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Physiological and Biochemical Evaluation of Barley (*Hordeum vulgare* L.) under Salinity Stress

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ABSTRACT

This study was conducted to investigate the response of four barley cultivars (Reyhan03, Yousef, Afzal, and Khatam) to salinity stress at 0 (control), 100, 200 and 300 mM levels as a factorial experiment, within the randomized complete block design in three replications in a greenhouse, using the Hoagland solution. The physiological and biochemical properties including dry weight and RWC, photosynthesis pigments, K^+/Na^+, osmotic adjustments (soluble sugars, glycine betaine, proline), hydrogen peroxide and antioxidants enzymes (catalase and peroxidase) in root and shoot of barley cultivars were evaluated in saline and non-saline conditions. To determine the relationship between growth performance and the physiological and biochemical properties, the correlation between the properties and causality analysis was examined. Results obtained from comparing the mean among the treatment combinations showed that the salinity stress reduced the dry weight, photosynthesis pigments, and K^+/Na^+, while it increased the soluble sugars, glycine betaine, proline, H_2O_2, catalase and peroxidase in the root and shoot of barley cultivars. Correlation analysis indicated that potassium in the shoot had the most positive and significant correlation coefficient (r= 0.86) with the dry matter of shoot. The stepwise regression analysis showed that the root dry weight, catalase of root and shoot, H_2O_2 of shoot and K^+/Na^+ of shoot contributed to the performance. Causality analysis revealed that the root dry weight, K^+/Na^+ of shoot, and catalase of shoot were highly important as they had a direct positive and significant impacts on the performance of shoot dry matter.

Keywords: Antioxidant enzymes, Glycine betaine, H_2O_2, K^+/Na^+ ratio, Proline, Stepwise regression.

INTRODUCTION

Barley (*Hordeum vulgare L*) from Poaceae family is one of the most important cereal grains that are cultivated in various weather conditions worldwide. After corn, wheat, and rice, barley ranks fourth concerning the production of dry matter in the world. In Iran, after wheat, it ranks second in terms of cultivated area. Barley is used for livestock, malt, drinking and alcohol industries (FAO, 2015).

One environmental stress constraining the performance of plants is salinity, which affects 20% of the global lands and water resources, reducing agricultural productions, ecological imbalance, and threatening human health. Soils with an electrical conductivity of more than 4 dS/m are saline soils that cause 0.2 MPa of the osmotic pressure, and significantly reduce the yield of cereal crops. Plants are classified into halophytes and glycophytes based on the ability to grow in saline soils, and most crops are considered as glycophyte. The critical concentrations of

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Na+ in a large proportion of glycophites is 50 mM or higher in plants. The saline environments influence the plant growth via different ways like decreasing the water intake, increasing the ionic toxicity, and decreasing the plant nutrient transfer (Munns and Tester, 2008; Tang *et al*., 2015)

The main problem associated with salinity for plants is the high content of sodium chloride in soil, which negatively affects the membranes and enzyme systems, such that damage intensity depends on climatic conditions, plant species, the duration of stress and growth stage of plants (Chen *et al*., 2007; Tang *et al*., 2015). Salinity stress further reduces the root length (Shelden *et al*., 2013), stomatal conductance (Rahnama *et al*., 2010), relative water content (Rivelli *et al*., 2002), chlorophyll index (Marcińska *et al*., 2013), photosynthetic electron transport system (Sun *et al*., 2016), and shoot and root dry weight (Shelden *et al*., 2013). Such reduction is significantly higher in salinity sensitive cultivars, compared with tolerant cultivars.

Under the salinity stress, plants show a wide range of responses in physiological, biochemical, and molecular levels. The physiological effects include loss of turgor pressure and osmotic adjustment, decrease in germination rates, reduced leaf water potential, reduced internal CO2 concentration, decrease in stomatal conductance to CO_2, and slowdown of net photosynthesis. The biochemical responses consist of accumulation of metabolites (sugar, proline, glycine betaine), decreased rubisco activity, increase in antioxidative enzymes, and lowered ROS accumulation. The molecular mechanism includes expression of genes inducible by stress, activation of genes of aba biosynthesis, expression of aba inducible genes, and production of specific proteins (Mbarki *et al*., 2018).

Exposure of plants to such undesired environmental conditions as salinity stress raises the production of active types of oxygen including superoxide radical, hydrogen peroxide and hydroxyl radical. These various active types may entail cellular damage via the oxidation of lipids, proteins, nucleic acids, and severely affect the metabolism, growth, and performance. (Yan *et al*., 2013). To overcome the induced oxidative effects by salinity, plants use a complicated antioxidant system, which includes non-enzyme antioxidants like carotenoid and enzyme antioxidants such as Superoxide Dismutase (SOD), Ascorbate Peroxidase (APX), Glutathione Reductase (GR), Glutathione Peroxidases (GPX), Catalase (CAT), and Peroxidase (POX). Studies have shown an increase in antioxidant enzymes like peroxidase under long-run salinity in wheat and barley (Ashraf and Akram, 2009). In barley, many features are involved in grain performance, directly and indirectly. Specifying these characteristics and determining their connection to performance is necessary for identifying the selection criteria and selecting high-yielding genotypes. (Negrao *et al*., 2017).

