

## Effects of Salinity on Photosynthetic Pigments, Respiration and Water Content in Two Barley Varieties

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**Abstract:** Salinity (NaCl Stress) was applied with 50, 100, 200, 300 and 400 mM NaCl. The shoot and root respiration of 2 barley cultivars (*Hordeum vulgare* L. var. Afzal and var. EMB 82-12) were determined in various concentrations of NaCl. Chlorophyll a,b and total chlorophyll content were significantly decreased, but carotenoids content increased under salinity. Decrease of chlorophyll content in EMB 82-12 was higher than Afzal, but carotenoids content in Afzal var. was higher than EMB 82-12. Relative Water Content (RWC) was used to indicate the degree of stress. Oxygen uptake declined in shoot and root with increasing NaCl concentrations. Decrease of oxygen uptake in shoot and root of EMB 82-12 variety was higher than Afzal variety. RWC decreased with increasing NaCl concentrations. Lowering of RWC reduced growth and increased shoot/root ratio. Decrease of water content in EMB 82-12 plants was higher than Afzal plants. Shoot/root ratio in EMB 82-12 var was higher than Afzal.

**Key words:** Salinity, carotenoids, chlorophyll, oxygen uptake, RWC

### INTRODUCTION

Soil salinization is one of the major factors of soil degradation. It has reached 19.5% of the irrigated land and 2.1% of the dry-land agriculture existing on the globe (FAO, 2000). Salinity effects are more conspicuous in arid and semi-arid areas where 25% of the irrigated land is affected by salts. The increase of salt-affected soils due to poor soil and water management in the irrigated areas, the salinity problem became of great importance for agriculture production in this region. Salinity inhibition of plant growth is the result of osmotic and ionic effects and the different plant species have developed different mechanisms to cope with these effects (Munns, 2002). In suppressing growth (net photosynthesis), salinity must decrease the rate of photosynthesis per unit leaf area, the utilization of photosynthate in growth, or both of these. In the case of barley, salinity affected the utilization of photosynthate rather than photosynthesis itself (Guach and Eatok, 1942). The rate of photosynthesis per unit leaf area may actually be increased in salt-affected plants (KLING, 1954). Under salt stress, Na concentration increase of plant tissue can result in an increase in oxidative stress, which causes deterioration in chloroplast structure. This leads to a decrease in the photosynthetic activity. Chlorophyll content decreased, but carotenoid content increased with increase of NaCl concentrations (El-Tayeb, 2005). Salt tolerance is associated with a high

rate of photosynthesis and a low rate of respiration (Shakhov, 1956). Respiration rates are often an order of magnitude lower than photosynthesis rates. However, since photosynthesis is limited temporally (i.e., daytime hours) and spatially (i.e., to green biomass), while respiration occurs continuously in every plant organ, the latter may be an equally important factor controlling productivity, particularly when photosynthesis is largely depressed, such as under salinity conditions (Nyawira *et al.*, 1987). The relative water content decreased significantly with salinity (El-Tayeb, 2005). Furthermore salinity decreased the relative water content in seedling of salt-sensitive cultivars.

### MATERIALS AND METHODS

This study was conducted at biochemistry Laboratory, Department of Biology, Urmia University, Iran, during the winter of 2007. Barley (*Hordeum vulgare* L. var. Afzal and var. EMB 82-12) seeds were surface sterilized in 0.5% sodium hypochloride solution for 20 min and grown in pots containing Vermiculite. Plants were watered every second day using one-half-strength Hoagland nutrient solution in controlled growth room for 4 days, then seedlings were subjected to treatment with 50, 100, 200, 300, 400 mM NaCl for 3 days. Shoots and roots to be used for biochemical determinations were frozen and stored in liquid nitrogen immediately after

harvest until enzyme extraction. MDA content was measured using a 2-thiobarbituric acid reaction (Heath and Packer, 1968). The chlorophylls and carotenoids (carotene and xanthophylls) content of shoot measured with Lichtenthaler and Wellbum (1983) method. The pigments of 0.1 g of shoot fresh weight extracted by acetone 80%. Extracts filtered by filter paper absorbance of samples was measured at 662, 645 and 470 nm by Uv-visible spectrophotometer (WPA model S2100). Chlorophyll a, b, total Chlorophyll and carotenoids content were measured with following equations:

$$\text{Chlorophyll a} = 11.75 A_{662} - 2.350 A_{645}$$

$$\text{Chlorophyll b} = 18.61 A_{645} - 3.960 A_{662}$$

$$\text{Total Chlorophyll} = \text{Chlorophyll a} + \text{Chlorophyll b}$$

$$\text{Total carotenoid} = (1000A_{470} - 2.270 \text{ Chl a} - 81.4 \text{ Chl b})/227$$

