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## Salinity Causes Increase in Proline and Protein Contents and Peroxidase Activity in Wheat Cultivars

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**Abstract:** In a pot experiment, 15 cultivars of Iranian wheat (*Triticum aestivum* L.) were evaluated at glasshouse for proline and protein concentrations, peroxidase (POD) activity, SSI and STI in response to salinity (NaCl and Na<sub>2</sub>SO<sub>4</sub> in 1:1 ratio). A Completely Randomized Design (CRD) with factorial treatments in three replications was used. Using three salt treatments: 1.26 (control), 6.8 and 13.8 dS m<sup>-1</sup>. Salinity caused increase in proline and protein and POD activity in wheat genotypes in two salinity treatments. Kavir, Niknejad and Marvdasht showed high increase in some of studied traits compared with Ghods, Zarin and Cross Adl (sensitive cultivars). Based on studied traits other genotypes may be considered as semi-tolerant cultivars. Furthermore, tolerant cultivars showed higher STI and lower SSI compared with non-tolerant cultivars. Result showed that salinity tolerances are associated with higher accumulation of proline and protein concentration and higher POD activity in wheat.

**Key words:** Glasshouse, peroxidase, proline, protein, salinity, wheat, Na<sub>2</sub>SO<sub>4</sub>, NaCl

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### INTRODUCTION

Some environmental stresses adversely affect plant growth and development and final crop yield. Salinity, drought, oxidative stress and nutrient imbalances are the major environmental stresses. It has been reported that only less than 10% of the world's arable lands are free from environmental stresses, with drought and salinity being the wide spread (Ashraf, 1994). Salinity stress remains one of the worlds oldest and the most serious environmental problem, which substantially hampers crop productivity in arid and semi arid area. Low precipitation, high surface evaporation, weathering of native rocks, irrigation with saline water and poor cultural practices are among the major contributors to the increasing salinity. Secondary salinization, in particular, exacerbates the problem where once productivity agricultural lands are becoming unfit to cultivation due to poor quality irrigation water. Plant salt tolerance has generally been discussed in relation to regulatory mechanisms of ionic and osmotic homeostasis over two decades (Ashraf and Harris, 2004; Chinnusamy *et al.*, 2005; Ehret and Plant, 1999; Hasegawa *et al.*, 2000; Poustini and Siosemardeh, 2004; Srivastava *et al.*, 1998; Yeo, 1998; Zhu, 2003, 2002). These problems have been addressed by expensive and energy depleting soil reclamation measures. Salt stress thus exposes the plant to secondary osmotic stress. In addition to its injurious osmotic effects, salt may injure plant by way of specific toxic effect (Dionisio-Sese and

Tobita, 1998). Accumulations of osmotic compounds such as proline have been reported for use as a parameter of selection for salt stress tolerance (Ashraf, 2004; Elshintinawy and Elshourbagy, 2001; Goudarzi and Pakniyat, 2008a; Jain *et al.*, 2001; Khatkar and Kuhad, 2000; Lee and Liu, 1999; Muthukumarasamy *et al.*, 2000; Pakniyat and Armion, 2007; Pakniyat *et al.*, 2003; Singh *et al.*, 2000; Wang *et al.*, 2003). Despite of a strong correlation between stress tolerance and accumulation of proline in higher plants, this relationship may not be universal (Lutts *et al.*, 1999). It has been reported that soluble proteins increase at salinity in many plants and decreased in some else (Agastian *et al.*, 2000; Meneguzzo and Navarilzzo, 1999; Parida *et al.*, 2004). Salinity also inhibits the synthesis of majority of shoot proteins (Lutts *et al.*, 1999). Plants defend against the reactive oxygen which is product of hyperosmotic oxygen species, by induction of activities of certain antioxidative enzymes such as catalase, peroxidase, glutathione reductase and superoxide dismutase, which scavenge reactive oxygen species. There are several reports on activity of antioxidative enzymes under salt stress in wheat (Hernandez *et al.*, 1999; Mittler, 2002; Rios *et al.*, 2002; Sairam *et al.*, 2002). It is now widely accepted that Reactive Oxygen Species (ROS) are responsible for various stress-induced damage to macromolecules and ultimately to cellular structure (Hernandez *et al.*, 2000; Imlay, 2003; Neill *et al.*, 2002). These compounds are responsible for the quenching of ROS that produced

during stress become very important (Menconi *et al.*, 1995). The objective of the present investigation was to study the effect of salinity on free proline content, total protein and peroxidase activity in wheat cultivars and selection of the salt tolerant cultivars based on changes in this parameters with attention to the stress susceptibility index (SSI) and Salt Tolerance Index (STI).

