# Proline Accumulation and Related Genes in Wheat Leaves under Salinity Stress

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#### ABSTRACT

This study was carried out to evaluate the effects of salinity stress  $(16 \text{ dS m}^{-1})$  on proline accumulation and expression pattern of three genes involved in the proline synthesis (P5CS, P5CR) and degradation (PDH) in the fourth and the flag leaves. The experimental treatments consisted of two levels of salinity stress including 1.6 (control) and 16 dS m<sup>-1</sup> and three wheat cultivars, namely, Hirmand and Chamran (as tolerant cultivars) and Atrak (as sensitive). These were arranged as factorial based on a Completely Randomized Design with three replications. There was an increase in Na<sup>+</sup> concentration and decrease in K<sup>+</sup> concentration in salinity stress compared to the control condition in the fourth and the flag leaves of all cultivars. However, the tolerant cultivars showed lower level of Na<sup>+</sup> concentration and higher level of K<sup>+</sup> concentration and K<sup>+</sup>/ Na<sup>+</sup> ratio. Seemingly, the increase in proline accumulation was due to P5CS, P5CR up-regulation and PDH downregulation. On the other hand, the higher proline degradation in the fourth leaf of the tolerant cultivars was probably due to the provision of a source for energy or nitrogen, which helped to enhance the yield of these cultivars under salinity stress.

Keywords: Proline, Salinity, Wheat, P5CS, P5CR, PDH.

#### **INTRODUCTION**

Salinity is one of the most important abiotic stresses and it limits the productivity and geographical distribution of plants. According to the FAO (2008) report, over 6% of the world's land (about 800 Mha) is affected by salinity. Most of this salinity-affected land has arisen from natural causes i.e. accumulation of salts over long periods of time in arid and semiarid zones, and also by human activities such as irrigation (Munns and Tester, 2008).

The adverse impacts imposed by salt stress are osmotic stress, ionic stress, nutrient imbalance, and the production of reactive oxygen species; then, the plants would display declining growth and photosynthesis rate, even death in the end (Wu *et al.*, 2014). Salinity affects many aspects of plant metabolism and the accumulation of various organic solutes that provide the turgor necessary for cell expansion. Among them, the accumulation of low molecular weight solutes and compatible osmolytes, such as proline and glycine betaine, function as osmoprotectants. In addition to osmotic role, it has been proposed that, in stress conditions, proline has different roles including a carbon and nitrogen storage component, ROS scavenger, buffer of cytosolic pH and cell redox balance status, stabilizer of proteins structure (Hare and Cress, 1997), and finally, signal of stress adaptive responses (Maggio *et al.*, 2002).

Proline accumulation has been demonstrated to be correlated with salinity tolerance in plants. Moreover, the increase of proline content in leaf is also significant in salt stressed sorghum (de Lacerda *et al.*, 2003), maize (Hajlaoui *et al.*, 2010), and

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soybean (Çelik and Atak, 2012). Its concentration has been shown to be generally higher in stress-tolerant than in stress-sensitive plants (Ashraf and Foolad, 2007), but in wheat cultivars, Poustini *et al.* (2007) have shown a larger concentration of proline in salt sensitive cultivars than salt tolerant ones in salinity stress condition.

Proline accumulation is mediated by its increased synthesis and reduced oxidation. Some studies demonstrate that, in stress condition, most of the proline accumulated in plants is the result of enhanced synthesis from glutamate (Delauney and Verma, 1993; Hare and Cress, 1997). It is a two-step process that glutamate is reduced to  $\Delta^1$ -pyrroline-5- $\Delta^1$ -pyrroline-5carboxylate (P5C) by carboxylate synthetase (P5CS) and then it is followed by the reduction of P5C to proline,  $\Delta^1$ -pyrroline-5-carboxylate catalvzed by reductase (P5CR). Proline degradation is also a two-step oxidation process that starts with the oxidation of proline to P5C by proline dehydrogenase (PDH) and subsequently the conversion of P5C to glutamate by pyrroline-5-carboxylate dehydrogenase (P5CDH) (Lehmann et al., 2010). It has been reported that proline accumulation is regulated by transcriptional changes of proline biosynthesis and degradation (Mattioli et al., 2009). Xue et al. (Xue et al., 2009) have indicated that stress-induced accumulation of proline in rapeseed results from the activated biosynthesis and also the inhibited proline degradation. They have also shown that the expression of BnP5CS1 and BnP5CS2 were enhanced under salt stress, ABA treatment and dehydration, but the expression of BnPDH was inhibited. The objective of this evaluate research was to proline accumulation and its biosynthesis and degradation in transcriptional regulation under salinity stress in tolerant and sensitive wheat cultivars.