This study aims at investigating the physiological and biochemical properties under salinity stress and non-stress conditions in barley cultivars and determining the direct and indirect relationship between the performance and other characteristics using causality analysis so that the most effective salinity tolerant characteristics are selected and applied in plant breeding and biotechnology.

MATERIALS AND METHODS

In order to investigate the effect of salinity stress on barley cultivars of Reyhan03, Yousef, Afzal, and Khatam (Table 1), an experiment was conducted in a factorial form within a fully random design with three replications. After being sterilized by hypochlorite 5%, seeds were cultivated in greenhouse conditions in perlite, and germinated seeds were irrigated by Hoagland solution (Hoagland and Arnon, 1950). In the four-leaf stage, salinity levels of 0, 100, 200, and 300 mM were applied. To avoid any shortage of calcium, salinity stress was applied by using sodium chloride and calcium chloride

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Table 1. Characteristics of the studied barley cultivars.

Cultivar	Origin	Introduction year	Seed row per plant	Growth habit	Maturing time	1000 Seed weight (g)	Grain yield (t ha^{-1})	Lodging	Disease resistance
Reyhan03	Iran	2006	6	spring	early maturing	44-48	7-8	Semi resistant	Semi resistant
Yousef	Iran	2009	6	spring	early maturing	42-44	5-6	Semi resistant	semi sensitive
Afzal	Iran	1996	6	spring	Semi-early maturing	38-42	4-5	resistant	sensitive
Khatam	Iran	2015	6	Facultative	Semi-early maturing	36	5	resistant	Semi sensitive

at a ratio of 2:1. After two weeks of salinity application, roots and shoots were cut and kept at -80°C for analysis.

Measurement of Relative Water Content

RWC was obtained by determining leaves Fresh Weight (FW), Dry Weight (DW), and Weight of turgid leaves (SW) and calculated as follows:

RWC= 100×(FW-DW)/(SW-DW), (Lara *et al*., 2003)

Na^+ and K^+ Contents

Further used by flame photometer was 0.2 gr of dry matter of root and shoot samples was used by flame photometer, as proposed by Patterson *et al*. (1984) to determine the values of Na^+ and Cl^-.

Measurement of Chlorophyll and Carotenoid

To determine chlorophyll a, chlorophyll b, and carotenoids, 0.5 g of fresh leaf samples were grounded in 0.5 mL of acetone (80% V/V). Values of chlorophylls a and b and carotenoids were read at 663, 645, and 470 nm wavelengths by the spectrophotometer (PG Instrument Ltd., UK). Photosynthetic pigment contents were calculated using the following equations (Lichtenthaler and Wellburn, 1983).

Chl a (mg g^{-1} FM)= 11.75×A663-2.35×A645

Chl b (mg g^{-1} FM)= 18.61×A645-3.96×A663

Carotenoids (mg g^{-1} FM) = 4.69×A470-0.268×(20.2×A645+8.02×A663)

Determination of Total Soluble Sugar, Glycine Betaine, and Proline

In order to determine amount of soluble sugar in the root and shoot, 0.5 g of fresh tissues with 15 mL of 80% ethanol were vortexed and centrifuged. Supernatants were

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kept at 50°C for 24 hours in an oven to evaporate ethanol. Five mL of zinc sulfate 5% and 4.7 mL of barium hydroxide were added and centrifuged. One mL of 5% Phenol and 5 mL sulfuric acid 98% was added to the supernatant. After 30 minutes, the absorbance of each solution was measured at 485 nm wavelength (Dubois *et al*., 1956). Glycine betaine content was measured according to Grieve and Grattan (1983). After stirring root and shoot samples in distilled water for 48 hours at 25°C and filtering, the solution was diluted using 2N H2SO4. Cold KII2 was added to the diluted liquid, and after centrifugation, the supernatant was mixed with 1, 2- dichloroethane. Absorption was recorded at 365 nm wavelength. In order to determine amount of proline in shoot and root, 0.5 g of fresh tissues were homogenized with 10 mL of 3% aqueous sulfosalicylic acid and briefly centrifuged. Two mL of the supernatant was blended with acid ninhydrin and glacial acetic acid (2 mL of each). The mixture in test tube was put in a water bath for 1 hour at 100°C. The reaction mixture was extracted with toluene (4 mL). Absorbance of the mixture was determined at 520 nm wavelength after being cooled down to room temperature (Bates *et al*., 1973).