Oxygen uptake of shoot and root were measured at 25°C using an oxygen meter (WTW model oxi 730). Shoot and root segments (approximately 0.5 g fresh weight) were placed in 4 mL reaction medium [0.25 M sucrose, 0.01 M tris, 0.01 M<sub>2</sub>HPO<sub>4</sub>, 0.005 M MgCl<sub>2</sub>, 0.005 M EDTA, 0.5 mg mL<sup>-1</sup> BSA] adjusted to PH =7.2 with HCl and O<sub>2</sub> measured in period of 2 min (John *et al.*, 1970). Relative water content was determined with following equation:

$$\text{RWC} = (\text{Fresh weight} - \text{dry weight} / \text{Turgid weight} - \text{dry weight}) \times 100$$

Fresh weight of the plants was measured and after that plants were dried at 105°C until reached constant weight for the determination of dry weight.

To determine the turgid weight, samples were soaked in distilled water for 4 h at room temperature (approximately 20°C) and then turgid weight was measured (Fletcher, 1988).

**Statically analysis:** Mean values were taken from measurements of 4 replicates and Standard Error of the means was calculated. Differences between means were determined by One-way ANOVA and Turkey's multiple range tests ( $p < 0.05$ ). Analyses were done using the Statistical Package for Social Sciences (SPSS) for windows (version 13.0).

## RESULTS

Salinity caused a reduction in chlorophyll a, b and total chlorophyll content in both varieties, but the decrease in EMB82-12 variety was higher than Afzal var. In 400 mM NaCl, chlorophyll a content was 0.44 fold in EMB82-12 var. and 0.41 fold in Afzal var. as compared to control plants. In highest salinity, chlorophyll b content was 0.24 fold in EMB 82-12 var. and 0.54 fold in Afzal var. as compared to control plants and the decrease in Afzal var. was gradually and in EMB82-12 var. in 400 mM NaCl was enormous. Total chlorophyll content decreased in both varieties and in 400 mM NaCl, this factor was 0.38 fold in EMB 82-12 var. and 0.44 fold in Afzal var. as compared to control (Fig. 1). Carotenoids content in both varieties increased, but the increase in Afzal var. was higher than EMB82-12 var. In 400 mM NaCl, carotenoids content was 4.11 fold in EMB 82-12 var. and 3.55 fold in Afzal var. as compared to control plants. It means that Afzal plants have higher carotenoids content and lower chlorophyll content than EMB 82-12 var. when salt stressed.

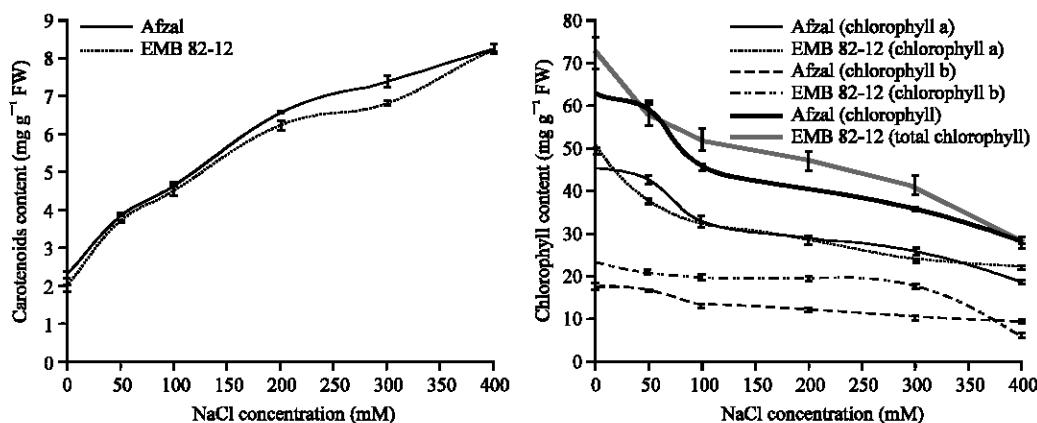


Fig. 1: Effects of different NaCl concentrations on photo synthetic pigments in shoots of 2 barley cultivars. Results are shown as mean±standard error ( $p < 0.05$ ), obtained from four replicates