#### MATERIALS AND METHODS

This study was carried out in greenhouse pot culture from the middle of October 2010

to the middle of February 2011. The air temperature ranged from 22 to 28°C during the day and 14 to 17°C during the night. The experimental treatments were arranged as factorial based on a completely randomized design with three replications. Treatments consisted of two levels of salinity i.e. 1.6 (control) and 16 dS m<sup>-1</sup> (salinity stress), and three wheat cultivars, namely, Hirmand and Chamran (as tolerance cultivars) and Atrak (as sensitive cultivar) (Tavakoli, 2011).

The pots contained 3.5 kg of soil (mixture of farm soil, sand, and farmyard manure in a 3:2:1 ratio). Ten grains were sown in each pot. They were daily irrigated with water until seedlings establishment (21days). Afterwards, the pots were watered with a solution of NaCl (150 mM) to reach the saline level of 16 dS m<sup>-1</sup> gradually in 3 days by adjustment of the soil water content close to the field capacity. Plants were sampled at two stages: anthesis (Zadoks; 61) and harvesting (Zadoks; 92). The flag and the fourth leaves of plants were separated for measuring proline and ions concentration and analyzing genes expression. Leaves were dried at 60°C for 72 h for measuring the concentration of leaf Na<sup>+</sup> and K<sup>+</sup>. The measurements were taken from the 2 N chloride acid extract of the samples(0.5 g)that had been burned at 600°C for 4 h, using and flame photometer (Poustini а Siosemardeh, 2004).

Proline content of the leaves and leaf discs was determined by homogenizing the samples in a mortar and pestle in 3% sulfosalicylic acid. After centrifugation at 13,000 rpm for 11 min, 2 mL supernatant was added to 2 mL glacial acetic acid and 2 mL ninhydrin solution (0.2 M). The mixture was kept at 95°C for 60 min, then, the reaction was stopped quickly by an ice bath. Toluene (3 mL) was added to the mixture and the organic phase was extracted and monitored at 520 nm by a spectrophotometer (Shimadzu, UV-160) (Bates *et al.*, 1973).

The mRNAs of the flag and fourth leaves were extracted using the Qiagen kit according to the manufacturer's protocol. Total cDNA was synthesized from mRNA (1 µg) by Fermentas kit according to the manufacturer's protocol. Quantitative realtime RT-PCR was carried out with wheat actin gene as the internal standard. The gene-specific primers were designed by PrimerQuest and evaluated by OligAnalyzer (Table 1). In the first step, amplification was started at 95°C for 10 min, followed by 40 cycles of PCRs: denaturation at 95°C for 30 s, annealing at 55°C for 30 s, and elongation at 72°C for 30 s. A melting curve was run after PCR cycles. Detection of PCR products was done using the SYBR Green Real-time PCR Master Mix. Reactions were repeated 5 times for each sample and relative gene expression was calculated using the  $2^{-\Delta\Delta C}T$ method (Livak and Schmittgen, 2001).

The analysis of variance of the data was done by SAS, 9.1 Software. The data mean comparisons were made following Least Significant Difference (LSD) test at 5% probability level.

