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Catalase and Metallothionein genes expression analysis in wheat cultivars under drought stress condition

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Abstract

Drought stress is one of the serious problems that restricted agronomic plant production worldwide. In molecular level, the harmful effect of drought stress is mostly caused by producing of large amount of reactive oxygen species (ROS). Catalase and Metallothionein genes have a crucial role to mope the hydrogen peroxide (H_2O_2) resulting reducing oxidative damage. In this research the gene expression pattern of Catalase and Metallothionein was studied in response to drought stress treatments. The treatments included - 0.3 bar, -0.9 bar, - 8 bar and -12 bar and wheat varieties included Zagros (drought tolerant), Moghan (semi- tolerant) and Tajan (drought sensitive). The amount of cellular oxidative levels (TBARM) increased steady by intensify of drought stress levels. Real time PCR analysis showed different expression pattern for catalase and metallothionein encoded genes. Catalase gene expression was increased during drought stress up to -8 bar and reduced in -12 bar treatment, in all cultivars specially in Tajan cultivar. Metallothionein gene expression was linearly reduced during different levels of drought treatments especially in Zagros and Tajan cultivars. The most activity for both genes has observed in Zagros cultivar at -0.9 bar treatment. Whereas, Moghan cultivar showed most transcription for both genes at -8 bar treatment. Overall gene activities, content of chlorophyll (a, b) and whole plants appearance declined by high level of drought stress e.g. -12 bar treatment in all cultivars particularly in Tajan variety. Whereas, the moderate levels of drought stress treatments induced genes activitiy.

Key words: Wheat, Reactive oxygen species, Gene expression, Chlorophyll, Drought stress**.**

Introduction

Drought is one of the major factors limiting crop production globally, with increasing global climate change making the situation more serious (Apel and Hirt., 2004; Glombitza *et al.,* 2004). Wheat is

the important crops that provide food for nearly half of world population (Shao *et al*., 2005b). During growth and development, a plant has to cope with a range of different internal and external stresses and the ability to adapt to metabolic and environmental changes is essential for plant survival. Plants acclimate to biotic and abiotic stresses by triggering a cascade or network of events that starts with stress perception and followed by the expression of target genes (Pastori and Foyer, 2002). Oxygen is a vital molecule for all plant activities. However the production of reactive oxygen species (ROS) and its derivatives such as hydrogen peroxide (H_2O_2) occurs all the times at cellular levels, but is increased when plants are exposed to various stresses e.g drought stress (Dat *et al*., 2000; Arora *et al*., 2002; Devarshi *et al*., 2004). These ROS have dual roles, at high level of concentration they can be quite detrimental and could oxidize proteins, fatty acids and nucleic acids resulting some chlorosis and necrosis. Whereas at low level of concentration they act as signaling molecule and could be useful for gene expression and metabolic cell (Navabpour *et al.,* 2003; Villalobos *et al*., 2004; Bayoumi *et al*., 2008). In addition, drought is also related to salt stress, cold stress, high temperature stress, pathological reactions, senescence, and so on (Puhakainen *et al*., 2004; Wang *et al*., 2004). Therefore, drought is connected with almost all aspects of biological reactions. Currently, drought study has been one of the main directions in global plant biology and biological breeding (Shao *et al*., 2005a). Plants have a number of different defense mechanisms by which they respond to oxidative stress. These include the production of both non-enzymatic antioxidants such as ascorbate and glutathione and enzymatic antioxidants such as catalase and superoxide dismutase (Vacca *et al*., 2004; Gechev *et al*., 2006). CATs (catalase) are main components of anti-oxidative machinery for drought tolerant in higher plants (Shao *et al*., 2005a). Many evidences suggest that the expression and activities of catalase gene always activated by stresses in *Arabidopsis* (Luna *et al*., 2005; Verslues *et al*., 2007; Xing *et al*., 2007) and interestingly the mRNA abundance of CAT gene is also accumulated in drought, ABA and salt treatment (Xing *et al*., 2007). Catalase is essential for the removal of H_2O_2 produced in the peroxisomes by photorespiration (Noctor *et al*., 2000). The researches suggest that various environmental stresses always enhance the transcription of CATs, and increase enzyme activity of catalase subsequently, which controlling redox homeostasis in plant cells. Another important gene in response to drought stress is metallothionein. Among the different metal binding factors, metallothioneins (MTs) play a major part in the processes of plant metal homeostasis and detoxification (Cobbett and Goldsbrough, 2000). Metals are essential components of enzymes which involved in detoxification processes, like

Zn and Cu in superoxide dismutases. In addition to their important functions in vivo, however, metals may have very harmful effects. When not cleaned properly, transition metals like Fe or Cu generate reactive oxygen species in the Fenton reaction (Dietz *et al*., 1999; Michaeli *et* al., 2001; Belles-Boix and Inze., 2002). In this study, we analyzed the CAT and MT genes expression in wheat leaves during natural leaf development, under water-deficiency conditions, and normal irrigation.

Materials and Methods

Plant material

Three wheat genotypes (Zagros, Tajan and Moghan) were used in this research. The genotypes were obtained from Agricultural Research Center in Gorgan-Iran.

Experimental fields

The experiment was carried out at research greenhouse of Gorgan University of Agricultural Science and Natural Resources, using plastic pots with 5 kg field soil. In each pot 15 plants were grown. Air condition and humidity were established during experiment.

