation of compatible solutes in the cytoplasm to balance the decrease of water potential (Di Martino et al., 2003). Biochemical studies have shown that plants under salinity stress accumulate a number of metabolites, which are termed compatible solutes because they do not interfere with biochemical reactions. These metabolites include carbohydrates, such as mannitol, sucrose and raffinose oligosaccharides, and nitrogen-containing compounds, such as amino acids and polyaminies (Bohnert et al., 1995).

The *Ocimum* genus, belonging to the *Lamiaceae* family, includes herbs and shrubs distributed in tropical and subtropical regions of Asia, Africa and the Americas. The most important species of *Ocimum* genus is *O.*

Table 1. Results of two-way analysis of variance (ANOVA) of salinity (S) and genotypes (G) and their interaction (S×G) for the variables listed.

Dependent variable	Independent variable		
	S	G	S × G
Fresh weight (FW)	0.002056*	0.001662^{ns}	0.00349*
Proline	648.972***	463.499***	99.449**
Soluble carbohydrate	0.001372^{ns}	0.01868^{ns}	0.00423^{ns}
Chlorophyll 'a'	0.021787*	0.003042^{ns}	0.039313*
Chlorophyll 'b'	0.004080*	0.00022^{ns}	0.008380*
Carotenes	0.007224*	0.001088^{ns}	0.010352*

*P < 0.0, **P < 0.01, ***P< 0.001. Numbers represent F values at 5% level, ns: not significant.

basilicum L.; this species, usually named common basil or sweet basil, is considered economically useful because of their basic natural characteristics as essential oil producers (Lawrence, 1993). Sweet basil is a popular culinary herb used in food and oral care products (Machale et al., 1997). The essential oil of the plant is also used as perfumery. Also, basil is well known as a plant of a folk medicinal used as carminative, galacto-gogue, stomachic and antispasmodic tonic and vermifugem, In addition, basil tea taken hot is good for treating nausea, flatulence and dysentery. Basil is used in pharmacy for diuretic and stimulating properties, in perfumes and cosmetics for its smell; in fact, it is a part of many fragrance compositions (Khatri et al., 1995).

Therefore, the objectives of this study were to study the effect of salinity levels on plant growth, photosynthetic pigments and osmotic components in two genotypes of basil.

MATERIALS AND METHODS

This study was conducted in a greenhouse at the University of Zabol, Iran during February to April, 2011. Two genotypes of basil were B_1: *O. basilicm* L. and B_2: *O. minimum L.* The experiment was laid-out in a complete randomized factorial design with three replicates. Surface-sterilized basil seeds were germinated in the dark on sand moistened with distilled water and the resulting seedlings were transferred to containers containing the following continuously aerated standard nutrient solution: 0.7 mM K_2SO_4, 0.1 mM KCl, 2 mM $Ca(NO_3)_2$, 0.5 mM $MgSO_4$, 0.1 mM KH_2PO_4, 10 µM H_3BO_3, 0.5 µM $MnSO_4$, 0.5 µM $ZnSO_4$, 0.2 µM Cu SO_4 and 0.01 µM $(NH_4)_6MO_7O_{24}$ (Melonid et al., 2001).

When the seedlings were seven days old they were canvassed for uniform size, and transferred to plastic containers and placed under hydroponic culture with 2 L of the earlier mentioned nutrient solution. The plants were grown under greenhouse conditions with a 12 h photoperiod of natural daylight, maximum and minimum temperatures of 26 and 18°C, respectively and relative humidity of 70% on average. Three salinity treatments (S_0 = 0 (control), S_1 = 3 and S_2 = 6 ds/m) were imposed to the nutrient solution after the plants were ten days old. The culture solution was weekly renewed and its pH was initially adjusted to 6.5. Twenty days after salt treatment, the plants were harvested.

Determination of proline, soluble carbohydrate and photosynthetic pigments

The extracts of the leaves were used to determine soluble carbohydrates (Irigoyen et al., 1992). Free proline was estimated according to Bates et al. (1973) in leaf samples, which were homogenized in 5 ml sulphosalycylic acid (3%) using mortar and pestle. With about 2 ml of extract in a test tube, 2 ml of glacial acetic acid and 2 ml of ninhydrin reagent were added. The mixture was boiled in a water bath at 100°C for 30 min and allowed to cool. When the reaction mixture was cool, 6 ml of toluene was added and the combination transferred to a separating funnel. After thorough mixing, the chromophore containing toluene was separated and the absorbance read at 520 nm in a spectrophotometer against a toluene blank.