#### RESULTS

#### **Growth and Ionic Characteristics**

Shoot biomass and grain weight were decreased by NaCl in all cultivars. This decrease was larger for Hirmand but it had the highest shoot biomass in the saline treatment (Table 2). Moreover, Chamran had the highest grain weight under salinity stress and it showed stability in grain weight. In contrast, the lowest value of grain weight was observed in Atrak with 76% decrease under salinity stress.

Results (Table 2) showed that  $K^+$  concentration generally decreased in both leaves in all cultivars under salinity stress conditions, except in Chamran which showed the highest  $K^+$  concentration in flag leaf under salinity. In the normal condition  $K^+$  concentration of flag leaves was higher than 4<sup>th</sup> leaves in Chamran and Hirmand but Atrak showed a lower  $K^+$  concentration in flag leaves. However  $K^+$  concentration in flag leaves rather than flag leaves was: 1. higher in Chamran 2. similar in Atrak and 3. lower in Hirmand (Table 2).

Distribution of Na<sup>+</sup> in leaves changed significantly with their age and NaCl concentration. Dealing with all treatments of salinity, the greatest accumulation was observed in the 4<sup>th</sup> leaf. The concentration of Na<sup>+</sup> in the sensitive cultivars was 2 folds or more in both leaves compared to the tolerant cultivars. Atrak showed the highest level and change percent of Na<sup>+</sup> in flag and 4<sup>th</sup> leaves. While there wasn't different between all cultivar in the normal condition.

In leaves,  $K^+/Na^+$  ratio in all cultivars decreased significantly with the increase in salinity level as compared to the control treatment (Table 2).  $K^+/Na^+$  ratio was higher in the flag leaf than the 4<sup>th</sup> leaf and in the tolerant cultivars (Chamran and Hirmand) than the sensitive cultivar (Atrak).

**Table 1**. List of the primers used for RT-PCR analysis of the wheat candidate genes.

Gene	Accession No	Primer	Sequence	TM
A	AD101001 1	Forw	GCCACACTGTTCCAATCTATGA	60.2 °C
Actin	AB181991.1	Reve	TGATGGAATTGTATGTCGCTTC	60.1 °C
DSCS	10062070 1	Forw	TCGGTGCTGAGGTTGGCATAAG	59.5 °C
PSCS	JQ003079.1	Reve	TTGTCACCATTCACCACTTGCCC	60.1 °C
D5CD	A V880317 1	Forw	CGGGTAAACATCCAGGGCAGC	60.8 °C
FJCK	A1000317.1	Reve	TCGGCATCTTGTTGTGGCAGC	60.9 °C
DDU	A 12220190 1	Forw	CGGGATCCTCGACTACGGCATC	61.3 °C
PDH	АКЗЗ2189.1	Reve	TGATCTTGATACACACGCTCGCCG	61.1 °C

	Shoot biomass $^{c}$
ons.	Proline concentration <sup>b</sup>
ntrol and salt stress condition	K/ <sup>+</sup> Na <sup>+</sup>
of three wheat cultivars under co	Sodium concentration <sup>a</sup>
2. Comparison of some traits in two leaves	Potassium concentration <sup><i>a</i></sup>