Experimental design

Each variety was conducted in four level soil water treatments controlled by matrix soil potential (- 0.3 as control, - 0.9, - 8 and - 12 bar, respectively). Each treatment has got three replications.

Chlorophyll extraction

Chlorophyll a and b were extracted and measured according to Porra *et al.,* (1989) in order to highly purifying and less contamination, leaves chlorophyll were extracted with 80% acetone and then estimated spectrophoto-metrically, by taking absorbances at 645 and 663 nm in WAP spectrophotometer model S2000 uv/vis. The chlorophyll content was calculated according to followed equations:

Chl a($mg.ml^{-1}$) = $12.25A_{663.6} + 2.55A_{646.6}$

Chl b($mg.ml^{-1}$) = $20.31A_{646.6} + 4.91A_{663.6}$

Measurement of thiobarbituric acid reactive material (TBARM)

TBARM (of which malondialdehydes (MDAs) are considered to be a significant component) was measured using a modified assay from Hagege *et al.,* (1990). Plant material (0.5 g) was homogenized with 1 ml trichloroacetic acid (10% w/v). The homogenate was washed with 10 ml acetone, vortexed then centrifuged at 4750 rpm for 15 min. The pellet was washed in 5 ml acetone, vortexed and then centrifuged at 4750 rpm for 10 min (4 times). The pellet was air dried and incubated at 100^{o}C for 30 min with 3 ml H_3PO_4 (1%) and 1 ml thiobarbituric acid (0.6%). The reaction was terminated by rapidly cooling the tubes on ice. Butan-1-ol (3 ml) was added and the mixture vortexed then centrifuged at 5500 rpm for 20 min to achieve separation of the phases. Absorbance of the aqueous phase was measured at 532

nm and 590 nm using Uvikon 930 Spectrophotometer.

Gene expression analysis

MT1, CAT1 transcript measurements of wheat heading stage was conducted by collection of eight leaves. Each treatment carried out three times at same condition. After that samples frozen in N_2 (liquid), then keeping at - 80° C in order to gene expression measurement by QRT-PCR method. At the same time we gathered data for all of the three samples which are at the same condition. The mean value of these three data series used for statistical test.

Reverse transcription-polymerase chain reaction was performed with Sina-Gene PCR master kit (according to the manufacturer's ecommendation, 5 l of total RNA extracted from leaves at heading stage after exposure to water treatments) and Biopars Syber Green as a dye for reactions detection (maximum fluorescent between 494-521nm). Actin gene was used as reference in reactions. The relative quantity of target gene transcripts was calculated using the comparative cycle threshold method. The affected samples were quantified relative to control (normal watering) at the same time points. The size of the PCR productions varied between 132 and 187 base pairs and the melting point varied between 51.4 and 60.0 C according to (G+C) percentage and length of bands. Actin as an endogenous control used to normalize the data for input RNA difference between the various samples. Fold changes in mRNA accumulation of MT1 and Cat1 genes in drought tolerant (Zagros), drought semi- tolerant (Moghan) and drought sensitive (Tajan) wheat at specified irrigation water treatments. Averages values and S.D for independent assays are shown. QRT-PCR conditions were carried out according to the cDNA protocol replication (with some changes primer melting temperatures) (Udvardi et al., 2008). Gene expression analysis was done by using REST software. The following oligonucleotides were used as primers (Table 1):

Table 1. CAT, MT and Actin Primers sequence and accession number in NCBI.

Gene	Primer sequence	PCR production length	Melting Tem (°C)	NCBI accession number
CAT1.F	5´ - CCATCTGGCTCTCCTACTGG - 3´	141 bp	60	E 16461
CAT1.R	5´ - AGAACTTGGACGACGGCCCTGA - 3´		57.9	
MT 1.F	5´ - ACACCAAGGGCAGAGCATAG - 3´	132 bp	51.4	L 11879
MT1.R	5´ - CACTCGTGTGATGGTGTGAG - 3´		53.9	
Actin.F	5´ - GTCGGTGAAGGGGACTTACA - 3´	187 bp	60	AB 181991.1
Actin.R	5´ - TTCATACAGCAGGCAAGCAC - 3´		60	

Results and Discussion

Living organisms use of the redox potential of oxygen while controlling oxidation. At first, oxygen derivations-reactive oxygen species (ROS) such as superoxide, hydroxyl radicals, hydrogen peroxide and singlet oxygen are in the shape of signals to make organism ready in front of weakly changes in temperatures extremes, pathogens, drought, or physical and chemical elements. If biotic and abiotic changes are too extreme to be tolerable to maintain vital fluxes through basic metabolism while preventing uncontrolled oxidation, then stress induced damage as a result (Apel and Hirt., 2004; Shao *et* al., 2005b). In this situation acclamatory changes in some especial genes expression and not in all of them (e.g. housekeeping genes (Actin) have no expression changes during stress) are induced to keep are not adequate or useful to extreme ROS productions, the primary metabolism is weakened, oxidative stress lead to increasing cell death and programmed senescence responses are happened (Harding *et al*., 2003; Chen and Gallie, 2004). It is observed that wheat in expose to different stresses had shown some of these responses. Therefore ROS are key components contributing to cellular redox state (Kreps *et al*., 2002). They participate as an alarm in all processes controlled by redox reactions such as protein synthesis or gene expression and more over (Zhu, 2003; Shao *et al*., 2005a, 2007). In this regard wheat responses to stresses are therefore directed to acclimate and repair damage. So, the control roles of catalase and metallothionein genes activity based on their functions would be quite important.