The changes in shoot and root respiratory rate could result from damage to the mitochondria themselves or in shoot which altered substrate availability due to inhibition of photosynthesis. Leaf respirations averaged  $18.21 \mu\text{mol O}_2 \text{g}^{-1} \text{FW min}^{-1}$  in control plants, EMB82-12 var. and  $15.91 \mu\text{mol O}_2 \text{g}^{-1} \text{FW min}^{-1}$  in Afzal var. Under severe salt stress (400 mM NaCl) respiration was lower EMB82-12 var. than Afzal var., although not significantly different ( $7.38 \mu\text{mol O}_2 \text{g}^{-1} \text{FW min}^{-1}$  in EMB82-12 var. and  $8.22 \mu\text{mol O}_2 \text{g}^{-1} \text{FW min}^{-1}$  in Afzal var.). In roots of control plants, respiration averaged  $11.0 \mu\text{mol O}_2 \text{g}^{-1} \text{FW min}^{-1}$  in EMB82-12 var. and  $10.13 \mu\text{mol O}_2 \text{g}^{-1} \text{FW min}^{-1}$  in Afzal var. Under severe salt stress (400 mM NaCl) respiration was lower than control ( $4.28 \mu\text{mol O}_2 \text{g}^{-1} \text{FW min}^{-1}$  in EMB82-12 var. and  $5.29 \mu\text{mol O}_2 \text{g}^{-1} \text{FW min}^{-1}$  in Afzal var.). Therefore, respiration decreased with increasing NaCl treatments (Fig. 2). In 50 mM NaCl, shoot respiration was 0.85 fold

in EMB 82-12 var. and 0.8 fold in Afzal var. as compared to control plants and in 400 mM NaCl, shoot respiration was 0.4 fold in EMB 82-12 plants and 0.51 fold in Afzal plants as compared to control plants. About root respiration, in 50 mM NaCl, this factor was 0.82 fold in EMB 82-12 var. and 0.95 fold in Afzal var. and in 400 mM NaCl, root respiration was 0.36 fold in EMB 82-12 var. and 0.52 fold in Afzal var. as compared to control plants. The decrease of respiration rates in roots and shoots in EMB 82-12 var. were higher than Afzal var. The percentage of oxygen consumption decreased gradually with increasing NaCl concentrations (Fig. 2). The percentage of oxygen consumption in 400 mM NaCl, in roots decreased to 42.27% in EMB82-12 var. and 50.16% in Afzal var. and in shoots decreased to 58.25% in EMB82-12 var. and 67.67% in Afzal var. The decrease of oxygen consumption in EMB82-12 var. was higher than Afzal var. and in roots was higher than shoots.

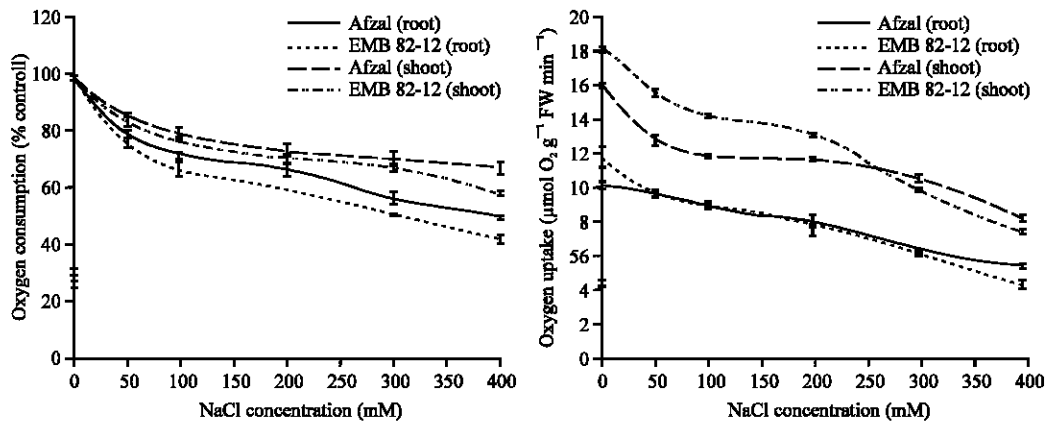


Fig. 2: Effects of different NaCl concentrations on oxygen uptake and oxygen consumption percent in roots and shoots of 2 barley cultivars. Results are shown as mean±standard error ( $p < 0.05$ ), obtained from four replicates

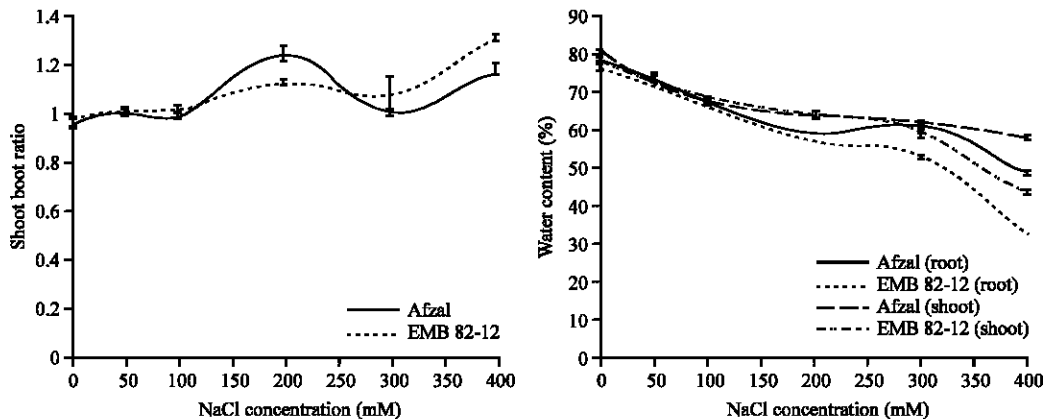


Fig. 3: Effects of different NaCl concentrations on water content and shoot/root ratio in roots and shoots of 2 barley cultivars. Results are shown as mean±standard error ( $p < 0.05$ ), obtained from four replicates