		Potassium	concentratic	<sup>p</sup> uc	Sodium	concentral	tion <sup>a</sup>		K/*Na*		Proline	concentrati	$^{q}$ uo	Sho	ot biom	ass <sup>c</sup>	Ū	ain weig	ht
Cultivars	Lear	Cq	s	Ch	C	s	Ch	C	s	Ch	C	s	c	C	s	Ch	C	s	Ch
-1 V	flag	23.2 ±0.9 <sup>g</sup>	20.8±0.6	-10.6	0.0∓ 9.0	2.4 ±0.3	300	39.2 ±0.9	8.6 ±0.3	-78.2	0.51±0.1	2.02±0.20	295.4	2.01±	1.51±		0.75±	0.25±	2
Auak	4th	30.7 ±0.8	20.6±0.9	-32.8	3.5 ±0.8	23.6±2	582.6	8.9 ±0.4	0.9 ±0.2	-90.4	$0.18\pm0.01$	1.65±0.01	798.2	0.8	0.2	- 24.9	0.35	0.12	0/-
	flag	21.9 ±1.2	30±0.8	37.3	0.6 ±0.1	1.6 ±0.1	158	35 ±1.8	19.7 ±0.5	-43.9	0.29±0.03	1.81±0.08	515.9	2.48±	1.97±	200	1.04±	0.74±	000
Cnamran	4th	20.6±1	14.8±1	-28.4	3.1 ±0.3	12.7 ±1.1	3116.4	6.8 ±0.1	$1.2 \pm 0.3$	-83	$0.4\pm0.08$	1.3±0.09	223.1	6.0	0.3	0.02-	0.46	0.2	0.02-
11	flag	32.1 ±0.7	22±0.8	-31.6	0.4 ±0.0	1.1 ±0.0	163	75.7±2.1	20.9 ±0.9	-72.4	0.16±0.02	$1.9\pm0.10$	1087.5	3.31±	2.06±		1.09±	0.67±	
Hirmand	4th	29.3 ±1.3	25.2±0.2	-14.2	4.7 ±0.2	6.9 ±0.2	48.4	6.3 ±0.8	3.7 ±0.2	-42.1	$0.31 \pm 0.02$	$1.1\pm0.05$	258.7	0.9	0.3	9.16-	0.91	0.12	1.16-
<sup>a</sup> mg g <sup>-1</sup> c	lry mat	ter. mg $g^{-1}$ , <sup>b</sup>	fresh weigh	ht. <sup>c</sup> g pl	ant <sup>-1</sup> . <sup>d</sup> C; cc	ontrol, S: sa	alinity an	d Ch: chang	ges made by	<pre>/ salinity</pre>	/ in percenta	ge, <sup>d</sup> Values	are mear	ı ± stand:	ard error	(n=3).			

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Figure1. Effect of salinity on (a) P5CS (b) P5CR (c)PDH expression in the flag and 4<sup>th</sup> leaves of wheat cultivars.



# Proline Concentration and Genes Expression Pattern

All three cultivars showed increase in the level of proline in their tissue upon salinity treatment. Proline concentration in flag leaves was larger than the 4<sup>th</sup> leaves and it also was higher in the sensitive cultivar (Atrak) compared to the tolerant ones (Hirmand and Chamran).

Under unstressed conditions, the P5CS gene was not different between cultivars, but, under salinity condition, both the flag and 4<sup>th</sup> leaves showed a dramatic increase (Figure 1). Sensitive cultivar (Atrak) showed a larger P5CS gene expression than salinity stress tolerant cultivars (Chamran and Hirmand). Expression of P5CS in the 4<sup>th</sup> leaf of Atrak was 1.5 fold more than the other cultivars and it was the same as the flag leaf expression under salinity stress (Figure 1).

Expression of P5CR increased in all cultivars under salinity stress and its pattern was similar to P5CS, but in terms of intensity they were different i.e. the maximum expression of P5CR was 3.5 fold and in P5CS it was 8.5 fold more than the control (Figure 2).

In contrast, expression pattern of PDH decreased in flag leaves of all cultivars and also increased in the 4<sup>th</sup> leaves of Chamran and Hirmand under salinity stress, but Atrak's 4<sup>th</sup> leaf did not show any change in PDH expression by NaCl (Figure 3). Proline content and level of P5CS and PDH expression in Atrak's 4<sup>th</sup> leaves was higher than other cultivar's 4<sup>th</sup> leaves. But level of P5CR expression in Atrak's 4<sup>th</sup> leaves didn't show any significant difference with other cultivar's 4<sup>th</sup> leaves.

