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Differential Gene Expression of Durum Wheat (*Triticum turgidum* L. var. durum) in Relation to Genotypic Variation under NaCl Salinity Stress

Nidal Odat*

Department of Biotechnology, Al-Balqa' Applied University, Al-Salt, 19117, Jordan

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Abstract

Variation in expression of important genes under environmental stress is suggested to play an important role in determining phenotypic diversity. Virtually little is known about the genotypic-specific expression variation in durum wheat as an important cereal plant. This study examines transcript levels in durum (*Triticum turgidum* L.) using real-time PCR analysis in relation to genotypic differences by contrasting two gene pools of durum wheat under NaCl salinity stress. Four important genes in relation to stress were analyzed, three of which showed differential down-regulation of expression at the RNA level. Specifically, it is found that the expression of *GAD* and *ASN* genes showed a different pattern in response to different levels of salinity. At 50 mM NaCl *GAD* was down-regulated in only (Sham 1) genotype, while *ASN* was down-regulated in (Sham1), (Um Qais), and (Akcad). Under 100 mM NaCl, both genes were down-regulated in the genotype (Um Qais), while salinity of 150 mM NaCl induced down-regulation of both genes in (Um Qais) and (Norseh). Genetic variation (cultivars vs. landraces) was found to show a significant relationship to salinity responses especially at 50 and 150 mM NaCl (one-way ANOVA; $P < 0.05$). *SOD* and *CAT* genes were also down-regulated by salinity, but the relationship to genetic variation seems to be statistically less important. The genetic difference in gene expression could be best attributed to transcriptional and post-transcriptional regulation processes that are involved in durum tolerance to stress. This work demonstrates that the durum is an excellent plant system to study genetic variation in relation to gene expression under environmental stress because it seems that alleles of different genotypes of durum are affected by genetic background or environmental factors. The results of this study may help to classify the studied genotypes into sensitive and tolerant varieties.

Keywords: Durum, qRT-PCR, Gene expression, Genetic difference, NaCl, Wheat, Jordan.

1. Introduction

Durum or pasta wheat (*Triticum turgidum* L. var. durum, 2n = 4x = 28; AABB genomes) is an important plant grown for a long time in the Middle and Near Eastern countries (Jaradat and Duwayri 1981; Belaid 2000). Durum production is largely determined by the habitat conditions such as drought and soil salinity (Munns *et al*., 2006; Carvalho *et al*., 2011). Genetic variation, relatively abundant, in durum wheat seems to be a main reason for the adaptation of this cereal in salt-affected areas especially in dry and semi-dry soils. Durum contains a high level of genetic variation that includes nucleotide polymorphisms and large variants of DNA fragments and polymorphisms (Elía *et al*., 2016; Laddomada *et al*., 2017) . Genetic variation is believed to influence the pattern and level of gene expression especially under environmental heterogeneity of stress (Saintenac *et al*., 2018). It has been shown that different genotypes of the same species are not expressed equally at the transcript level, and such variation is linked to important regulatory mechanisms involved in stress tolerance and adaptation to stress factors, such as soil salinity (Müller *et al*., 2018).

Soil salinity is an important factor that reduces plant growth and productivity in several parts of the world, particularly arid and semiarid regions (Ren *et al*. 2005). Salinity can potentially induce changes in genetic variation in transcript pattern and levels of various regulatory mechanisms including transcriptional and post-transcriptional regulations (Tirosh *et al*., 2006).

Salinity has been shown to cause osmotic and water stress by increasing the accumulation and the up-regulation of important enzymes that largely show differential responses in varied genotypes of a species (Zhand *et al*., 2017). This indirect influence of genetic variation through the modulation of gene expression can ultimately lead to phenotypic variation (AL-Quraan *et al*., 2010; Giunta *et al*., 2018). The response to salinity typically involved both enzymatic and no-enzymatic antioxidants defense mechanisms (Sharma *et al*., 2012). These mechanisms play a role in combating the accumulation of reactive oxygen species (ROS) by a

* Corresponding author. e-mail: nidalodat@gmail.com.

diverse set of enzymes such the superoxide dismutase (SOD), which dismutase the O_2^- radicals to H_2O_2 (Luis *et al*., 2018). Additionally, catalase (CAT), glutamic acid decarboxylase (GAD), and asparagine synthetase (ASN) are among the other enzymes that scavenge H_2O_2 and reduced its accumulation to toxic levels. These enzymes were shown to have different reactivity to H_2O_2; CAT, for example, has low affinity to H_2O_2, and it was shown that it is important for scavenging most of the H_2O_2 (Harb and Samarah 2015; Duan *et al*., 2018; Klein *et al*., 2018;).