Estimation of H_2O_2, Activity Catalase (CAT), and Peroxidase (POX)

In order to determine H_2O_2 content in shoot and root, 0.5 g fresh tissues were homogenized with 5 mL of 0.1 % w/v Trichloroacetic Acid (TCA) and centrifuged. Then, supernatant (0.5 mL) was supplemented to 0.5 mL of potassium phosphate ($KHPO_4$) buffer (10 mM, pH 7.0) and 1 mL of potassium iodide (1 M). The upper phase was aliquoted to read its absorbance at 390 nm wavelength. H_2O_2 was used for graphing calibration curve in order to calculate H_2O_2 concentration (Velikova *et al.,* 2000). Half a gram of fresh root and shoot tissues were homogenized in 1 mL of extraction buffer and centrifuged at 15,000g for 15 minutes at 4°C. Then, 25 µL supernatant was added to 1 mL of the reaction mixture. (The reaction mixture for CAT included 50 mM sodium acetate buffer (pH= 7), 25 mM H_2O_2, deionized water, and for POX included 50 mM sodium acetate buffer (pH= 7), 25 mM guaiacol, 25 mM H_2O_2, deionized water). In order to measure the CAT activity, the reduction in absorbance was recorded at 240 nm wavelength for 1 minute, and for POX activity, the absorbance was measured at 470 nm wavelength. All stages were done on ice (Venisse *et al*., 2001).

Statistical Analysis

Statistical analyses including variance analysis, comparison of means by Duncan test, determination of simple correlation coefficients, stepwise regression and causality analysis were conducted to specify the direct and indirect effects of important characteristics on shoot growth using SAS and MSTSTC software.

RESULTS AND DISCUSSION

Variance analysis showed that there was a significant relationship between barley cultivars and salinity level regarding the studied characteristics at the 1% level. The interaction between the cultivar and salinity was significant (at 1% level) concerning all characteristics. The coefficient of variation characteristics was 0.02 – 12.83, indicating the high accuracy of the present research. Given the significant interaction between cultivar and salinity, the means of all characteristics at a 5% level were compared by Duncan test (Table 2).

Qasim *et al*. (2003) studied leaf relative water content and Munns and James (2003) introduced seedling dry weight as a reliable trait for studying plant response to salinity stress. In the present study, there was a significant difference between the relative water content of leaves in different cultivars

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Table 2. Factorial variance analysis of studied traits.

		Mean of Squares (MS)												
SOV[a]	df	RWC	Root DW	Shoot DW	Chla	Chlb	Total chlorophyll	Carotenoid	Root Na^+	Shoot Na^+	Root K^+	Shoot K^+	Root K^+/Na	Shoot K^+/Na^+
A	3	342.82**	0.001**	0.146**	0.012**	0.005**	0.027**	0.02**	0.719**	0.037**	750.25**	110.09**	896.34**	168.01**
B	3	275.09**	0.002**	0.275**	0.137**	0.032**	0.292**	0.279**	0.104**	2.72**	812.01**	559.75**	2549.67**	5373.60**
A×B	9	221.03**	0.0002**	0.019**	0.008**	0.007**	0.011**	0.007**	0.557**	0.028**	403.83**	3.99**	1381.56**	138.69**
E	32	18.69	0.00005	0.003	0.00003	0.00002	0.00002	0.0001	0.000017	0.000003	0.009	0.0006	0.045	0.007
CV (%)		6.86	8.82	12.83	2.09	3.92	1.37	2.32	0.36	0.13	0.36	0.07	0.49	0.28

		Mean of Squares (MS)											
SOV	df	Root sugar	Shoot sugar	Root Proline	Shoot Proline	Root glycine betaine	Shoot glycine betaine	Root H_2O_2	Shoot H_2O_2	Root CAT	Shoot CAT	Root POX	Shoot POX
A	3	0.016**	0.247**	220.44**	597.89**	1720.36**	619.94**	4.91**	3.69**	3.99**	2.19**	17.21**	14.64**
B	3	0.048**	1.033**	576.69**	3431.51**	260.03**	1836.84**	27.17**	15.28**	1.46**	1.51**	34.03**	4.52**
A×B	9	0.006**	0.062**	24.73**	248.21**	325.49**	1418.50**	1.65**	0.86**	0.64**	0.21**	4.65**	10.65**
E	32	0.00001	0.006	0.011	0.009	0.012	0.009	0.007	0.003	0.001	0.001	0.023	0.009
CV (%)		0.02	0.33	0.61	0.26	0.30	0.27	4.09	1.44	2.00	3.09	2.18	1.85

[a] A: Cultivar; B: Stress ; A×B: Cultivar×Sress, CV: Change Coefficient. ns, *, **: Are non-significant and significant at 5 and 1% probability level, respectively.

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of barley, under severe salinity. Salinity in all barley varieties resulted in dry weight loss and relative water content in comparison with non-stress conditions, which is in line with Poustini and Siosemardeh (2004) results. Afzal had the highest rate of RWC in 200 mM salinity and the highest dry weight values of the root and shoot under salinity conditions, indicating its ability to tolerate salinity (Table 3).