# DISCUSSION

Salt tolerance reflects the mechanisms associated with tissues tolerance to the accumulation of Na<sup>+</sup> as well as the ability of the plants to exclude Na<sup>+</sup> (Benderradji *et al.*,

2011). Based on the results obtained, Chamran showed a relative salt tolerance in grain yield and biomass production, and Hirmand showed to be an absolute salt tolerant cultivar. Chamran showed superior shoot biomass and yield (less reduction), Hirmand showed only less reduction in yield, but higher reduction in shoot biomass compared to Atrak. Therefore, maybe it is proper to say that Hirmand is salt tolerant during reproductive period, but not during vegetative growth stage.

The difference between the sensitive and tolerant groups can be related to their difference in response to high available Na<sup>+</sup> Under salinity stress conditions, the relatively salt tolerant cultivars try to exclude Na<sup>+</sup> and keep high K<sup>+</sup>/ Na<sup>+</sup> ratio, especially in the flag leaf (Husain *et al.*, 2003). Atrak was considered salt sensitive cultivar because it showed high loss of yield and biomass, and low ability in Na<sup>+</sup> exclusion, and low K<sup>+</sup>/ Na<sup>+</sup> ratio in flag leaf.

Under salinity, the K<sup>+</sup>/Na<sup>+</sup> ratio falls dramatically, therefore, one of the key features of salt tolerant plants is the ability of cells to maintain optimal  $K^+/Na^+$  ratio in the cytosol (Tester and Davenport, 2003). This occurs as the result of both excessive Na<sup>+</sup> accumulation in the cytosol and also enhanced  $K^+$  leakage from the cell, the latter resulting from NaCl-induced membrane depolarization under saline conditions (Chen et al., 2005). Therefore, K<sup>+</sup>/Na<sup>+</sup> ratio in plant tissues has often been suggested as a potential screening tool for plant breeders (Asch et al., 2000; Asch et al., 1999; Poustini and Siosemardeh, 2004). (Asch et al., 2000) indicated a highly significant correlation between leaves K<sup>+</sup>/Na<sup>+</sup> and salinity-induced grain yield reduction. They showed that the most sensitive cultivars had the lowest leaves  $K^+/Na^+$  and the largest yield reductions. The accumulation of free proline under salinity stress is reported in Pisum sativum (Najafi et al., 2007), Brassica juncea (Rais et al., 2013) and Triticum aestivum (Ashfaque et al., 2014) as an osmoprotectant that reduce adverse effects of salinity by regulating redox potentials, scavenging oxygen radicals, reducing the acidity in the cell and acts as storage compound and nitrogen source. It that the increase in proline seems accumulation was due to P5CS, P5CR updown-regulation. regulation and PDH Verslues and Sharma (2010) also proposed a model for proline metabolism in Arabidopsis under salinity stress that attributed proline accumulation to PDH and P5CDH downregulation and P5CS up-regulation. Also, Xue et al. (2009) reported that the expression of BnP5CS1and BnP5CS2 was induced, while the expression of BnPDH was inhibited under salt stress, ABA treatment, and dehydration, prior to proline accumulation in Brassica napus. PDH inhibition by knockout or antisense repression resulted in no or only marginal increases in Pro levels under normal conditions. However, during stress, Pro levels were several-fold higher as in the wild type(Mani et al., 2002; Nanjo et al., 2003). Exceptions to the standard model of upregulated Pro synthesis and down-regulated catabolism leading to Pro accumulation have been reported. For example, Kaplan et al. (2007) saw increased expression of PDH1 in longer term (24-96 h) cold treatments even while Pro contents were high and increasing. Stines et al. (1999) found high levels of Pro despite low P5CS1 expression in grape (Vitis vinifera) berries.

Results of this study showed that the salt sensitive cultivar had higher proline accumulation than the salt tolerant ones. Higher proline accumulation as well as Na<sup>+</sup> in the sensitive cultivar than in the tolerant ones has been indicated by previous studies (Aziz et al., 1998; Poustini et al., 2007). Rout and Shaw (1998) attributed the higher proline levels to higher injury which indictes more  $Na^+$ accumulation in sensitive wheat cultivars. Although there was a positive correlation between proline and Na<sup>+</sup> in each leaf, proline in flag leaves was more than in the 4<sup>th</sup> leaves, which had higher Na<sup>+</sup> concentration. Also, proline accumulation in flag leaf was more in tolerant cultivars than in salt sensitive one, while it is the opposite for 4<sup>th</sup> leaf. This makes sense since flag leaf the young organ that needs more protection

during stress and tolerant cultivars play this role perfectly.