Metallothionein gene activity

Metallothioneins are defined as low-MW Cystein-rich proteins that bind heavy metals. MTs are widely distributed in eukaryotic and prokaryotic organisms (Coyle *et al*., 2002; Cobbett and Goldsbrough, 2002). So it can be used as a factor in stresses that affected the amount of solvent element in plant. In our research, according to Figure.1, under level -0.9 bar soil water treatment, Zagros has the higher *MT1* activity. Under level -8 bar, Moghan possessed the higher *MT1* activity and it seems that more researches needed to find its behavior. Zagros and Tajan express no obvious difference and similar changing trend under -8 to -12 bar. Comparing Zagros, Moghan and Tajan, we found that Zagros has the most *MT1* activity and showed lower reducing *MT1* activity in -8 and -12 bar in compared to control, indicating it has high ability to respond to reducing soil water content in terms of *MT1* activity. More precisely the most gene activity occurred at -0.9 bar whereas, the least at -12 bar for all cultivars. It seems, the -12 bar is far beyond the tolerant threshold for the *MT1* gene activity that may be sign of high toxicity of some elements, concentrating during drought stress that result in structural deformation of the gene product (enzyme). In animals and fungi, MTs have been shown to play a

role in the detoxification of heavy metals too (Yang *et al*., 2009; Cobbett and Goldsbrough., 2002). Many studies suggest that MTs have specialized functions in different tissues. Some of the functions proposed for plant MTs include a role during cell growth and development (Haq *et al.,* 2003) in senescence (Heise *et al.,* 2007; Breeze *et al*., 2004;) and in protection against oxidative stress (Coyle *et al.,* 2002) as it is mentioned in our results. The pattern of *MT1* expression has reflected by physical damaged appearance and almost decreasing chlorophyll content because of enzymatic metal patterns involved in photosynthesis (data not shown).

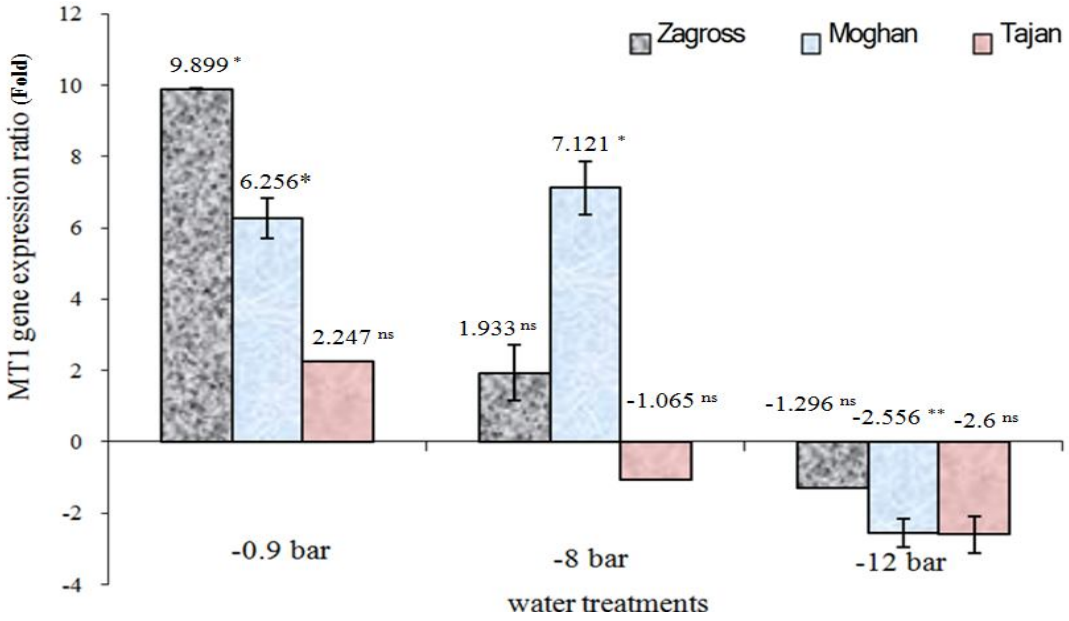

Figure 1. Metallothionein gene activity in different treatments Asterisks indicate significant difference between control and affected samples: *:P< 0.05; **:P< 0.01 and **ns**: not significant.

Catalase gene activity

Catalase is one of the major systems in the plant for the enzymatic removal of hydrogen peroxide (H_2O_2) in peroxisomes (Hirt and shinozaki, 2004). Despite moderate concentration of H_2O_2 is important as signaling molecule and can serve in lignin precursors of cell wall proteins, but high level of H_2O_2 is toxic for plant cell and cause oxidative damage. According to the role of catalase in photo-respiratory enzymatic system, its activity as a critical factor for the protection of photosynthesizing cells against oxidative stress is inevitable. This is supported by the observation that a barley mutant with reduced catalase activity is impaired in growth under photorespiratory conditions (Fath *et al.,* 2001).

Our experiment results showed that different wheat cultivars clearly responded to soil water deficiency

differently in term of *CAT* activity but not significantly (very close to significant α=0.58). At the first levels of watering, all of cultivars increase *CAT1* gene expression especially at -8 bar and they had not shown any especial diverse from their controls and each other, whereas obvious drop in *CAT* activity observed at -12 bar treatment (Figure. 2). At water potentional of -8 bar we can see that Moghan promoted its expression more than other varieties (especially Zagros). The amount of *CAT* gene activity in Zagros during increase pressure to -8 bar was slightly more than its amount at -0.9 bar. Du to this result, in field condition there is good possibility to reduce irrigation level up to 50% with less harmful damage on agronomic traits and significant decline in genes expressions especially in tolerant and partly in semi-tolerant varieties.