Chlorophyll 'a' and 'b' of leaves were extracted with 80% aceton and determined according Arnon's method (1949), and spectrum absorption was measured at 645 and 663 nm. Carotenes were estimated at 440 nm.

Statistical analyses

All data were analyzed using the SAS Institute Inc. Version 6.12 Software. Initially, the data were analyzed in an analysis-of-varance (ANOVA) test to determine significance ($P \leq 0.05$) of the treatment effects. Significant differences between individual means were determined using Fisher's Protected Least Significant Difference Test.

RESULTS

Effect of salt stress on growth and photosynthetic pigments

The results reveal that the growth of the basil plants as measured by fresh weight, was significantly different between salt and non salt stressed or control during the exposure to stress treatment (Table1). By increasing salinity level from 0 (control) to 6 ds/m, fresh weight of two basil genotypes decreased. The reduced rate for B_1: *O. basilicm* was 17.8% and B_2: *O. minimum* was 28.2% (Figure 1).

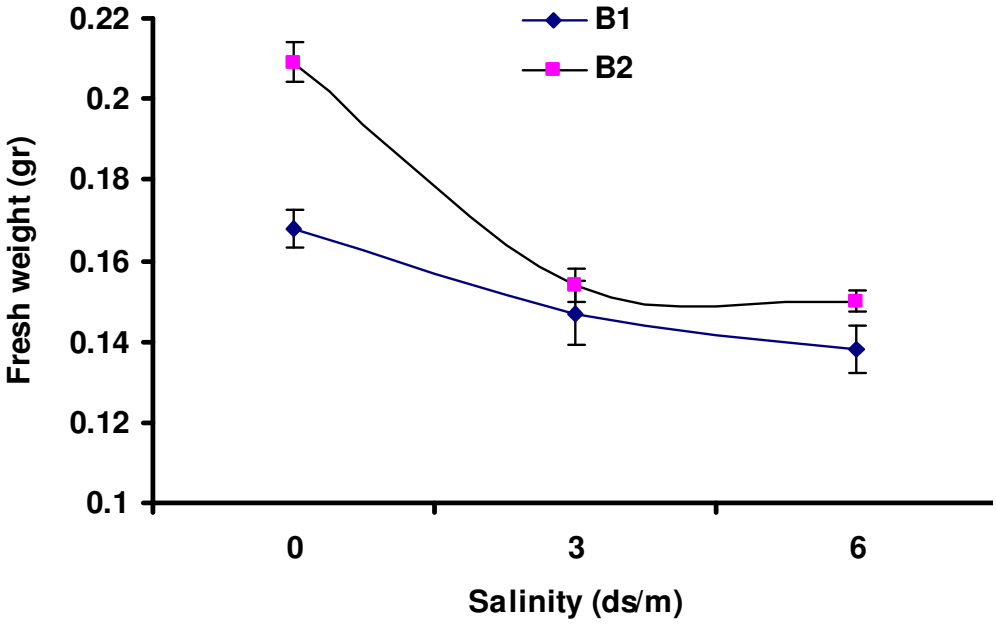

Figure 1. Effect of salinity on the fresh weight of basil genotypes.