Under salinity conditions, a high accumulation of toxic ions, such as Na^+ and Cl^-, occurs in chloroplasts and has a toxic effect on photosynthesis processes and pigments. It has been reported that salinity disables electron transfer system and photophosphorylation in tilacoid membrane and leads to disruption of rubisco, a key enzyme in photosynthesis. The high concentration of Na^+ in the shoots leads to reduced stomatal conductance and mesophyll, while Cl^- ion accumulation destroys chlorophyll and disrupts the PSII (Tang *et al*., 2015; Mbarki *et al*., 2018). The decrease in dry weight and plant growth under salt stress conditions can be attributed to the reduction in the number of photosynthetic pigments, as well as induced dryness due to salt stress, which reduces the osmotic potential in the growth medium, and ultimately forces the plant to use ionic compounds for osmoregulation (Munns and Tester, 2008). Carotenoids are a large group of isoprenoid molecules, and key pigments of antioxidant system in plants. These molecules, on the other hand, are highly susceptible to oxidative degradation. Salinity stress reduces chlorophyll content by increasing the activity of chlorophylls and inducing the destruction of the chloroplast structure and the imbalance of protein-pigment complexes (Noreen and Ashraf, 2009). Accordingly, in this study, salt stress reduced the photosynthetic pigmentation of chlorophyll a, b, and carotenoids in all barley cultivars (Table 3), which is in accordance with Noreen and Ashraf study on radish. Zhao *et al*. (2007) studied Oat figures and reported that salinity stress significantly reduced chlorophyll due to the increase in sodium concentration in the leaf tissue.

Cavalcanti *et al*. (2007) reported that the ratio of K^+/Na^+ conduces to determining the salinity tolerant cultivars of barley. If cation exchange capacity of the soil is saturated more than 40 to 50 percent with sodium, nutrition disorders are caused. Increasing sodium reduces calcium, magnesium, and potassium in the plant and disrupts cationic equilibrium (Yan *et al*., 2013). In the present study, by increasing the salinity stress, sodium concentration increased in the shoot and root of all barley cultivars, and the concentration of potassium and K^+/Na^+ ratio showed a downward trend that matched the results of Gu *et al*. (2016) (Table 3). The increase in the sodium intake of cytoplasm, under salinity conditions, causes sodium to substitute potassium and produce ionic toxicity. An increase in sodium concentration and a decrease in K^+/Na^+ ratio in response to salinity have also been reported in previous studies (Shelden *et al*., 2013). Cavalcanti *et al*. (2007) reported a negative correlation between the accumulation of sodium ion in barley leaf and root and the dry weight of these organs. They cited the main reason for the decrease in leaf growth and the root of the barley in the degradation of cell membranes, reduction of photosynthesis and leaves turgor under the influence of sodium ion.

It seems that accumulation of organic solution materials such as carbohydrates, proline and glycine betaine in response to salinity stress is involved in maintaining mechanisms such as restoration and compensation of the mass of the cell and its swelling, the reduction in the damage caused by free radicals to cells, and the protection of enzymes and membrane structures (Schmid, 1989). Prado *et al*. (2000) proposed an increase in carbohydrates to reduce the effects of osmotic and ionic stresses and ultimately adapt plants to such conditions. In this experiment, salinity stress led to an increase in soluble sugar in the root and shoot of barley cultivars (Figure 1). The accumulation of soluble sugars in leaves for

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Table 3. Means comparison among treatment combinations for studied traits by DMRT at Alpha= 0.05.[a]

Cultivar	Salinity (mM)	RWC±SE	Root DW±SE (g plant^{-1})	Shoot DW±SE (g plant^{-1})	Chla±SE (mg g^{-1} fw)	Chlb±SE (mg g^{-1} fw)
Yousef	0	68.50 ± 3.28 abc	0.091 ± 0.01 d	0.48 ± 0.09 cd	0.46 ± 0.0005 a	0.21 ± 0.0006 a
	100	70.01 ± 6.63 ab	0.095 ± 0.02 cd	0.32 ± 0.07 ef	0.31 ± 0.001 d	0.21 ± 0.001 a
	200	49.36 ± 9.78 f	0.089 ± 0.003 d	0.28 ± 0.05 f	0.18 ± 0.0005i	0.12 ± 0.0006 d
	300	66.32 ± 1.97 bc	0.067 ± 0.005 f	0.16 ± 0.02 g	0.15 ± 0.002 j	0.00001 ± 0.003i
Reyhan03	0	63.47 ± 5.48 bcd	0.081 ± 0.004 e	0.58 ± 0.05 b	0.36 ± 0.001 c	0.18 ± 0.002 b
	100	61.06 ± 3.23 cde	0.077 ± 0.007 e	0.44 ± 0.04 cd	0.31 ± 0.002 d	0.15 ± 0.005 c
	200	51.04 ± 3.06 f	0.063 ± 0.001 fg	0.23 ± 0.02 fg	0.25 ± 0.02 f	0.06 ± 0.007 g
	300	51.05 ± 2.67 f	0.060 ± 0.007 fg	0.23 ± 0.05 fg	0.20 ± 0.0006 h	0.02 ± 0.006 h
Afzal	0	74.29 ± 2.15 a	0.111 ± 0.005 a	0.78 ± 0.06 a	0.38 ± 0.003 b	0.08 ± 0.002 f
	100	56.81 ± 4.37 def	0.102 ± 0.003 b	0.62 ± 0.03 b	0.24 ± 0.001 g	0.12 ± 0.001 d
	200	76.30 ± 2.64 a	0.099 ± 0.012 bc	0.62 ± 0.06 b	0.12 ± 0.004 k	0.09 ± 0.001 e
	300	71.29 ± 2.68 ab	0.059 ± 0.002 g	0.24 ± 0.07 fg	0.12 ± 0.001 k	0.09 ± 0.002 e
Khatam	0	74.51 ± 1.34 a	0.095 ± 0.002 cd	0.54 ± 0.02 bc	0.31 ± 0.0006 e	0.13 ± 0.01 d
	100	53.18 ± 0.89 f	0.089 ± 0.004 d	0.39 ± 0.05 de	0.36 ± 0.0006 c	0.08 ± 0.001 f
	200	66.17 ± 5.61 bc	0.093 ± 0.008 cd	0.29 ± 0.05 ef	0.19 ± 0.001 i	0.09 ± 0.003 e
	300	54.97 ± 3.98 ef	0.062 ± 0.005 fg	0.29 ± 0.04 ef	0.10 ± 0.001 l	0.05 ± 0.002 g