Proline homeostasis is important for actively dividing cells as it helps to maintain sustainable growth under long-term stress. It also underpins the importance of the expansion of the proline sink during the transition from vegetative to reproductive growth and the initiation of seed development (Kishor et al., 2014). Because of these two reasons, it seems that proline catabolism is a very important mechanism of tolerance in the 4<sup>th</sup> leaf. This leaf has little energy due to senescence and stress. When proline is degraded, NAD(P)H will be released and cell energy will be higher. This leaf also is the source of nitrogen for grain and other young parts of plant. In this situation, proline degradation along with the increase of glutamate, a plant transportable nitrogen, will help to increase nitrogen source strength. According to this hypnosis, the expression pattern of PDH increased in the 4<sup>th</sup> leaves of Chamran and Hirmand under salinity stress, but Atrak's 4<sup>th</sup> leaf did not show any change in PDH expression in salinity treatment. It seems that the tolerant cultivars show higher degradation in the 4<sup>th</sup> leaf to increase cell energy and transportable nitrogen level.

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# REFERENCES

- 1. Asch, F., Dingkuhn, M., Dörffling, K. and Miezan, K. 2000. Leaf K/Na Ratio Predicts Salinity Induced Yield Loss in Irrigated Rice. *Euphytica*, **113**:109-118.
- 2. Asch, F., Dingkuhn, M., Wittstock, C. and Doerffling, K. 1999. Sodium and Potassium



Uptake of Rice Panicles as Affected by Salinity and Season in Relation to Yield and Yield Components. *Plant and Soil*, **207**:133-145.

- Ashfaque, F., Khan, M. I. R. and Khan, N. A. 2014. Exogenously Applied H2o2 Promotes Proline Accumulation, Water Relations, Photosynthetic Efficiency and Growth of Wheat (*Triticum Aestivum* L.) under Salt Stress. Annual Research & Review in Biology, 4:105-120.
- 4. Ashraf, M. and Foolad, M. 2007. Roles of Glycine Betaine and Proline in Improving Plant Abiotic Stress Resistance. *Environmental and Experimental Botany*, **59**:206-216.
- Aziz, A., Martin□Tanguy, J. and Larher, F. 1998. Stress□Induced Changes in Polyamine and Tyramine Levels Can Regulate Proline Accumulation in Tomato Leaf Discs Treated with Sodium Chloride. *Physiologia Plantarum*, **104**:195-202.
- Bates, L., Waldren, R. and Teare, I. 1973. Rapid Determination of Free Proline for Water-Stress Studies. *Plant and soil*, 39:205-207.
- Benderradji, L. et al. 2011. Sodium Transport in the Seedlings of Two Bread Wheat (*Triticum Aestivum* L.) Genotypes Showing Contrasting Salt Stress Tolerance. *Australian Journal of Crop Science*, 5:233-241.
- Çelik, Ö. and Atak, Ç. 2012. Evaluation of Proline Accumulation and Δ1-Pyrroline-5-Carboxylate Synthetase (P5cs) Gene Expression During Salinity Stress in Two Soybean (Glycine Max L. Merr.) Varieties. *Polish Journal of Environmental Studies*, 21:559-564.
- Chen, Z., Newman, I., Zhou, M., Mendham, N., Zhang, G. and Shabala, S. 2005. Screening Plants for Salt Tolerance by Measuring K<sup>+</sup> Flux: A Case Study for Barley. *Plant, Cell & Environment*, 28:1230-1246.
- De Lacerda, C. F., Cambraia, J., Oliva, M. A., Ruiz, H. A. and Prisco, J. T. n. 2003. Solute Accumulation and Distribution During Shoot and Leaf Development in Two Sorghum Genotypes under Salt Stress. *Environmental and Experimental Botany*, 49:107-120.
- 11. Delauney, A. J. and Verma, D. P. S. 1993. Proline Biosynthesis and Osmoregulation in Plants. *The Plant Journal*, **4**:215-223.