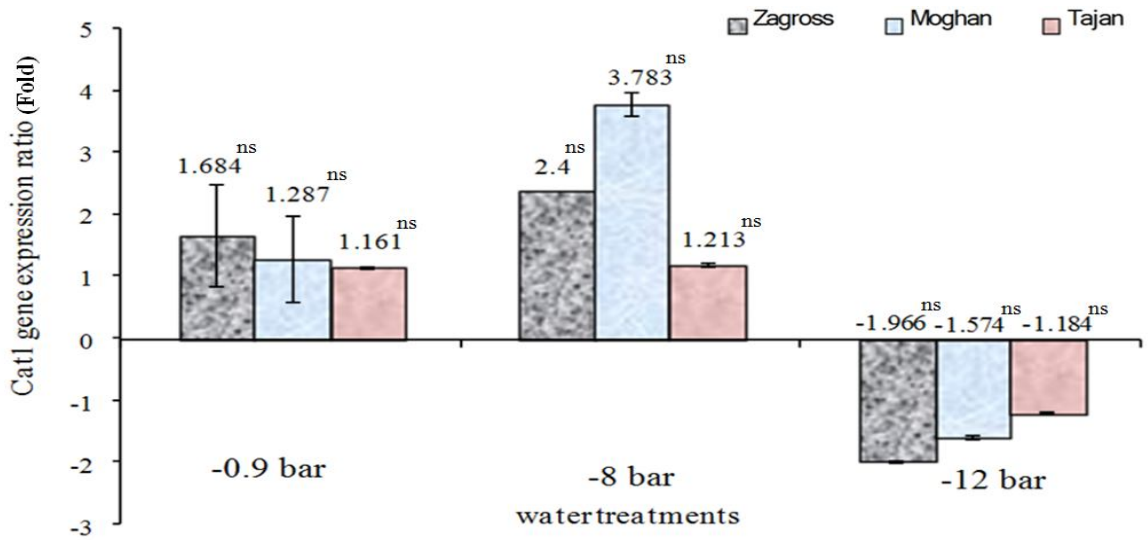

Figure 2. Catalase gene activity in different treatments Asterisks indicate significant difference between control and affected samples: **ns**: not significant.

Chlorophyll a and b content

Chlorophyll is the substantial basis for photosynthesis, so the content of chlorophyll can be useful index for evaluating photosynthesis. Many reports showed that drought could lead to lower photosynthesis and efficiency (Munns, 2002; Chandler and Bartels, 2003; Chaves *et al*., 2003; Dhanda *et al*., 2004). According to Figure.3, we found that Zagros had the highest chlorophyll (a+b) content in all levels of water treatments. Soil water treatments influence on chlorophyll ‘’a’’ and ‘’b’’ contents in all cultivars and reflecting the same reducing trend with each other. However, Tajan showed more sensitivity in response to soil water deficiency and had the lower chlorophyll (a+b) content (reducing nearly ½ in chl a and less in chl b) in all water treatments among other cultivars.

Interestingly for all cultivars, especially Zagros, there was no evidence of physical damage by -0.9 bar (data not shown). Since total chlorophyll content (a+b) in Moghan and Tajan was lower than Zagros (tolerant cultivar) that could imply chlorophyll content in is a tolerant factor against drought stress.

Comparison between *Cat1* and *MT1* in different cultivars separately implied various relations in their trends (Figure.4, 5, 6). Gene expressions in all of them were reduced by increasing pressure up to -12 bar. Also we found the same manner at -0.9 bar, all of them possess higher gene expression than controls level. Tajan and Zagros had a same inclination in gene expression but with different quantities. In Zagros, *MT1* had a high expression at pressure of -0.9 bar, then turned down rapidly as pressure increased to -8 bar and we see the same manner for *Cat1* expression too. At pressure of -12 bar the *Cat1* expression of Zagros is less than *MT1* (Figure.4). In Moghan different behavior was seen, both of genes increased expression at pressure of -8 bar and this modification disagree notably from other water treatments (Figure.5). The point that we can notice for Moghan is the relative amount of gene reduction in compare with Zagros. For both of genes, Moghan expressed smaller amount than Zagros at pressure of -12 bar. In Tajan we can report different gene expression for *MT1* at pressure of -8 bar, there was no visible increasing and it also lowered than control against *CAT1* (Figure.6). According to this result there is possible justification to conform visible link between the levels of *CAT1* and *MT1* activities and the tolerance to drought stress.