**MATERIALS AND METHODS**

**Plant material, sowing and salinity treatments:** Fifteen wheat cultivars (Table 1) were compared at 3 salinity levels (1.26 (control), 6.8 and 13.8 dS m<sup>-1</sup>) for their proline, protein and POD activity and two salinity indices (Stress Susceptibility Index (SSI) and Salt Tolerance Index (STI)), in a completely randomized design with three replications. The experiment was conducted in glasshouse at Agricultural College, Shiraz University in Badjgah, Iran, 2006. These cultivars were random regarding their salinity tolerance and some were known for their tolerance to salinity by local farmers in Iran. Cultivars Kavir and Ghods were known as salt-tolerant and sensitive cultivars, respectively and they were used as check cultivars in this experiment. Ten seeds of each cultivar that became disinfested by Vitavax fungicide were sown at glasshouse condition in pots each in approximately 5 kg of a clay loam soil. The plants were watered according to field capacity using tap water. After germination (15 days following sowing) three plants were retained in each pot. The plants were subjected to three conditions: no salt (control) and two salinity levels (2.5 and 5 g salt (NaCl and Na<sub>2</sub>SO<sub>4</sub> in 1:1 ratio) per kg of soil). Salt stress treatments were applied 4 weeks after planting (at 2 leaf stage). Salt stock solution (25 g of both salts in 1:1 ratio dissolved per liter

of deionized water) was applied to appropriate pots in split and in 4 stages within 4 weeks to final concentrations by irrigation based on soil field capacity.

**Sample preparation for proline:** Proline was determined according to the method described by Bates *et al.* (1973). Approximately 0.5 g of fresh leaf material was homogenized in 10 mL of 3% aqueous sulfosalicylic acid and then this aqueous solution was filtered through Whatman's No. 2 filter paper and finally 2 mL of filtrated solution was mixed with 2 mL acid-ninhydrin and 2 mL of glacial acetic acid in a test tube. The mixture was placed in a water bath for 1 h at 100°C. The reaction mixture was extracted with 4 mL toluene and the chromophore containing toluene was aspirated, cooled to room temperature and the absorbance was measured at 520 nm with a Spectrometer. Appropriate proline standards were included for calculation of proline in the sample.

**Protein assay:** Protein content in the enzyme extracts was determined according to Bradford (1976) using Bovine Serum Albumin as a standard. It should be emphasized that in both standard curve of proline and protein the coefficient of determination (R<sup>2</sup>) should be more than 98%.

**Peroxidase assay:** Leaves (0.5 g) were powdered with Microdismembrator and homogenized in 50 mM sodium phosphate buffer (pH 7.0) containing 1% soluble polyvinyl pyrrolidone (PVP). The homogenate was centrifuged at 13,000 x g for 15 min at 4°C and the supernatant used for assays of the activities of POD. The activity of POD was assayed by adding aliquot of the tissue extract (100 µL) to 3 mL of assay solution, consisting of 3 mL of reaction mixture containing 13 mM guaiacol, 5 mM H<sub>2</sub>O<sub>2</sub> and 50 mM Na-phosphate (pH 6.5)

**Table 1: Mean values for traits of 15 wheat cultivars grow under control (ECe = 1.26 dS m<sup>-1</sup>) and saline condition (ECe = 13.8 dS m<sup>-1</sup>)**