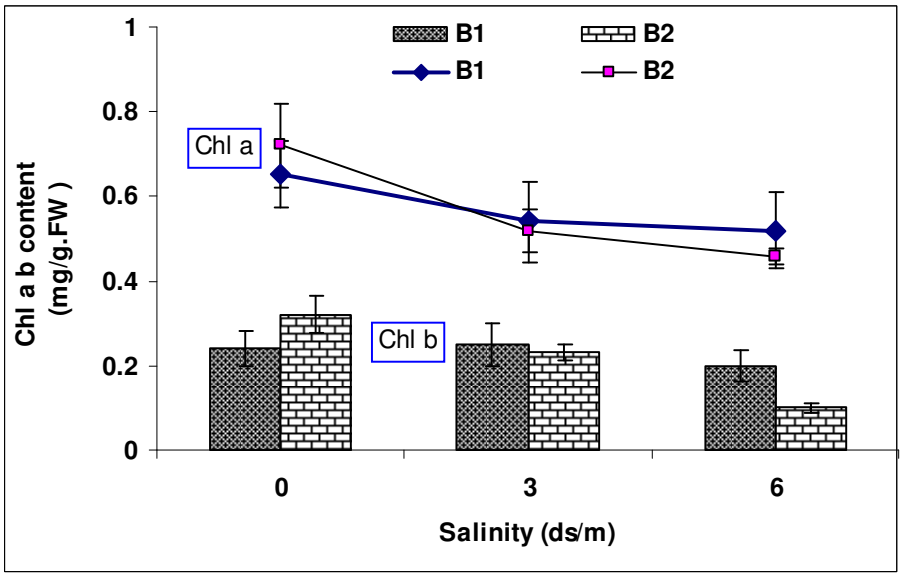

Figure 2. Effect of salinity on the chlorophyll a and b of basil genotypes.

Data regarding chlorophyll content is presented in Table 1. Statistical analysis of the data revealed that different salinity levels and the interaction between salinity and various genotypes had a significantly effect on chlorophyll a, b and carotenes content. By increasing salinity levels from 0 to 6 ds/m, these three photosynthesis pigments reduced. Maximum reduction was observed when plants were exposed to high salinity level (that is 6 ds/m). Mean values of data showed that B_2: *O. minimum* had the maximum reduction of chlorophyll a, b and carotenes under salinity stress (Figures 2 and 3).

Soluble carbohydrate and proline

Significant differences were observed between the genotypes of basil plants for proline in leaves (Table 1). In this study, the proline content varied among the basil

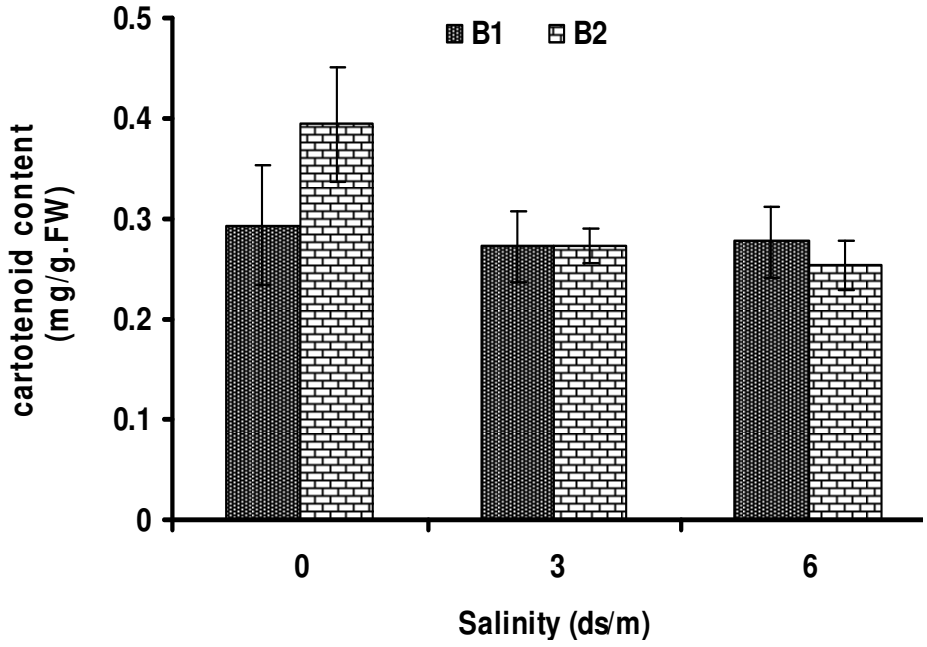

Figure 3. Effect of salinity on the carotenes of basil genotypes.