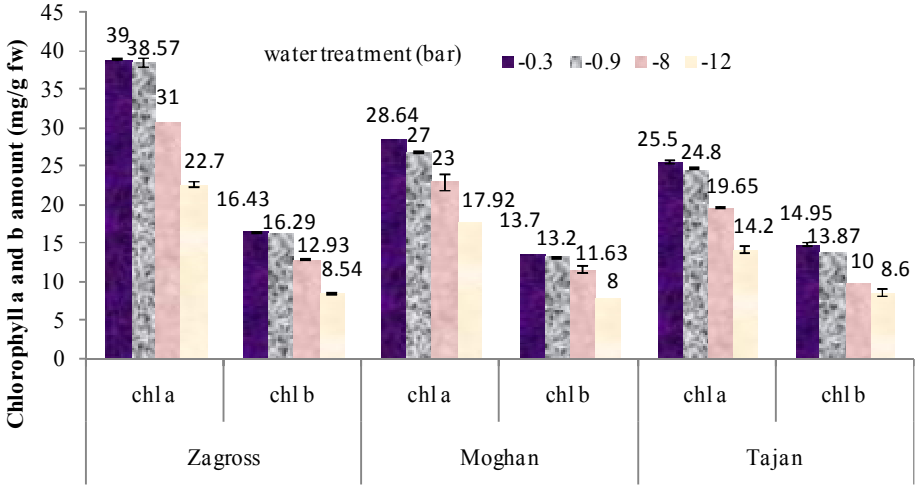

Figure 3. The average amount of chlorophyll "a" and "b" at different treatments for 3 cultivars. Arrow bar is used for standard error (n=3).

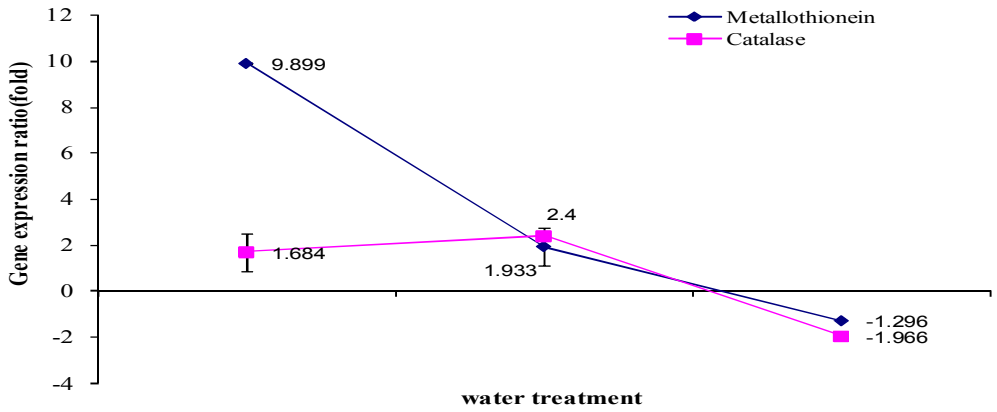

Figure 4. Interaction between catalase and Metallothionein genes activity for Zagros cultivar. Average amount are shown. Arrow bar is used for standard error (n=3).

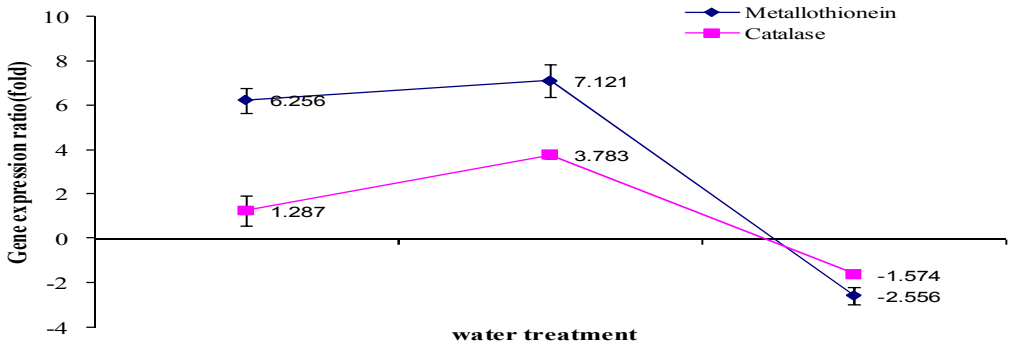

Figure 5. Interaction between catalase and Metallothionein genes activity for Moghan cultivar. Average amount are shown. Arrow bar is used for standard error (n=3).

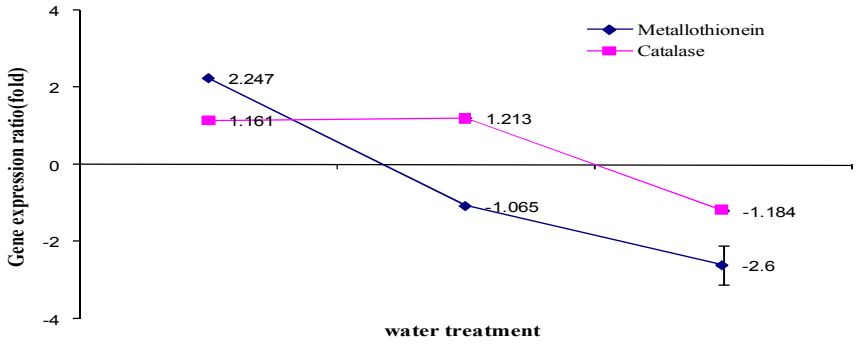

Figure 6. Interaction between catalase and Metallothionein genes activity for Tajan cultivar. Average amount are shown. Arrow bar is used for standard error (n=3).