Cultivar	Salinity (mM)	Total chlorophyll±SE (mg g^{-1} fw)	Carotenoid±SE (mg g^{-1} fw)	Root Na^+±SE (mg g^{-1} DW)	Shoot Na^+±SE (mg g^{-1} DW)
Yousef	0	0.67 ± 0.000001a	0.76 ± 0.03 a	0.32 ± 0.002 l	0.89 ± 0.002 k
	100	0.53 ± 0.0006 b	0.58 ± 0.01 d	0.87 ± 0.004 d	1.46 ± 0.001 h
	200	0.30 ± 0.002 h	0.40 ± 0.002 g	0.49 ± 0.000001j	1.56 ± 0.000001g
	300	0.15 ± 0.002 l	0.31 ± 0.005 i	0.92 ± 0.002 c	1.82 ± 0.002 c
Reyhan03	0	0.54 ± 0.001 b	0.62 ± 0.02 c	1.68 ± 0.000001a	0.52 ± 0.002 n
	100	0.46 ± 0.003 c	0.57 ± 0.01 d	0.55 ± 0.003 h	1.32 ± 0.001 g
	200	0.31 ± 0.013 g	0.44 ± 0.001 f	0.84 ± 0.000001e	1.62 ± 0.002 f
	300	0.22 ± 0.006 j	0.36 ± 0.002 h	0.83 ± 0.000001e	1.88 ± 0.002 a
Afzal	0	0.46 ± 0.000001c	0.64 ± 0.005 b	0.66 ± 0.002 f	0.77 ± 0.002 l
	100	0.36 ± 0.0006 f	0.44 ± 0.02 f	1.57 ± 0.003 b	1.46 ± 0.000001h
	200	0.21 ± 0.003 jk	0.39 ± 0.0005 g	0.55 ± 0.000001h	1.70 ± 0.002 d
	300	0.21 ± 0.001 k	0.31 ± 0.001 i	0.59 ± 0.002 g	1.86 ± 0.002 b
Khatam	0	0.43 ± 0.011 e	0.61 ± 0.005 c	0.21 ± 0.0005 m	0.72 ± 0.002 m
	100	0.44 ± 0.001 d	0.49 ± 0.002 e	0.36 ± 0.000001k	1.39 ± 0.002 i
	200	0.28 ± 0.003 i	0.38 ± 0.0006 h	0.56 ± 0.007 h	1.66 ± 0.002 e
	300	0.16 ± 0.002 l	0.23 ± 0.002 j	0.52 ± 0.001 i	1.67 ± 0.001 e

Cultivar	Salinity (mM)	Root K^+±SE (mg g^{-1} DW)	Shoot K^+±SE (mg g^{-1} DW)	Root K^+/Na^+±SE (mg g^{-1} DW)	Shoot K^+/Na^+±SE (mg g^{-1} DW)
Yousef	0	21.48 ± 0.09 j	38.31 ± 0.01 d	67.26 ± 0.35 b	42.85 ± 0.06 d
	100	24.12 ± 0.13 h	28.21 ± 0.01 k	27.86 ± 0.09 m	19.32 ± 0.02 h
	200	11.77 ± 0.02 p	27.25 ± 0.03 l	23.13 ± 0.04 n	17.40 ± 0.02 k
	300	35.07 ± 0.000001b	25.73 ± 0.01 o	38.13 ± 0.10 i	14.04 ± 0.02 o
Reyhan03	0	32.16 ± 0.09 e	39.95 ± 0.000001c	19.10 ± 0.06 p	76.27 ± 0.21 a
	100	34.37 ± 0.11 c	28.57 ± 0.04 j	62.45 ± 0.44 c	21.62 ± 0.02 g
	200	18.76 ± 0.15 l	27.05 ± 0.03 m	22.43 ± 0.13 o	16.66 ± 0.02 l
	300	29.46 ± 0.16 f	25.58 ± 0.04 p	35.46 ± 0.19 j	13.58 ± 0.03 p
Afzal	0	33.31 ± 0.03 d	47.62 ± 0.01 a	50.79 ± 0.25 d	61.74 ± 0.13 b
	100	69.70 ± 0.16 a	36.09 ± 0.03 e	44.24 ± 0.05 f	24.66 ± 0.02 e
	200	17.11 ± 0.02 m	36.32 ± 0.01 f	31.32 ± 0.05 k	18.99 ± 0.02 i
	300	27.83 ± 0.11 g	29.86 ± 0.01 i	47.14 ± 0.09 e	16.01 ± 0.02 m
Khatam	0	23.15 ± 0.03 i	43.05 ± 0.03 b	110.15 ± 0.44 a	59.65 ± 0.18 c
	100	15.20 ± 0.02 o	31.88 ± 0.03 g	42.65 ± 0.05 g	22.88 ± 0.04 f
	200	15.96 ± 0.10 n	30.08 ± 0.03 h	28.47 ± 0.19 ± l	18.11 ± 0.04 j
	300	20.24 ± 0.000001k	26.01 ± 0.01 n	38.74 ± 0.09 h	15.53 ± 0.01 n