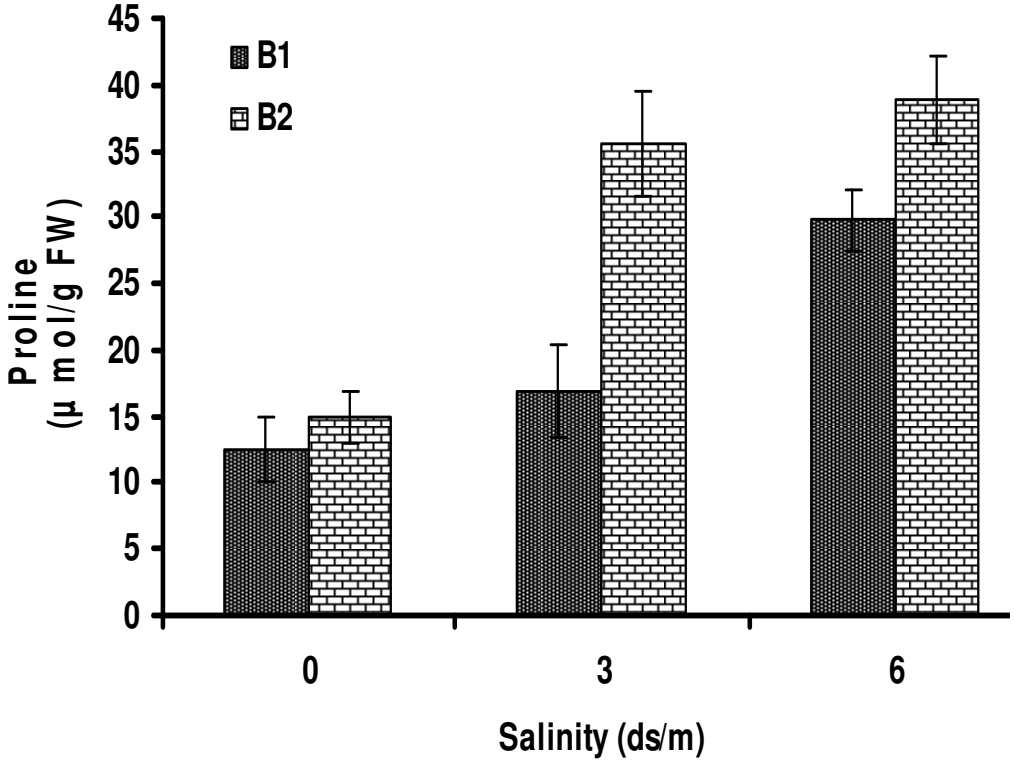

Figure 4. Effect of salinity on proline content of basil genotypes.

genotypes whether the plants were grown with or without salinity stress. With 6 ds/m NaCl applied, proline content was enhanced in the two genotypes (Figure 4). B_2: *O. minimum* had the maximum proline content in leaves when plants were exposed to high salinity level (that is 6 ds/m).

Statistical analysis of the data revealed that salinity had no significant effect on soluble carbohydrate content in leaves (Table 1). However, by increasing salinity levels from 0 to 6 ds/m, carbohydrate accumulation in leaves of

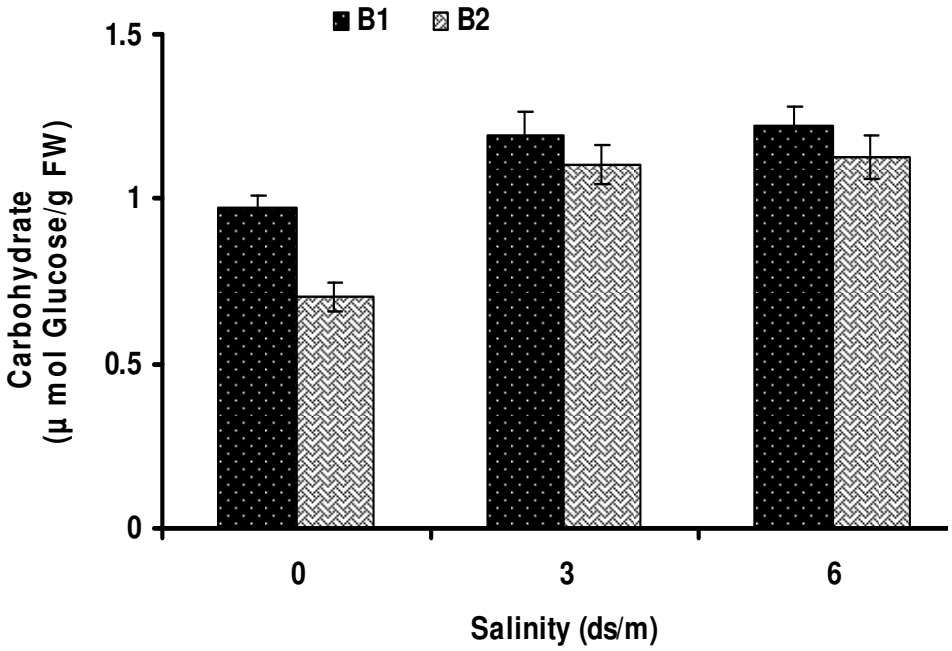

Figure 5. Effect of salinity on soluble carbohydrate content of basil genotypes.

basil plants increased (Figure 5).