Measurement of cellular oxidative level

The concept of drought stress is an oxidative process has been strongly supported by the result of TBARM assay. Basically oxidative stress results in an increase in lipid peroxidation and the extent of this can be measured by assaying the levels of the end products of lipid peroxidation. The TBARM assay measures the thiobarbituric acid reactive materials which are the stable end products of lipid peroxidation (Hodges *et al.,* 1999) and this assay was used to determine the oxidative damage caused to all cultivars by drought treatments. Drought stress increasing resulted in TBARM levels (Figure.7). There is a significant difference between levels of TBARM at -0.3 bar and other treatments according to statistical analysis. At -12 bar there is significant high level of TBARM accumulation especially in Tajan and its lowest amount was observed in -0.3 bar. The physical appearance of plant showed no damage until -0.9 bar treatment especially for Zagros cultivar but changing become visible during -8 and -12 bar (data not shown). Basically intensified drought stress resulted high level of ROS lead to phytotoxicity while relatively mild drought stress produced low levels of ROS that can be used for acclamatory signaling. This has supported by some researches (Dat *et al.,* 2000; Navabpour *et al.,* 2003). Aside from their destructive nature, ROS can also be used in a beneficial way by the plant. ROS acting as a beneficial signals to inducing protection enzymatic system such as catalase and metalothionein in order to preventing soon cell-death and its afterward problems. But in high water stresses its role become more harmful and if not controlled and reducing, could be damaging and ruined enzymatic system as seen in treatment -12 bar.

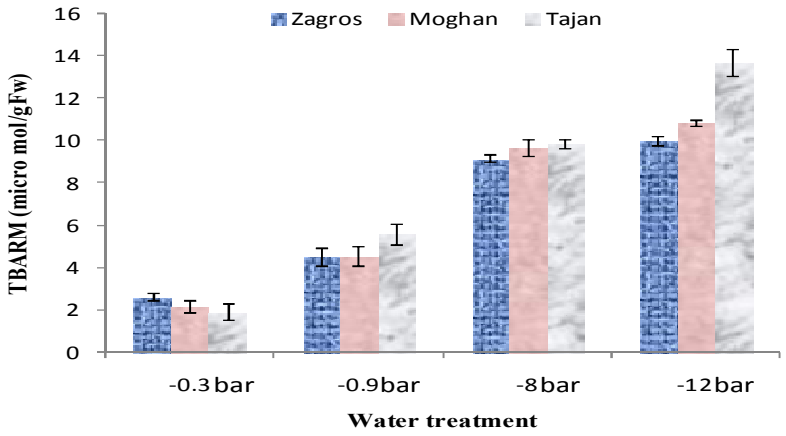

Figure 7. Levels of TBARM measured at different treatments for 3 cultivars. Arrow bar is used for standard error (n=3).

Conclusion

The most emphasize of our experiment was on two important genes expression were involved in drought stress. Plant oxidative circle occurs in all of different growing stages but in various level during different soil water stresses and environmental changes (Chaves *et al*., 2003; Plaut., 2003; Shao *et al*., 2007). Our results showed that different wheat genotypes differently responded to soil water stress. Chlorophyll content as a reliable index for stability of metabolism to keep photosynthesis level ongoing and delay senescence, has showed positive correlation with tolerance to drought stress as Zagros (tolerant cultivar) had the most chlorophyll content. This study provides a useful description about negative trend between chlorophyll content and TBARM that showed when the cellular oxidative level increased; the amount of chlorophyll would reduce, consequently. It should be noted that, comprehensive comparing between the levels of *CAT1*, *MT1* transcript and chlorophyll (a+b) with TBARM content, could be good justification to imply that as an especial enzymatic and physiological indexes to identify tolerant wheat genotypes under drought stress. The results presented here can serve benefit acknowledgments for future studies and suggested Zagros as an identified and confirmed drought tolerant variety that has fairly adaptation to Gorgan region.

Acknowledgment

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References

Apel, K., Hirt, H. 2004. Reactive oxygen species: metabolism, oxidative stress, and signal transduction, Annu. Rev. Plant Biol. 55: 373-399.

Arora, A., Sairam, R.K., Srivastava, G.C. 2002. Oxidative stress and antioxidative system in plants. Rew. Curr. Sci. 82(10): 1227-1238.

Bayoumi, T.Y., Eid, M.H., Metwali, E.M. 2008. Application of physiological and biochemical indices as a screening technique for drought tolerance in wheat genotypes. African Journal of Biotech. 7 (14): 2341-2352.

Belles-Boix, E., Inze, D. 2002. The role of active oxygen species in plant signal transduction. p. 46-73. *In* D. Scheel and C. Wasternack (ed.) Plant signal transduction. Oxford Univ. Press, New York.

Breeze, E., Wagstaff, C., Harrrison, E., Bramke, I., Rogers, H., stead, A., Thomas, B., Buchanan-Wollaston, V. 2004. Gene expression patterns to define stages of post-harvest senescence in Alstroemeria petals. Plant Biotech. 2 (2): 155-168.

Chandler, J.W., Bartels, D. 2003. Drought avoidance and drought adaptation. In: Encyclopedia of Water Science. Pp: 163165.

Chaves, M.M., Maroco, J., Pereira, J. 2003. Understanding plant responses to drought from genes to the whole plant, Funct. Plant Biol. 30: 239-264.

Chen, Z., Gallie, D.R. 2004. The Ascorbic acid redox state controls guard cell

signaling and stomatal movement. Plant Cell. 16: 1143-1162.

Cobbett, C., Goldsbrough, P. 2002. Phytochelatins and metallothioneins: roles in heavy metal detoxification and homeostasis. Annu. Rev. Plant Biol. 53: 159-182.

Cobbett, C.S., Goldsbrough, P.B. 2000. Mechanisms of metal resistance: Phytochelatins and metallothioneins. p. 247-269. *In* I. Raskin and B.D. Ensley (ed.) Phytoremediation of toxic metals: Using plants to clean up the environment. John Wiley & Sons, New York.

