



Changes on Proline, Growth, Chlorophyll Content and Osmotic Components in *Lepidium sativum* L under Salt Stress

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ABSTRACT

Salt stress as a major adverse factor can lower leaf water potential, leading to reduced turgor and some other responses, and ultimately lower crop productivity in arid and semi arid zones. To better understand salt stress responses in crop plants, we compared effects of salinity stress on growth, chlorophyll content and osmotic components in cresses that were grown in controlled environment in Hoagland nutrient solution containing 0, 50, 100, 150 and 200 mM NaCl, respectively. Proline, soluble carbohydrates, chlorophyll 'a, b' and carotenes of leaves were determined 30 days after initiation of salinity stress. The results reveal that salinity caused significant decreases in growth of cress plants as measured by fresh weight. By increasing NaCl levels from 0 to 200 mM, the content of chlorophyll a and b, and carotenes reduced. Maximum reduction was observed at 200 mM of NaCl. Mean values of data showed that *Lepidium sativum* had the maximum reduction of chlorophyll a and b, and carotenes under salinity stress. In this study, salinity had no significant effect on soluble carbohydrate but the proline content varied among the cresses whether the plants were grown with or without salinity stress.

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Introduction

Cress (Imperial) is a plant belonging to the family of a year's Brassicaceae scientific name *Lepidium sativum* L. Garden cress which is known in English. This crop is economically important medicinal and edible plant. 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 CO₂ 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 polyamines (Bohnert et al., 1995).

Materials and Methods

This study was conducted in a greenhouse at the University of Zabol, Iran during January to March, 2015. cress seeds were

surface-sterilized for 5 min in sodium hypochlorite solution (0.5%) and then were rinsed with distilled water. Then fifteen seeds were sowed in a plastic pot (20 × 25) contained nonsaline sandy loam soil. Pots were transferred to green house under conditions of 26/18°C day/night temperature and natural light. The pots were irrigated by distilled water. Treatments supplied in five NaCl levels (0, 50, 100, 150 and 200 mM). Treatment was applied to each pot when second leaf was completely expanded and plants were harvested after thirty days.

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 sulphosalicylic 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% acetone 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

The experiment was as factorial based on a randomized complete block design with three replications. All data were analyzed using the SAS Institute Inc. Version 6.12 Software. Means were compared by Duncan Multiple Rang Test (P ≤ 0.05).

Results

Effect of salt stress on growth and photosynthetic pigments

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The results reveal that the growth of the cress plants as measured by fresh weight, was significantly different between salt and non salt stressed or control during the exposure to stress treatment. By increasing salinity level from 0 (control) to 200 mM, fresh weight of two cress decreased. (Figure 1).

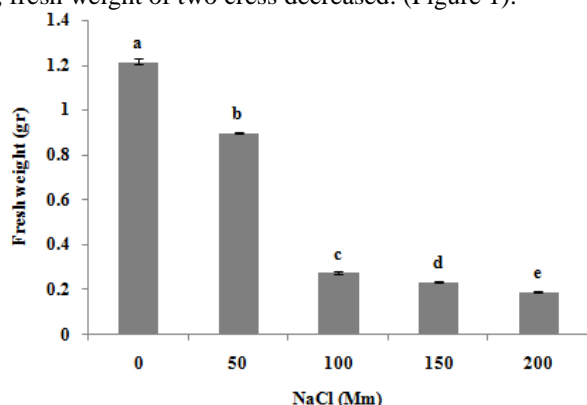


Figure 1. Effect of salinity on the fresh weight of cress

Statistical analysis of the data revealed that different salinity levels had a significantly effect on chlorophyll a, b and carotenes content. By increasing salinity levels from 0 to 200 mM, these three photosynthesis pigments reduced. Maximum reduction was observed when plants were exposed to high salinity level (that is 200 mM). minimum had the maximum reduction of chlorophyll a, b and carotenes under salinity stress (Figures 2 and 3).