DISCUSSION

The growth variation obtained here for basil plants may be attributed to the physiological scarcity of water due to increased osmotic pressure which is so common in saline soil (Munns et al., 2006). In this present study, salinity significantly affected the growth of the basil plants as measured by fresh weight (Table 1). Non-salinized basil plants had the greater fresh weight (Figure 1). Munns et al. (2006) indicated that salt in the soil water inhibits plant growth for two reasons. Firstly, the presence of salt in the soil solution reduces the ability of the plant to take up water; this leads to slower growth. This is the osmotic effect of salinity. Secondly, excessive amounts of specific salts entering the transpiration stream will eventually injure cells in the transpiring leaves, and this may further reduce photosynthesis and growth. Several studies have shown reductions in photosynthesis due to salt stress, which has been attributed to decrease in stomatal and mesophyll conductance of CO_2.

The negative effect of salinity on plant growth and water content may be due to the occurring of defect metabolism in plant cells. Since high osmotic pressure resulted from high salinity restricted plant cells to uptake water and some mineral nutrients dissolved in the culture medium (Cicek and Cakirlar, 2002). Rhodes and Samaras (1994) described that growth inhibition under osmotic condition might be mainly due to the reduction in cytoplasmic volume and the loss of cell turgor as a result of osmotic outflow of intracellular water.

In this present study, chlorophyll content in leaves was also affected by salinity (Table 1), and this effect depends on the levels of salinity. By increasing salinity levels from 0 to 6 ds/m, chlorophyll a, b and carotenes content in two basil genotypes decreased (Figures 2 and 3). The loss of chlorophyll under salt stress could be related to photoinhibition or ROS formation (Kato and Shimizu, 1985). The reduction in photosynthesis under salinity can also be attributed to a decrease in chlorophyll content. Salinity reduces the chlorophyll content in salt susceptible plants and increases it in salt tolerant plants. Salinity reducing growth in radish (*Raphanus sativus* L.) at high salinity level could be attributed to a reduction in leaf area expansion and hence to a lower light interception (Marcelis and Hooijdonk, 1999).

Osmotic adjustment in plants subjected to salt stress can occur by the accumulation of high concentrations of either inorganic ions or low molecular weight organic solutes. Although, both of these play a crucial role in higher plants grown under saline conditions, their relative contribution varies among species, among cultivars and even between different compartments within the same plant (Melonid et al., 2001). In this present study, the proline measured in the leaves of basil varied significantly with salinity (Table 1). Salinity had only significant effect on proline content and had no significant effect on soluble carbohydrate content in leaves (Table 1). By increasing salinity levels from 0 to 6 ds/m, proline accumulation in leaves of basil plants increased (Figure 4). Proline, sucrose, and other organic sugars in quinoa contribute to osmotic adjustment during stress and protect the

structure of macromolecules and membranes during extreme dehydration (Prado et al., 2000). Melonid et al. (2001) suggested that proline also serves as an important source of nitrogen in plant metabolism, as a readily available source of energy and as a reducing agent.

Conclusion

The results of this study showed that salinity stress had significant effect on growth, photosynthetic pigments and osmotic compounds of basil genotypes. However, salinity decreased the amount of Chlorophyll a and b, and carotenoid contents in both genotypes, but the B_2 genotype (*O. minimum*) had the greatest reduction of fresh weight, chlorophyll a and b. During salinity stress, between two osmotic components, proline greatly increased in leaves but salinity had no significant effect on carbohydrate content. In between two genotypes, B_1: *O. basilicm* was more resistance than B_2: *O. minimum* because it had the lowest reduction in chlorophyll, fresh weight loss and the accumulation of proline and carbohydrate in its leaves.

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