Alterations in gene expression is an important defensive response to salinity (Rabbani *et al.*, 2003). Variation in gene expression in various plant species such as in maize (Wang *et al*., 1999) and the tomato (*Lycopersicon esculentum*) (Cong *et al*., 2002) has been shown to be related to plant species. However, studies on the response of different genotypes of the same species to salinity response in relation to genetic variation and gene expression are limited. Accordingly, this study used quantitatively real-time PCR to evaluate genetic variation in relationship to gene expression of six local durum genotypes of two genepools, i.e., landraces and cultivars.

2. Materials and Methods

2.1. Durum Seeds and NaCl Treatments

Durum wheat seeds of six Jordanian varieties (Um Qais, Norseh, Dar Alla, Sham 1, Hourani, Akcad) were obtained from the National Center for Agricultural Research and Extension (NCARE) of Jordan. The studied varieties of wheat are of two genepools: cultivars (Deir alla 6, Acsad 65, and Sham 1) and local landraces (Um-qais, Hourani 27, and Noorseh). Seeds of a uniform size of each genotype were utilized in this study. The seeds were surface-sterilized with 70 % (v/v) ethanol and then with 2 % commercial bleach (sodium hypochlorite) for 3-5 minutes, and finally rinsed thoroughly in sterile distilled water. The seeds were then subjected to a stratification for a few days at approximately 4°C. Seeds from each genotype were germinated on 12 × 12 cm petri dishes using a wet filter paper (Whatman No 1, Kent, UK) at 25°C in dark conditions with or without salt treatment at four NaCl levels (0 mM as a control, 50 mM, 100 mM, 150 mM). The four levels of salinity were chosen as they have been shown to cause several growths and physiological responses to cereal cultivars (Salama *et al*. 1994). After three days of treatment, leaves from the wheat genotypes were collected, and were quickly frozen in liquid nitrogen at -20°C until RNA isolation.

2.2. RNA Isolation and cDNA Synthesis

RNA was isolated from wheat tissues using an IQeasy™ Plus plant RNA isolation kit (iNtRON Biotechnology, Korea). The concentration of isolated RNA in the samples were estimated spectrophotometrically (260 nm/280 nm; Biochrom, Cambridge, UK). The first-strand cDNA was synthesized by mixing 2 µg of RNA with 4 µL of Prime Script™ RT reagent (Takara, Japan), and the final volume of the mixture was adjusted to 20 µL with RNase-free water (0.1% (v/v); diethylpyrocarbonate-treated water). The mixture was then placed in a thermocycler (Biometra, Germany) for forty-five minutes at 37°C, followed by fifteen seconds at 85°C, and finally at 4°C for approximately five minutes. The resultant amplified products were then diluted to 50 ng/µL with sterile RNase-free water and stored at -20°C for gene expression analysis by quantitative real-time PCR (qRT-PCR).

2.3. Primer Design and Quantitative Real-Time PCR

qRT-PCR was used to examine the relative expression of the four genes in durum wheat genotypes after exposure to different levels of salinity. The gene-specific primers for qRT-PCR were designed on the basis of the sequences existent in GenBank using the software Primer3 (http://bioinfo.ut.ee/primer3-0.4.0/primer3/input.htm) as described in Thornton and Basu (2011). The primers used showed a high specificity as revealed by gel electrophoresis. Differential gene expression analysis of (CAT, GAD, ASN, SOD) genes was studied using qRT-PCR. The PCR reactions were made in a volume of 25 µL prepared from 10 µL of Kappa Syber Fast qPCR reagent (KAPA Biosystems, USA), 50 ng/µL template cDNAs, and 10 µM aliquots of each primer of the genes (Table 1). The cycling conditions utilized were: 2 min/95°C, 10 s/ 95°C, 25 s/57°C, 25 s/60°C, and a final extension step of 2 min/60°C using a CFX96 touch real-time PCR system (Bio-Rad) machine. The experimental procedure was conducted at least three times. The threshold cycle (Ct) values of the triplicate PCRs were averaged, and the relative quantification of the transcript levels was analyzed using the comparative Ct method (Schmittgen and Livak 2008).