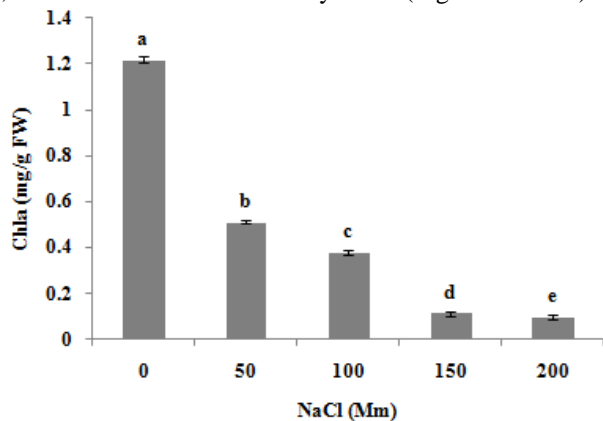


Figure 2. Effect of salinity on the chlorophyll a and b of cress

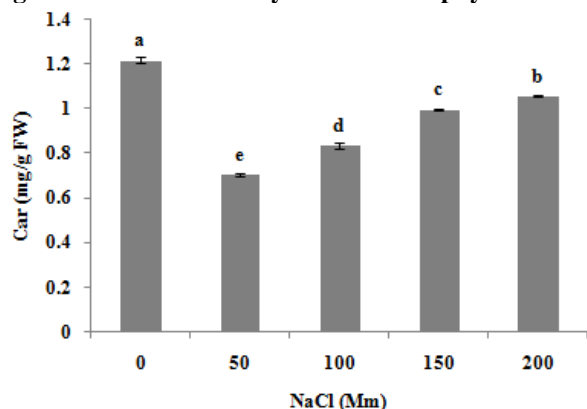


Figure 3. Effect of salinity on the carotenes of cress

Soluble carbohydrate and proline
Significant differences were observed for proline in leaves. In this study, the proline content varied among the cresses were grown with or without salinity stress. With 200 mM NaCl applied, proline content was enhanced in the cress (Figure 4). Statistical analysis of the data revealed that salinity had no significant effect on soluble carbohydrate content in leaves.

However, by increasing salinity levels from 0 to 200 mM, carbohydrate accumulation in leaves of cress plants increased (Figure 5).

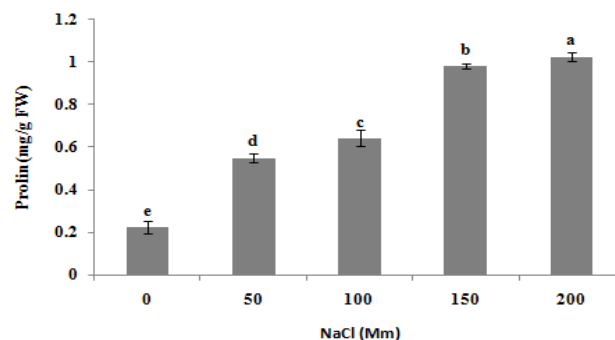


Figure 4. Effect of salinity on proline content of cress

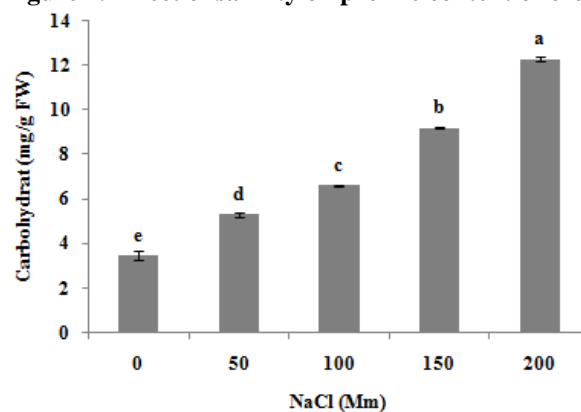


Figure 5. Effect of salinity on soluble carbohydrate content of cress

Discussion

The growth variation obtained here for cress 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 cress plants as measured by fresh weight. Non-salinized cress 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 CO₂. 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. By increasing salinity levels from 0 to 200 mM, chlorophyll a, b and carotenes content in two cress 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 cress varied significantly with salinity. Salinity had only significant effect on proline content and had no significant effect on soluble carbohydrate content in leaves. By increasing salinity levels from 0 to 200 mM, proline accumulation in leaves of cress 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.

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