[a] RWC: Relative Water Content; DW: Dry Weight, SE: Standard Error.

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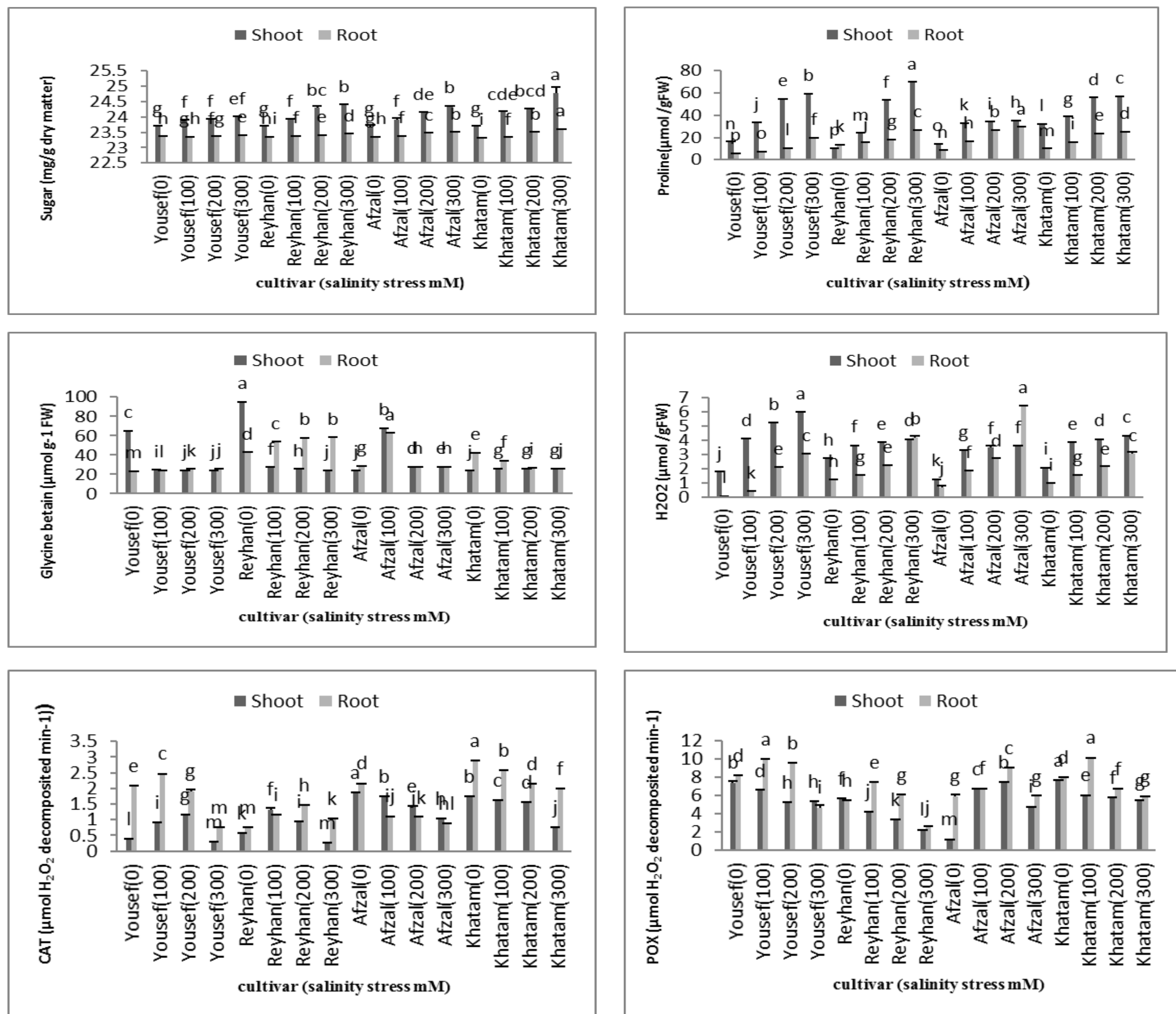

Figure1. Means comparison among treatment combinations for sugar, proline, glycine betaine, H_2O_2, CAT, and POX.

osmotic equilibrium when salt stress occurs causes a disruption in photosynthesis and reduces its performance. For this reason, in our study, a negative correlation was observed between the accumulation of soluble sugar and dry weight and photosynthetic pigments.