2.4. Statistical Analysis

Statistical analysis was done with the help of SPSS for Mac (v20, SPSS Inc., Chicago, IL, USA). The differential response to salinity for the studied genes were estimated by one- and two-way analyses of variance (ANOVAs) and Duncan's Multiple-Range test (DMRT). The values shown in the results are the arithmetic mean ± standard error (S.E.; n=3), with p value ≤ 0.05 regarded as a statically significant difference. Before the analysis, normality was checked (Shapiro-Wilk test), and when normality and homogeneity of variance were violated, the logarithmic transform was taken for the raw data. To measure the level of gene expression of all genes, the actin gene was used as an internal control to quantify the relative transcript level of each target gene (Goidin *et al*., 2001).

Table 1. Primer sequences, accession numbers and the amplicon size of the primers used in this study.

Gene name	Sequence from 5' to 3 '	Gene Bank accession #	Amplicon size (bp)
ASN-F/R	TGTCCTTTATACATGGGCTGGG/ ATCATAAGGGGCTGAGGGAATG	KC193248.1	192
Cat-F/R	AACTTCCCCGTCTTCTTCATCC/ AGAGGAAGGTGAACATGTGGAG	KP696753.1	154
GAD-F/R	ATCTTCCACATCAACTACCTCGG/ TTCTCCATGATGTTCCTGTACCC	AK355055.1	137
MnSOD-F/R	TGCTGCTTTACAAGGATCTGGA/ TCCCAGACATCAATTCCCAACA	KP696754.1	144
Actin-F/R	CTCCATCATGAAGTGTGACGTG/ GACGACCTTGATCTTCATGCTG	AY145451.1	151