No.	Cultivars	POD activity (µ mg <sup>-1</sup> )		Changes		Proline (mg g <sup>-1</sup> )		Changes		Protein (mg g <sup>-1</sup> )		Changes	
		Control	Saline	%	S <sup>a</sup>	Control	Saline	%	S <sup>b</sup>	Control	Saline	%	S <sup>b</sup>
1	Adl	4.03d-f	11.00c-f	273	**	1.14b-d	2.10f-h	184	**	3.50cd	5.20bc	149	**
2	Azadi	3.75d	10.67d-e	285	**	1.10b-d	3.10cd	282	**	2.30e	4.70cd	204	**
3	Bayat	10.45a	11.33b-e	108	ns	0.50f	3.30c	660	**	1.10f	2.60g	236	**
4	Chamran	6.12c	11.00c-f	180	**	1.20bc	3.10cd	258	**	1.90e	5.50b	289	**
5	Cross Adl	2.39g	12.46b	521	**	0.83de	1.81h	226	**	4.10bc	2.20g	-54	**
6	Darab-2	5.00cd	11.00c-f	220	**	1.30b	2.25e-g	173	**	3.10d	3.70f	119	ns
7	Falat	2.48g	10.00e-g	403	**	1.65a	2.45e	148	**	3.40cd	4.50c-e	132	ns
8	Ghods	4.28de	7.66h	179	**	1.25bc	1.98gh	158	**	3.60cd	2.40g	-67	**
9	Kavir	2.36g	13.93a	590	**	0.90cd	4.38a	471	**	4.70a	7.40a	157	**
10	Marvdasht	3.10e-g	10.33e-f	333	**	0.44f	2.10f-h	477	**	3.60cd	7.20a	200	ns
11	Niknejad	2.79fg	12.25bc	439	**	0.98bd	3.90b	398	**	3.50cd	5.70b	163	ns
12	Pishtaz	7.63b	10.67d-f	140	**	1.10bd	2.86d	260	**	2.10e	5.60g	267	**
13	Shiraz	2.39g	9.96fg	405	**	1.15b	2.14e-g	186	**	4.40ab	3.90ef	-89	ns
14	Star	3.56d-g	11.67b-d	328	**	0.60ef	2.90d	483	**	2.30e	4.80cd	209	**
15	Zarin	4.85d	8.67gh	179	**	1.2bc	2.40ef	200	**	3.50cd	4.20d-f	120	ns
Mean		4.35ab	10.82b			1.02a	2.72b			3.14a	4.64b		

In each column means followed by the same small letter(s) are not significant (DMRT, p<0.05), <sup>a</sup>Significance between control and saline condition (\*\*Significant at 1% levels of probability and ns not significant), <sup>b</sup>Significant at 1% level of probability between control and saline conditions for each character

(Chance and Maehly, 1955). An increase of the optical density at 470 nm for 1 min at 25°C was recorded using spectrophotometer. POD activity was expressed as change in absorbance min/mg/protein. The increase in ΔA470 was measured for 3 min and activity expressed as ΔA470 mg<sup>-1</sup> protein min.

**Salinity indices:** The Stress Susceptibility Index (SSI) was calculated (Fischer and Maurer, 1978) for the grain yield of each cultivar as:

$$SSI = \frac{1 - (GY_s / GY_p)}{1 - D}$$

where, GY<sub>s</sub> is the mean of a cultivar under salt stress and GY<sub>p</sub> the mean of a cultivar under control conditions, D the ratio of the overall mean of all cultivars under stress to the overall mean of all cultivars in control conditions. Salt Tolerance Index (STI) was calculated for the grain yield of each cultivar as:

$$STI = Y_s / Y_c$$

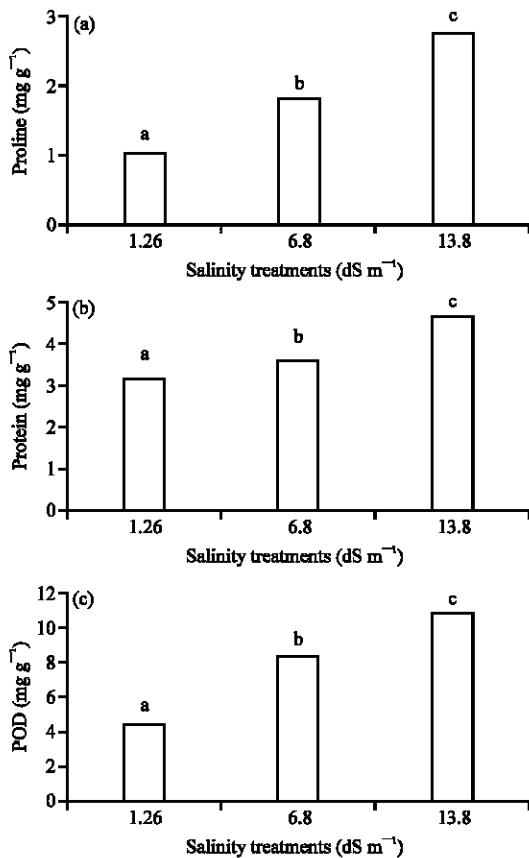


Fig. 1: Mean leaves proline and protein concentrations and POD activity of 15 wheat genotypes at 3 salt levels

where, Y<sub>s</sub> is the mean of the cultivar under salt stress and Y<sub>c</sub> the mean of cultivar under control condition.