By increasing salinity stress, the concentration of proline in the root and shoot of all barley cultivars augmented significantly, where the maximum amount of proline was related to the shoot of Reyhan03 (69.68 µmol/gFW) and Yousef (59.49), and the root of Afzal (29.25) (Figure 1). Such high level of proline in the root and shoot may be due to the expression of the coding genes of the key enzymes of proline synthesis (pyrroline-5-carboxylate synthetase and pyrroline-5-carboxylate reductase) and the low activity of the oxidizing enzymes (proline dehydrogenase) (Tavakoli *et al*., 2016). Proline also supplies the energy required to accommodate ions in the vacuole. In the case of salinity, glutamate, which is the precast material of chlorophyll and proline, is used to produce

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proline (Molazem *et al*., 2010). For this reason, in the present experiment, proline had a significantly negative correlation with dry weight, chlorophyll, and carotenoid, while a positive and meaningful correlation with soluble sugar.

Glycine betaine is an amphoteric composition that is neutral in terms of Conductivity and operates at different physiological pH. Studies have shown that glycine betaine contributes to the stability and strength of the structure and activity of enzyme and protein compounds, it further helps the stability of the cell wall against the effects of excessive damage to salt, cold, heat and freezing (Raza *et al*., 2012). With the increase in salinity stress, the concentration of glycine betaine increased in the shoot of Afzal and Khatam cultivars and in the root of Afzal, Reyhan03 and Yousef cultivars (Figure 1). Also, the correlation between the shoot of glycine betaine and chlorophyll was positive and meaningful, while chlorophyll had a significantly negative relationship with proline in shoot.

It has been observed that osmotic and ionic effects involved in salinity stress can disrupt photosynthesis and respiration, leading to an increase in the production of Reactive Oxygen Species (ROS), which are responsible for secondary oxidative stress and damage the cell structure and metabolism (Yan *et al*., 2013). In this study, the increase in salt stress augmented hydrogen peroxide in the root and shoot of all barley cultivars (Figure 1) and the correlation between H_2O_2, dry weight, and photosynthetic pigments was negative and meaningful, while H_2O_2 had a significantly positive correlation with soluble sugar and proline, similar to results of Nxele *et al*., (2017).

The peroxidase enzyme plays a role in metabolic processes such as hormone catabolism, defense against pathogens, phenol oxidation, and the formation of transplantation of cellular structural proteins and cell wall polysaccharides. Ashraf and Ali (2008) observed that salinity stress increased the peroxidase activity in canola leaves, thereby reducing the destructive effects of salinity stress on these plants. Considering the important role of peroxidase in eliminating H_2O_2 enzymes, reducing malondialdehyde and maintaining the integrity of the cell membrane, increasing this enzyme in plants under salt stress is quite reasonable (Shakeri and Emam, 2018). In this study, it was observed that the increase in salinity stress augmented the activity of enzyme peroxidase in the shoot of Afzal cultivar and the root of all barley cultivars (Figure 1). The catalase enzyme together with superoxide dismutase convert anion superoxide and H_2O_2 radicals into water and oxygen and reduce cell damage caused by various stresses including salinity. Certain researchers believe that protein synthesis is reduced by extreme salt stress, so, catalase activity may also be reduced under severe salt stresses (Reddy *et al*., 2000). In this study, the activity of catalase enzyme in Reyhan03 and Yousef cultivars increased under mild salinity stress, but decreased under severe salt stress of 300 mM. Also, the correlation between shoot catalase and root and shoot dry weight was significantly positive.

To determine the influence of those properties on performance, stepwise regression analysis was used. In this regard, five properties were established as the most effective for performance. Dry matter of shoot, as the dependent variable, was analyzed against other independent variables, the results of which are given in Table 4. The linear relationship of the regression model is obtained as:

$$Y= 0.221-0.062X_1+5.072X_2-0.088X_3+0.068X_4+0.002X_5$$

Where, Y is shoot dry matter, X_1 is H_2O_2 of shoot, X_2 is the root dry weight, X_3 is the root catalase, X_4 is the shoot catalase and X_5 is the shoot K^+/Na^+. In the regression model, root dry weight, H_2O_2 and K^+/Na^+ ratio of shoot, root and shoot catalase were included in the model, where they were totally responsible for 86% of the changes (R^2= 0.861). The linear model was significant at a probability level of 1% (Table 4).

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Causality analysis showed that the direct impact of root dry weight on the increase in dry matter was positive, indirectly increasing the dry matter through the shoot H_2O_2, shoot catalase, and shoot K^+/Na^+. Therefore, root dry matter is selected directly and indirectly under salinity stress conditions leading to an increase in shoot dry matter. The direct impact of shoot H_2O_2 on the dry matter of the shoot was negative and significant. This property indirectly reduced the shoot dry matter via the root dry weight, shoot catalase, and shoot K^+/Na^+. The direct impact of shoot catalase on the increased dry matter was positive, indirectly augmenting the dry matter through the shoot H_2O_2, shoot K^+/Na^+, and root dry weight (Table 5).