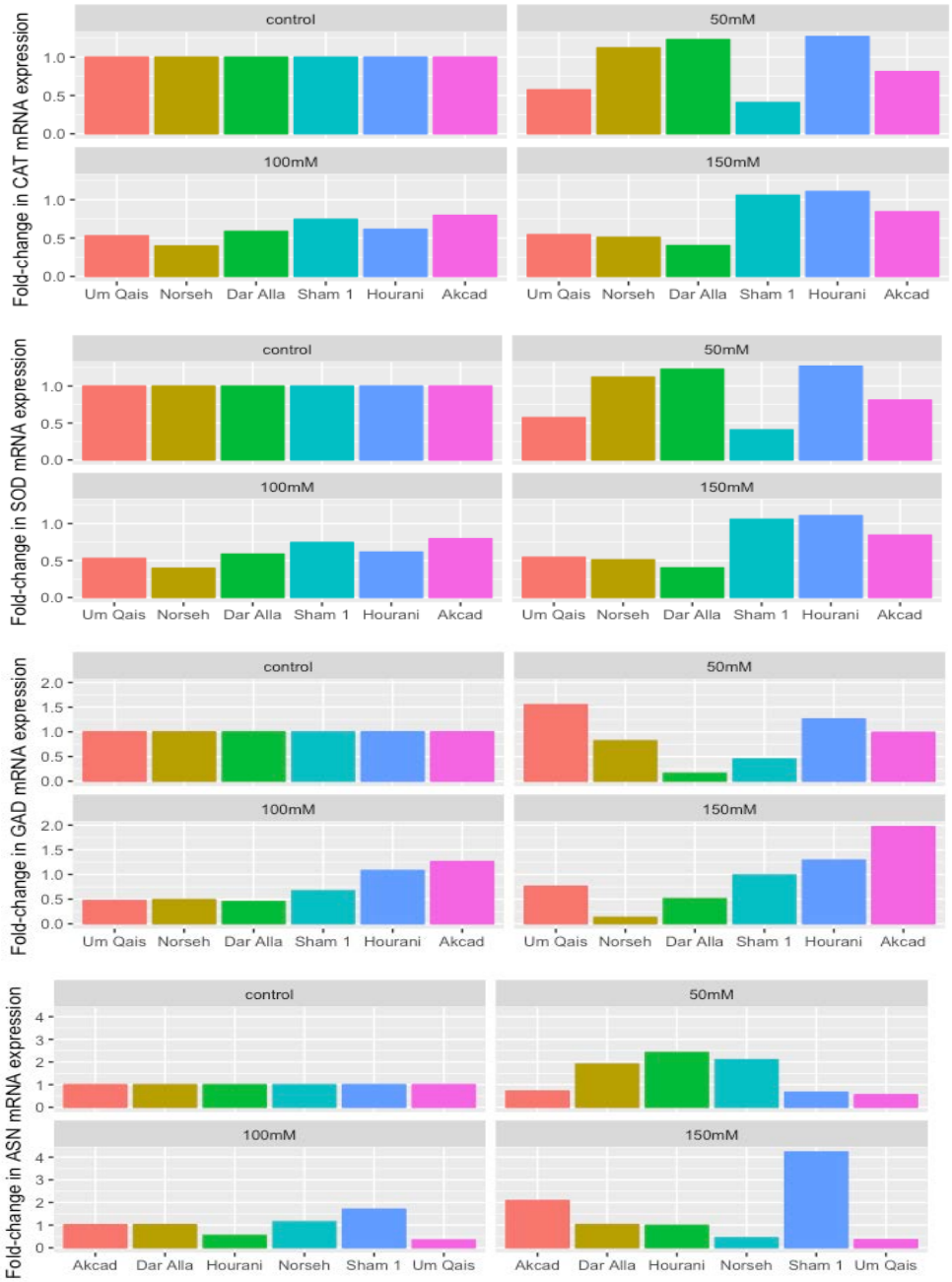

Figure 1. Relative expression levels of the four genes studied in six durum genotypes exposed to different concentrations of NaCl salinity. Fold changes greater than one (> 1) indicate up-regulation, and (< 1) down-regulation.

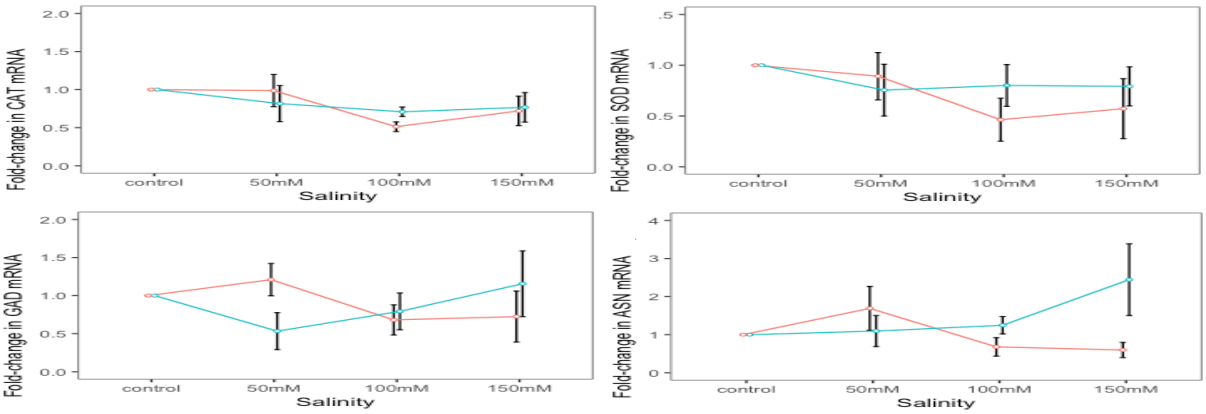

Figure 2. The relative expression levels of the four genes studied in landraces (Um-qais, Hourani 27, and Noorseh; blue line) genotypes and cultivars (Deir alla 6, Acsad 65, and Sham 1; orange line) exposed to varied salinity levels.