Coyle, P., Philcox, J.C., Carey, L.C., Rofe, A.M. 2002. Metallothionein: the multipurpose protein. Cell and Mol Life Sci. 59: 627-647.

Dat, J., Vandenabeele, F., Vranova, M., Van Montagu, M., Inze, d., Van Breusegem, F. 2000. Dual action of the active oxygen species during plant stress responses. Cell and Mol life Sci. 57: 779-795.

Devarshi, S.S., Bharti, S., Khanna-Chopra, R. 2004. Drought acclimation reduces O_2 accumulation and lipid peroxidation in wheat seedlings. Biochemical and Biophysical Research Communications: 314: 724-729.

Dhanda, S.S., Sethi, G.S., Behl, R.K. 2004. Indices of drought tolerance in wheat genotypes at early stages of plant growth, J. Agron. Crop Sci. 190 (1): 6-12.

Dietz, K.J., Baier, M., Krämer, U. 1999. Free radicals and reactive oxygen species as mediators of heavy metal toxicity in plants. p. 73-97. *In* M.N.V. Prasad and J. Hagemeyer (ed.) Heavy metal stress in plants.

Fath, A., Bethke, P.C., Jones, R.J. 2001. Enzymes That Scavenge Reactive Oxygen Species Are Down Regulated Prior to Gibberellic Acid-Induced Programmed Cell Death in Barley Aleurone. Plant Physiol. 126:156-166.

Gechev, T.S., Breusegem, F.V., Stone, J.M., Denev, I., Laloi, Ch. 2006. Reactive oxygen species as signals that modulate plant stress responses and programmed cell death. Bio Essays. 28 (11): 1091-1101.

Glombitza, C., Dubuis, P.H., Thulke, O. 2004. Crosstalk and differential response to abiotic and biotic stressors reflected at the transcriptional level of effector genes from secondary metabolism. Plant Mol. Biol. 54(6): 817-835.

Hagege, D., Nouvelot, A., Boucard, J., Gaspar, T. 1990. Malondialdehyde titration with thiobarbiturate in plant extracts: avoidance of pigment interference. Phytochem Anal 1: 86-89.

Haq, F., Mahoney, M., Koropatnick, J. 2003. Signaling events for metallothionein induction. Mutation Res. 533: 211-226.

Harding, H.P., Zhang, Y.H., Zeng, H.Q. 2003. An integrated stress response regulates amino acid metabolism resistance to oxidative stress. Mol Cell. 11: 619-633.

Heise, J., Krejci, S., Miersch, J., Krauss, G.J., Humbeck, K. 2007. Gene expression of metallothioneins in barley during senescence and heavy metal treatment. Crop Sci. 47:1111-1118.

Hirt, H., Shinozaki, K. 2004. Plant Responses to Abiotic Stress. Springer, Vienna, Austria. 297 pp.

Hodges, D.M., Delong, J.M., Forney, C.F., Prange, R.K. 1999. Improving the thiobarbituric acid-reactive-substances assay for estimating lipid peroxidation plant tissues containing anthocyanin and

other interfering compounds. Planta. 207:604-611.

Kreps, J.A., Wu, Y.J., Chang, H.S. 2002. Transcriptome changes for Arabidopsis in response to salt, osmotic, and cold stress, Plant Physiol. 130: 2129-2141.

Luna, C.M., Pastori, G.M., Driscoll, S., Groten, K., Bernard, S., Foyer, C.H. 2005. Drought controls on H_2O_2 accumulation, catalase (CAT) activity and CAT gene expression in wheat. J. Exp. Bot. 56: 417-423.

Michaeli, R., Philosoph-Hadas, S., Riov, J., Shahak, Y., Ratner, K., Meir, S. 2001. Chilling-induced leaf abscission of Ixora coccinea plants. III. Enhancement by high light via increased oxidative processes. Physiol Planta. 113: 338-345.

Munns, R. 2002. Comparative physiology of salt and water stress. Plant Cell Environ. 25 (2); 239-252.

Navabpour, S., Morris, K., Allen, R., Harrison, E., A.H.Mackerness, S., Buchanan Wollaston, V. 2003. Molecular and Biochemical analyses pf Oxidative stress and leaf senescence. Imperial College of Science, Technology and Medicine at Wye University of London. 54(391):55-56.

Noctor, G., Veljovic-Jovanovic, S., Foyer, C.H. 2000. Peroxide processing in photosynthesis: antioxidant coupling and redox signalling. Philosophical Transactions of the Royal Society of London B. 355: 1465-1475.

Pastori, G.M., Foyer, C.H. 2002. Common Components, Networks and Pathways of Cross-Tolerance to Stress. The Central Role of "Redox" and Abscisic Acid-Mediated Controls1 . Plant Physiol. 129: 460-468.

Plaut, Z. 2003. Crop plants: critical development stages of water. In: Encyclopedia of Water Science. Pp: 95-100.

Porra, R.J., Thompson, W.A., Kriedmann, P.E. 1989. Determination of accurate extinction coefficients and simultaneous equations for assaying chlorophylls a and b extracted with four different solvents: vertification of the concentration of chlorophyll standards by atomic absorption spectroscopy. Biochem Biophys Acta. 975: 384-394.

Puhakainen, T., Hess, M.W., Makela, P. 2004. Overexpression of multiple dehydrin genes enhances tolerance to freezing stress in Arabidopsis. Plant Mol. Biol. 54: 743-753.