It was observed that in spite of the direct negative impact of root catalase on the shoot performance, its indirect effects via other properties, particularly the root dry weight, was highly important. The direct impact of shoot K^+/Na^+ on the increase in dry matter was positive, indirectly increasing the dry matter through the shoot H_2O_2, shoot catalase, and root dry weight. Selecting shoot K^+/Na^+ under salinity stress conditions will, directly and indirectly, lead to increased shoot dry matter.

It was found that using the statistical method of causality analysis can be efficient in understanding the essential relationship among the variables and it is not enough to rely on the correlation relations for justifying the relations among the variables. In this regard, the results of causality analysis showed that the performance of shoot was indirectly affected by the root dry weight, shoot catalase, and shoot K^+/Na^+. A significant point in this study was that shoot H_2O_2 had the highest direct and negative effect on the shoot performance, and needs to be heeded sufficiently in selecting the cultivars for the given corrective properties. It is necessary to mention that studies on the analysis of direct and indirect effects of biochemical properties with shoot performance have not been conducted through causality analysis. On the other hand, numerous studies have been done on physiological properties with grain performance, including Rihan and Abdullah (2018).

Table 4. Analysis of stepwise regression of shoot dry weight on other traits of barley.

ANOVA			
Model	df	Mean Square	F
Regression	5	0.268	59.47**
Residual	42	0.005	
Total	47		

** Highly significant at 1% level of significance.

CONCLUSIONS

Results of this research revealed that, due to the highest root and shoot dry weight in salinity conditions, Afzal cultivar is the most tolerant to salinity stress. This property in Afzal is due to the aggregation of compatible solutions in the cytoplasm and the significant increase in peroxidase as the

Table 5. Direct and indirect effects of traits on shoot dry matter (yield) in barley cultivars.

traits	Indirect effect						Correlation of dependent variable with independent variables
	direct effect	Shoot H_2O_2	Root DW	Root CAT	Shoot CAT	Shoot K^+/Na^+	
Shoot H_2O_2	-0.402	-	-0.246	0.098	-0.072	-0.156	-0.788**
Root DW	0.491	0.202	-	-0.158	0.134	0.089	0.765**
Root CAT	-0.336	0.118	0231	-	0.086	0.037	0.127**
Shoot CAT	0.200	0.145	0.328	-0.145	-	0.035	0.561**
Shoot K^+/Na^+	0.204	0.308	0.214	-0.061	0.035	-	0.706**

** Highly significant at 1% level of significance.

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antioxidant enzyme in root and shoot. Results of causality analysis also showed that certain properties have high direct impacts (positive and negative) on performance; therefore, a lot of care should be given to the aforementioned properties in plant breeding.

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ارزیابی فیزیولوژیک و بیوشیمیایی جو (*Hordeum vulgare* L.) تحت تنش شوری

ط. نریمانی، م. تورچی، ع. ر. تاری‌نژاد، س. ا. محمدی، و ح. محمدی

چکیده

این مطالعه به منظور بررسی پاسخ چهار رقم جو (ریحان ۰۳، یوسف، افضل و خاتم) به تنش شوری در سطوح صفر (شاهد)، ۱۰۰، ۲۰۰ و ۳۰۰ میلی مولار، به صورت آزمایش فاکتوریل و در قالب طرح بلوک‌های کامل تصادفی در سه تکرار در شرایط گلخانه ای با استفاده از محلول هوگلند انجام شد. صفات فیزیولوژیک و بیوشیمیایی شامل وزن خشک و RWC، رنگدانه‌های فتوسنتزی، K^+/Na^+، تنظیم کننده‌های اسمزی (قندهای محلول،گلایسین بتائین، پرولین)، پراکسید هیدروژن و آنزیم‌های آنتی اکسیدان (کاتالاز و پراکسیداز) در ریشه و اندام هوایی ارقام جو در شرایط غیر شور و شور مورد ارزیابی قرار گرفت. جهت تعیین روابط میان عملکرد و ویژگی‌های فیزیولوژیک و بیوشیمیایی، همبستگی بین صفات و تجزیه علیت اجرا گردید. نتایج مقایسه میانگین بین ترکیبات تیماری نشان داد که تنش شوری باعث کاهش وزن خشک، رنگدانه‌های فتوسنتزی، K^+/Na^+ و افزایش قندهای محلول، گلایسین بتائین، پرولین، H_2O_2، کاتالاز، پراکسیداز در ریشه و اندام هوایی ارقام جو شد. تجزیه همبستگی نشان داد که پتاسیم اندام هوایی دارای بیشترین ضریب همبستگی مثبت و معنی دار ($r=0.86$) با ماده خشک اندام هوایی بود. تجزیه رگرسیون گام به گام مشخص نمود که وزن خشک ریشه، کاتالاز ریشه و اندام هوایی، H_2O_2 اندام هوایی و K^+/Na^+ اندام هوایی در عملکرد سهیم بودند. نتایج تجزیه علیت نشان داد که وزن خشک ریشه، K^+/Na^+ اندام هوایی و کاتالاز اندام هوایی به لحاظ داشتن اثرات مستقیم مثبت و قابل توجه بر عملکرد ماده خشک اندام هوایی از اهمیت چشمگیری برخوردار هستند.

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