3. Results and Discussion

The present study investigates the expression levels of four important genes involved in a wide range of physiological and biochemical processes that are important for the tolerance of plants against environmental stresses (Herzog *et al*., 2018). Results present in this study revealed that at 50 mM NaCl the *CAT* gene was down-regulated in (Um Qais), (Sham 1), and (Akcad). Under 100 mM NaCl (Um Qais), (Norseh), (Dar alla), (Sham 1), and (Akcad) genotypes were found to be down-regulated, whereas at 150 mM NaCl induced gene down-regulation in the genotypes (Um Qais), (Norseh), (Dar Alla), and (Akcad) (Figure 1). This variation in gene expression of *CAT* in different genotypes of durum wheat may suggest that the genotypes (Um Qais), (Dar Alla), (Akcad) seem to be more sensitive to salt compared to the other studied genotypes. The expression levels of *SOD* gene were largely similar to *CAT* gene in all studied genotypes. Specifically, under 50 mM NaCl the *SOD* was down-regulated in (Um Qais), (Sham 1), and (Akcad), and under 100 mM NaCl the genotypes SOD was found to be down-regulated in (Um Qais), (Norseh), (Dar alla), (Sham 1), and (Akcad). However, the high concentration of salinity, i.e., (150 mM NaCl), induced downregulation of *SOD* in the genotypes (Um Qais), (Norseh), (Dar Alla), and (Akcad) (Figure 1). The similar expression pattern of *CAT* and *SOD* genes indicated that salinity of different levels induce similar responses in durum wheat independent of their genetic background. In fact, one-way ANOVA did not signify any important difference ($P > 0.05$; Figure 2) between cultivar or landrace nature of the genotypes. Moreover, these results perhaps suggest that both *CAT* and *SOD* genes are involved in rather similar salinity tolerance mechanisms. These results are similar to those found in barley and wheat in some defense-related genes (Herzog *et al*., 2018; Karlik and Gözükırmızı 2018), implying that the expression of the *CAT* and *SOD* genes are closely related to salt tolerance, and thus may play an essential role in plant defense.

The expression of *GAD* and *ASN* genes showed a different pattern in response to varied concentration of salinity. Under 50 mM NaCl *GAD* was down-regulated in only (Sham 1) genotype, while *ASN* was down-regulated in (Sham1), (Um Qais), and (Akcad) (Figure 1). Under 100 mM NaCl both genes were down-regulated in the genotype (Um Qais), while salinity of 150 mM NaCl induced down-regulation of both genes in (Um Qais) and (Norseh). Perhaps, this suggests that (Um Qais), (Sham 1), (Norseh), and (Akcad) seem to be more sensitive compared to (Dar alla) and (Horani) at all salinity concentrations possibly due to their genetic variation. In fact, genetic variation (cultivars vs. landraces) was found to show a significant relationship to salinity responses especially at 50 and 150 mM NaCl (Figure 2).

Additionally, the results of this study showed an up-regulation pattern of gene expression in some of the studied genes in relation to genetic variation and in response to salinity. Namely, the genotypes (Dar alla), (Norseh), and (Horani) showed up-regulation expression of the genes *CAT*, *SOD*, *ASN* under salinity of 50 mM NaCl, and under 150 mM NaCl (Sham 1) and (Horani) showed up-regulation of the same genes, i.e., *CAT*, *SOD*, and *ASN* (Figure 1). On the other hand, the 100 mM NaCl induced up-regulation of *ASN* and *GAD* only in (Sham 1), (Akcad), and (Horani). This genetic difference in gene expression could be resulting from a number of regulatory processes, including, for example, transcriptional and post-transcriptional regulations. Moreover, variation of the expression of the studied genes in this study could be also attributed to the decreased level of transcript abundance caused by salinity (Ramirez-Parra *et al*., 2004; Zhand *et al*., 2017).

In summary, this study shows that the gene expression variation occurred differentially in durum wheat in response to different levels of salinity genetic background. Although only few genes were analyzed, distinct expression patterns were found between a landraces and cultivars. This work demonstrates that the durum is an excellent plant system to study genetic variation in gene expression under environmental stress. It can be concluded then that gene expression in durum wheat is differentially determined by the combined effects of genetic and environmental factors.

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