- 12. Fao. 2008 Plant Nutrition Management Service.
- Hajlaoui, H., Ayeb, N. E., Garrec, J. P. and Denden, M. 2010. Differential Effects of Salt Stress on Osmotic Adjustment and Solutes Allocation on the Basis of Root and Leaf Tissue Senescence of Two Silage Maize (*Zea Mays L.*) Varieties. *Industrial Crops and Products*, **31**:122-130.
- 14. Hare, P. and Cress, W. 1997. Metabolic Implications of Stress-Induced Proline Accumulation in Plants. *Plant Growth Regulation*, **21**:79-102.
- 15. Husain, S., Munns, R. and Condon, A. T. 2003. Effect of Sodium Exclusion Trait on Chlorophyll Retention and Growth of Durum Wheat in Saline Soil. *Crop and Pasture Science*, **54**:589-597.
- 16. Kaplan, F., Kopka, J., Sung, D. Y., Zhao, W., Popp, M., Porat, R. and Guy, C. L. 2007. Transcript and Metabolite Profiling During Cold Acclimation of Arabidopsis Reveals an Intricate Relationship of Cold Regulated Gene Expression with Modifications in Metabolite Content. *The Plant Journal*, **50**:967-981.
- Kishor, K., Polavapapu, B. and Sreenivasulu, N. 2014. Is Proline Accumulation Per Se Correlated with Stress Tolerance or Is Proline Homeostasis a More Critical Issue? *Plant, cell & environment,* 37:300-311.
- Lehmann, S., Funck, D., Szabados, L. and Rentsch, D. 2010. Proline Metabolism and Transport in Plant Development. *Amino Acids*, **39**:949-962.
- 19. Livak, K. J. and Schmittgen, T. D. 2001. Analysis of Relative Gene Expression Data Using Real-Time Quantitative PCR and the  $2^{-\Delta\delta ct}$  Method. *methods*, **25**: 402-408.
- 20. Maggio, A. et al. 2002. Does Proline Accumulation Play an Active Role in Stress□Induced Growth Reduction? The *plant journal*, **31**:699-712.
- Mani, S., Van De Cotte, B., Van Montagu, M. and Verbruggen, N. 2002. Altered Levels of Proline Dehydrogenase Cause Hypersensitivity to Proline and Its Analogs in Arabidopsis. *Plant Physiology*, **128**:73-83.
- 22. Mattioli, R., Falasca, G., Sabatini, S., Altamura, M. M., Costantino, P. and Trovato, M. 2009. The Proline Biosynthetic Genes P5cs1 and P5cs2 Play Overlapping Roles in Arabidopsis Flower Transition but

Not in Embryo Development. *Physiologia Plantarum*, **137**:72-85.

- 23. Munns, R. and Tester, M. 2008. Mechanisms of Salinity Tolerance. *Annu. Rev. Plant Biol.*, **59**:651-681.
- 24. Najafi, F., Khavari-Nejad, R., Rastgar-Jazii, F. and Sticklen, M. 2007. Growth and Some Physiological Attributes of Pea (Pisum Sativum L.) as Affected by Salinity. *Pakistan journal of biological sciences: PJBS*, **10**:2752-2755.
- Nanjo, T., Fujita, M., Seki, M., Kato, T., Tabata, S. and Shinozaki, K. 2003. Toxicity of Free Proline Revealed in an Arabidopsis T-DNA-Tagged Mutant Deficient in Proline Dehydrogenase. *Plant and Cell Physiology*, 44:541-548.
- Poustini, K. and Siosemardeh, A. 2004. Ion Distribution in Wheat Cultivars in Response to Salinity Stress. *Field Crops Research*, 85:125-133.
- Poustini, K., Siosemardeh, A. and Ranjbar, M. 2007. Proline Accumulation as a Response to Salt Stress in 30 Wheat (*Triticum Aestivum L.*) Cultivars Differing in Salt Tolerance. *Genetic Resources and Crop Evolution*, 54:925-934.
- Rais, L., Masood, A., Inam, A. and Khan, N. 2013. Sulfur and Nitrogen Co-Ordinately Improve Photosynthetic Efficiency, Growth and Proline Accumulation in Two Cultivars of Mustard under Salt Stress. *J Plant Biochem Physiol*, 1:101-101.
- 29. Rout, N. and Shaw, B. 1998. Salinity Tolerance in Aquatic Macrophytes: Probable