Shao, H.B., Chuc, L.Y., Wu, G., Zhang, J.H., Lua, Z.H., Hu, Y.C .2007. Changes of some anti-oxidative physiological indices under soil water deficits among 10 wheat (*Triticum aestivum* L.) genotypes at tillering stage. Colloids and Surfaces B: Biointerfaces. 54: 143-149.

Shao, H.B., Liang, Z.S., Shao, M.A. 2005a. Changes of anti-oxidative enzymes and MDA content under soil water deficits among 10 wheat (*Triticum aestivum* L.) genotypes at maturation stage. Colloids and Surfaces B: Biointerfaces. 45: 7-13.

Shao, H.B., Liang, Z.S., Shao, M.A., Wang, B.C. 2005b. Changes of anti-oxidative enzymes and membrane peroxidation for soil water deficits among 10 wheat genotypes at seedling stage. Colloids and Surfaces B: Biointerfaces. 42: 107-113.

Udvardi, M.K., Czechowski, T., Scheible, W.R. 2008. Eleven Golden Rules of Quantitative RT-PCR. Plant Cell. 20:1736-1737.

Vacca, R.A., Pinto, M.C., Valenti, D., Passarella, S., Marra, E., Gara, L.D. 2004. Production of Reactive Oxygen Species, Alteration of Cytosolic Ascorbate Peroxidase, and Impairment of Mitochondrial Metabolism Are Early Events in Heat Shock-Induced Programmed Cell Death in Tobacco Bright-Yellow 2 Cells. Plant Physiol. 134 (3): 1100-1112.

Verslues, P.E., Batelli, G., Grillo, S., Agius, F., Kim, Y.S., Zhu, J. 2007. Interaction of SOS2 with NDPK2 and catalases reveals a point of connection between salt stress and H2O2 signaling in Arabidopsis. Mol. Cell. Biol: 27: 7771-7780.

Villalobos, M.A., Bartels, D., Iturringa, G. 2004. Stress tolerance and glucose insensitive phenotypes in Arabidopsis overexpressing the CpMYB10 transcription factor gene. Plant Physiol. 135: 309-324.

Wang, H., Huang,Z., Chen, Q. 2004. Ectopic overexpression of tomato JERF3 in tobacco activates downstream gene expression and enhances salt tolerance. Plant Mol. Biol. 55: 183-192.

Xing, Y., Jia, W., Zhang, J. 2007. AtMEK1 mediates stress-induced gene expression of 403 *CAT1* catalase by triggering H2O2 production in Arabidopsis. J. Exp. Bot. 58(404): 2969-2981.

Yang, Z., Wu, Y., Li, Y., Ling, H.Q., Chu, C. 2009. OsMT1a, a type 1 metallothionein, plays the pivotal role in zinc homeostasis and drought tolerance in rice. Plant Mol. Biol. 70 (1-2): 219-229.

Zhu, J.K. 2003. Regulation of ion homeostasis under salt stress. Curr. Opin. Plant Biol. 6 (5): 441-445.

ارزیابی بیان ژن‌های کاتالاز و متالوتاینین تحت شرایط تنش خشکی در گندم

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چکیده

تنش خشکی یکی از معضلات جدی در ایجاد محدودیت تولید محصولات زراعی در سطح جهانی می‌باشد. از منظر مولکولی، خسارت ناشی از تنش خشکی ناشی از تأثیر سوء رادیکال‌های فعال اکسیژن است. ژن‌های کاتالاز و متالوتاینین با مهار میزان فزاینده این رادیکال‌ها واجد نقش تأثیر گذاری در کنترل تنش اکسیداتیو هستند. در این تحقیق میزان بیان افتراقی ژن‌های کاتالاز و متالوتاینین تحت شرایط تیمارهای خشکی در ارقام گندم بررسی گردید. تیمارهای تنش خشکی شامل ۰/۳- بار، ۰/۹- بار، ۸- بار و ۱۲- بار، و ارقام مورد مطالعه شامل زاگرس (متحمل)، مغان (نیمه متحمل) و تجن (حساس) بودند. میزان شاخص اکسیداسیون سلولی (TBARM) با افزایش شدت تنش خشکی و به صورت خطی بالا رفت. بیان افتراقی متفاوتی با استفاده از تکنیک اندازه گیری بیان ژن در زمان واقعی (RT-PCR) برای ژن‌های مورد مطالعه حاصل گردید. بیان ژن کاتالاز تا تیمار ۸- بار افزایش و پس از آن در تیمار ۱۲- بار کاهش معنی‌داری در تمام ارقام به ویژه رقم تجن نشان داد. روند بیان ژن متالوتاینین در شرایط تنش خشکی با کاهش خطی مواجه بود. در مورد هر دو ژن بیش‌ترین بیان در تیمار ۰/۹- بار خشکی و در رقم زاگرس ملاحظه شد. در حالیکه در رقم مغان حداکثر سطح رونوشت‌برداری برای هر دو ژن در تیمار ۸- بار حاصل گردید. به طور کلی فعالیت ژن‌های کاتالاز و متالوتاینین، میزان کلروفیل (a, b) و وضعیت ظاهری گیاهان با افزایش شدت تنش افت چشمگیری نشان داد (این مسئله در تیمار ۱۲- بار کاملاً مشهود بود). با این حال سطوح میانی تنش سبب تحریک فعالیت ژن‌های مورد مطالعه گردید.

کلمات کلیدی: گندم، رادیکال‌های فعال اکسیژن، بیان ژن، کلروفیل، تنش خشکی.