**Statistical analysis:** Data were analyzed using MSTATC Software and the mean comparisons were made using Duncan's Multiple Rang Test. Graphical representations and diagram were made from EXCEL software.

**RESULTS**

**Proline accumulation:** There was significant increase in proline concentration under salinity stress (Fig. 1a). The total content of proline at S<sub>2</sub> showed 2.66 fold higher than control (S<sub>0</sub>) (Table 1). At control (S<sub>0</sub>) Falat showed the highest proline content while Marvdasht and Bayat had the lowest amount. At S<sub>2</sub> Kavir and Niknejad accumulated the highest proline and Cross Adl and Ghods (Fig. 2a) accumulated the lowest amount of it.

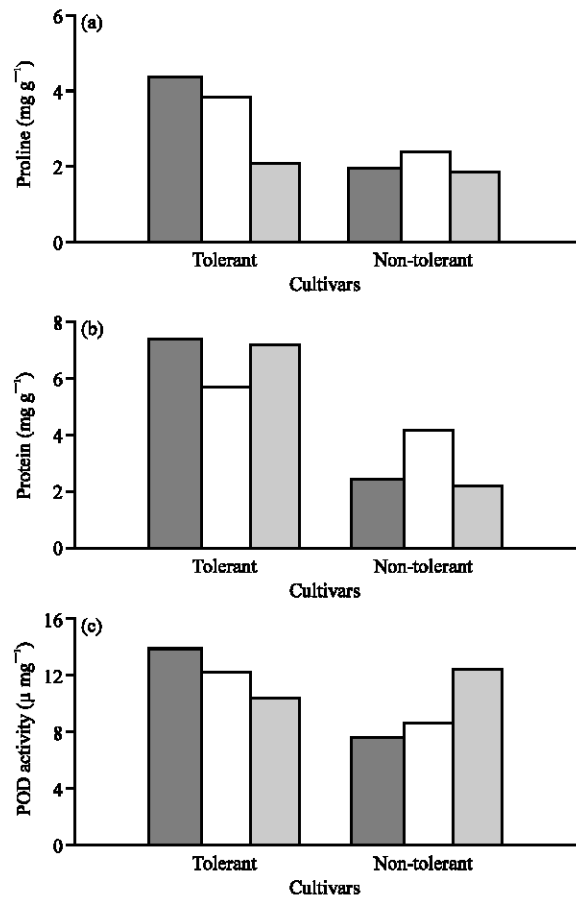


Fig. 2: Variation in mean proline, protein concentration and POD activity between tolerant (Kavir, Niknejad and Marvdasht) and non-tolerant (Ghods, Zarin and Cross Adl) wheat genotypes at S<sub>3</sub> salinity level

Table 2: Comparison between wheat cultivars based on changes in STI and SSI at S<sub>2</sub>

Cultivars	1	2	3	4	5	6	7	8	9	10	11	12	13	14	15
STI*	0.80	0.70	0.60	0.80	0.80	0.80	0.80	0.60**	0.90**	0.70	0.80	0.70	1.10	1.40	0.70
SSI*	0.80	1.10	1.60	0.90	0.80	0.90	0.80	1.60*	0.40*	0.80	0.80	1.20	0.70	0.70	1.20

\*STI and SSI are salt tolerance index and salinity susceptibility index, respectively

**Protein concentration:** Salinity treatments, protein caused protein increase in all genotypes (Fig. 1b), (Table 1). The highest protein content at control (S<sub>0</sub>) was belonged to Kavir and Shiraz, while, Bayat showed the lowest amount of it. At S<sub>2</sub> Kavir, Marvdasht, Niknejad and Pishtaz produced the highest protein content while, Ghods, Cross Adl and Bayat (Fig. 2b) produced the lowest amount.