Role of Proline, the Enzymes Involved in Its Synthesis and C 4 Type of Metabolism. *Plant Science*, **136**:121-130.

- 30. Stines, A. P., Naylor, D. J., Høj, P. B. and Van Heeswijck, R. 1999. Proline Accumulation in Developing Grapevine Fruit Occurs Independently of Changes in the Levels of  $\Delta$ 1-Pyrroline-5-Carboxylate Synthetase Mrna or Protein. *Plant Physiology*, **120**:923-923.
- 31. Tavakoli, M. 2011. Some Physiological Traits Associated with Salinity Stress and Related Genes Expression Pattern in Bread Wheat (*Triticum Aestivum* L.). University of Tehran, Master thesis,
- 32. Tester, M. and Davenport, R. 2003. Na<sup>+</sup> Tolerance and Na<sup>+</sup> Transport in Higher Plants. *Annals of Botany*, **91**:503-527.
- Verslues, P. E. and Sharma, S. 2010. Proline Metabolism and Its Implications for Plant-Environment Interaction. *The Arabidopsis Book/American Society of Plant Biologists*, 8: 344-342.
- 34. Wu, G., Zhou, Z., Chen, P., Tang, X., Shao, H. and Wang, H. 2014. Comparative Ecophysiological Study of Salt Stress for Wild and Cultivated Soybean Species from the Yellow River Delta, China. *The Scientific World Journal*, **45**:321-340.
- 35. Xue, X., Liu, A. and Hua, X. 2009. Proline Accumulation and Transcriptional Regulation of Proline Biothesynthesis and Degradation in Brassica Napus. *BMB reports*, **42**:28-34.

# تجمع پرولین و ژنهای مرتبط با آن در برگهای گندم تحت تنش شوری

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# چکیدہ

این مطالعه به منظور ارزیابی اثر تنش شوری(۱۶ دسی زیمنس بر متر) بر تجمع پرولین و الگوی بیان سه ژن دخیل در سنتز(P5CR و P5CR) و تجزیه (PDH) پرولین در برگهای چهارم و پرچم گندم صورت پذیرفت. تیمارهای آزمایش شامل دو سطح شوری ۱.۶ و ۱۶ دسی زیمنس بر متر و سه رقم گندم هیرمند و چمران (متحمل) و اترک (حساس) که به صورت فاکتوریل در قالب طرح کاملا تصادفی با سه تکرار



آرایش یافتند. تیمار شوری موجب افزایش غلظت سدیم و کاهش غلظت پتاسیم در برگهای چهارم و پرچم همه ارقام نسبت به تیمار شاهد شد. اگرچه ارقام مقاوم غلطت سدیم کمتری را نسبت به رقم حساس نشان دادند، غلطت پتاسیم و نسبت پتاسیم به سدیم در این ارقام بیشتر بود. چنین به نظر میرسد که تجمع پرولین از طریق افزایش بیان دو ژن PSCS و PSCR و کاهش بیان ژن PDH بوده است. از طرفی تجزیه پرولین بالای برگهای چهارم در ارقام مقاوم احتمالا به دلیل مهیا کردن منبعی از انرژی و نیتروژن است که میتواند به افزایش عملکرد در این ارقام تحت تنش شوری کمک کند.