In roots and shoots in control plants, EMB82-12 var. have higher water content than Afzal var., but in severe salinity, Afzal plants roots and shoots have higher water content than EMB82-12 plants (Fig. 3). Relative water content in roots and shoots decreased with increasing NaCl concentrations. In 400 mM NaCl, this decrease was enormous in roots and shoots in both varieties. In 400 mM NaCl, RWC was 0.4 fold in EMB82-12 var. and 0.62 fold in Afzal var. in roots and 0.53 fold in EMB 82-12 var. and 0.75 fold in Afzal var. in shoots as control plants. With increase of NaCl concentrations, the shoot/root ratio was increased and shoot/root ratio in EMB 82-12 plants was higher than Afzal plants (Fig. 3). In 400 mM NaCl, shoot/root ratio was 1.34 fold in EMB 82-12 plants and 1.22 fold in Afzal plants as compared to control.

### DISCUSSION

Reductions of chlorophyll content under salinity conditions were observed for some salt-sensitive plant species (Delfine *et al.*, 1999; Ashraf *et al.*, 2000; Jungklang *et al.*, 2003; Lee *et al.*, 2004). The decrease of chlorophyll content was dependent on the salinity level, the time of exposure to salts and the species. In contrast, Chl. content in salt-tolerant plants either does not decline or else rises with increasing salinity (Brugnoli and Björkman, 1992; Qui and Lu, 2003). Chlorophyll concentration can be used as a sensitive indicator of the cellular metabolic state; thus its decrease signifies toxicity in tissues due to accumulation of ions. In our experiments, chlorophyll a and b contents and total chlorophyll decreased with increasing NaCl supply (Yeo and Flowers, 1983). In EMB 82-12 plants chlorophyll content in shoots decreased higher than Afzal plants, but in severe salinity, EMB 82-12 plants have higher total chlorophyll content than Afzal plants. Increase of carotenoids content in Afzal var. was higher than EMB 82-12 plants. Therefore, Afzal plants have a better protection than EMB 82-12 plants, because carotenoids have a protective role and protect chlorophyll from photo oxidation. Increasing NaCl concentration clearly depressed both roots and shoots respiration. Leaf respiration rates decreased under salinity in most species, but the decline was always smaller than that of photosynthesis, therefore resulting in decreased photosynthesis to respiration ratio (indicative of shoot carbon balance). The decline in respiration in response to salinity seems to be part of a systemic metabolic response, which occurs under conditions where salinity severely restricts CO<sub>2</sub> availability inside leaf cells, therefore, creating the risk of a secondary oxidative stress

(Flexas *et al.*, 2004). Our original objective from this study was finding the changes in respiration rates in shoots and roots with increasing NaCl treatments. We found that with increase of salinity, respiration rates decreased in both shoots and roots. These results supported previous findings (Valentini *et al.*, 1999). In 400 mM NaCl, respiration rate in EMB 82-12 plants in roots and shoots were not only lower than the control, but were also lower than in Afzal var. It means that in leaves and roots the decrease of oxygen uptake in EMB 82-12 var. was higher than Afzal var. With increase of NaCl concentrations, oxygen consumption percentage decrease in both roots and shoots. The decrease of oxygen consumption percentage in EMB 82-12 plants was higher than Afzal plants. Therefore, salinity has a higher effect in EMB 82-12 plant's respiration than Afzal plants. Oxygen consumption percentage in high salinity in roots was lower than shoots and roots were more sensitive than shoots. Salinity probably acted directly on roots, because the roots were immersed in NaCl solutions and salinity in roots was higher than shoots, whereas shoots could reduce oxygen consumption by stoma regulations. There were a positive and strong correlation between respiration rates and water content. The decreased respiration rate was positively correlated to decrease of relative water content. Respiration rates affected by a decrease in water content. It means that a decrease in water content caused a decreased in oxygen consumption and respiration rates (Kasumov and Abbasova, 1998). The decrease of water content in EMB 82-12 plants was higher than Afzal plants in roots and shoots. Afzal plants have higher water content than EMB 82-12 plants in roots and shoots. The shoot/root ratio was increased in salinity and this factor in EMB 82-12 var. was higher than Afzal var.

The above results suggest that plants of the Afzal variety have a better tolerance to salinity as compared to EMB82-12 variety.

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