**Peroxidase activity:** Leaves enzyme activities are presented in Table 1. POD activity increased significantly under salinity stress at two salinity levels (Fig. 1c). Higher enzyme activity was observed at S<sub>2</sub> salinity level and for cultivars: Kavir, Marvdasht and Cross Adl, although these cultivars had low activities at control (S<sub>0</sub>). The cultivars Ghods and Zarin (Fig. 2c) maintained the lowest POD activity at S<sub>2</sub> when compared with other genotypes.

**Salinity indices:** There was a variation between cultivars in regard to SSI and STI under saline conditions (Table 2). Kavir had the lowest SSI and the highest amount of STI. In contrast Ghods had the highest SSI and the lowest of STI values.

## DISCUSSION

Accumulation of proline and protein under stress in many plant species has been correlated with stress tolerance and the concentrations has been shown to be generally higher in stress-tolerant than in stress-sensitive plants (Fig. 2a). Proline is known to accrue widely in higher plants and normally its accumulation is in large quantities in response to salinity to protect the cell by balancing the osmotic strength of cytosol with that vacuole and external environment (Kavi *et al.*, 2005; De-Lacerda *et al.*, 2003). Kavir and Niknejad had high concentration of proline, while Cross Adl and Ghods accumulated the lowest levels of this component. Furthermore, this component may be contributed to stabilizing sub-cellular structures (e.g., proteins and enzymes) which present in Kavir. Salinity stress caused a significant increase in protein content in salt stressed plants. Proteins accumulations are particularly important for cell survival under salt stress and causes membranes stabilization under salt stress. Kavir and Marvdasht produced the highest protein, while Ghods and Cross Adl had the lowest protein content (Fig. 2b). In response to salinity, plants make new proteins that help them to grow

and develop under saline condition. One may speculate that, salt tolerant cultivars producing higher protein concentration is due to higher efficiency of osmotic regulation mechanism in these plants which in turn causes decreasing sodium toxicity in cytoplasm compared to susceptible ones and the result is to prevent proteins reduction under salt stress (Flowers and Yeo, 1995).

It has been demonstrated that both osmotic and ionic effects are involved in salinity, can limit photosynthesis and respiration, leading to an increase in ROS production, which are responsible for a secondary oxidative stress that can damage cellular structure and metabolism. Antioxidant enzyme (e.g., POD) are responsible for quenching of single oxygen hence their comparative levels in a variety may determine its relative tolerance and helps the plants to maintain their growth compared to salt sensitive cultivars. The higher activity of these antioxidant enzymes has a role in importing tolerance to these cultivars and protects them against the oxidative reactions. In the present investigation, we noticed that POD activity has been increased when plants subjected to salinity. Wheat cultivars were different in regard to POD activity. Kavir, Cross Adl and Niknejad had the highest POD activity and they may be considered as salt tolerant cultivars. Ghods and Zarin cultivars showed the lowest POD activity and therefore they may be considered as salt sensitive cultivars (Fig. 2c). In addition, Cross Adl produced high Na<sup>+</sup> content (Goudarzi and Pakniyat, 2008b), indicating salt sensitivity of cultivar and the increase in POD activity is only a reaction to salt stress and not the plant response to tolerance, therefore other parameters (e.g., protein and proline) may be involved in this regard. Increase in antioxidant enzymes such as POD under long term salinity in tolerant wheat has been reported by Sairam *et al.* (1998) and Hernandez *et al.* (2000). The present finding showed that the higher proline and protein contents and also higher POD activity are associated with SSI and STI. The tolerant cultivars had a higher and lower STI and SSI values compared to sensitive cultivars.

## CONCLUSION

Salinity stress is a complex system and the plant responses to salt stress are multigenic and no single parameter could be suggested as the sole factor responsible for salinity stress tolerance of wheat genotypes, therefore a combination of parameters are

contributing to salinity stress tolerance. It is possible that better salinity resistance of Kavir, Niknejad and Marvdasht was associated with their ability to maintain higher activity of POD and having higher STI and lower SSI value. High concentration of proline and protein may provide better performance under saline conditions. The studied parameters in this research showed that Ghods, Cross Adl and Zarin (salt sensitive cultivars) had the lowest amount of these components.

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