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Analysis of Salt Stress-Responsive Transcriptome in Barley Root *(Hordeum vulgare* L.)

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ABSTRACT

Salinity stress is one of the most important environmental stresses that decrease crop growth and yield. Barley is an important crop known as the salt-tolerant plant in cereals. In this study, the salt stress-responsive root transcriptome of tolerant (Afzal) and susceptible (Yusef) cultivars was investigated. The sequencing of mRNA transcripts (termed RNA-Seq) was performed using the Illumina HiSeq platform after filtering for RNA with 3' polyadenylated tails to include only mRNA. The Tuxedo pipeline was used to identify the altered expression of transcripts. Sequencing results showed that, after initial trimming of the reads, more than 20 million reads (92%) remained for all samples, of which 88% were aligned with the barley genome. Bioinformatics analysis showed the altered genes expressions in various processes such as membrane antiporter and transporter activity, an antioxidant, wide range of kinase and phosphatase cascades, internal signal transduction, metabolism of carbohydrates, amino acids, and lipids, binding processes, response to plant hormones, catalytic activity, and cell wall organization. Gene network analysis revealed that key genes, including proteins involved in systemic acquired resistance, peroxidase family proteins, cyclin-dependent protein kinase, phosphatidylinositol kinase, auxin-carrying proteins, mannose 6 phosphate isomerase, helicases and transcription factors play an important role in salt tolerance. These data can be used as a valuable source in future studies for genetic manipulation of barley and development of salinity tolerant cultivars.

Keywords: Gene network, Gene ontology, RNA-Seq, Salinity tolerance.

INTRODUCTION

The global warming phenomenon threatens food security along with a reduction in water resources and increase in salinity of agricultural lands (Parihar *et al*., 2015). Salinity stress is one of the most important environmental stresses that affect all stages of growth from germination to physiological maturity. The plant Responses to salinity varies with plant genotype/species, plant development stage, intensity and duration of stress (Arzani and Ashraf, 2016). Salinity stress causes osmotic stress during the first phase and ion toxicity during the second phase resulting in disruption of metabolic processes, cell dehydration, membrane dysfunction, nutrient imbalances, and oxidative stress in plant cells (Arzani, 2008; Munns and Tester, 2008).

Barley (*Hordeum vulgare* L.) is the fourth most important crop in the world and the second crop in Iran. It is used in the baking and bakery industries, confectionery, malt and animal feed (Gozukirmizi and Karlik, 2017). In addition, barley is more tolerant to drought, salinity and alkalinity than other cereal crops. It is important to study the metabolome of this crop and identify traits related to salinity tolerance. Therefore, barley is an ideal model cereal to elucidate the

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underlying mechanisms responsible for tolerance induction under saline conditions (Ahmed *et al*., 2015; Ebrahim *et al*., 2020).

A better understanding of the principles and mechanisms governing gene expression networks is essential to explore gene expression in plant tissues at particular growth stages in response to a variety of environmental signals (Marguerat and Bähler, 2010). Transcriptome is a set of mRNA molecules that are expressed in a cell or in a population of cells under different conditions (Brown, 2018). In recent decades, plant transcriptome has emerged as a novel tool with the advent of Next Generation Sequencing (NGS) methods and the availability of reference genomes, a better and more accurate evaluation of molecular mechanisms involved in tolerating non-biological stress is being generated (Hiremath *et al*., 2011). The introduction of NGS and new computational algorithms have led to large-scale sequencing becoming commonplace in research (Mardis, 2008).

RNA-Seq is currently one of the most popular topics in the field of NGS, which has rapidly replaced other methods of large-scale transcriptome profiling such as microarray and SAGE and discovered transcripts, new gene models and different variants of small RNAs without new coding (Willenbrock *et al*., 2009). RNA-Seq method yields millions of cDNA sequences that are matched using computer programs on the reference genome or transcript prepared from de novo assembly and the transcript structure and expression of each gene can be evaluated (Mortazavi *et al*., 2008). The AgriGO website was also used to draw pathways to further examine the gene ontology (GO) obtained. AgriGO is a web-based software and database for analyzing and interpreting genes expressed. This website focuses on identifying agricultural cultivars pathways and has a user-friendly environment designed to support the community of agricultural researchers in the field of GO analysis (Tian *et al*., 2017)

The use of the new generation of cost-effective sequences with high repeatability and flexibility, and quantitative and qualitative information, will allow identifying different isoforms of a gene and splicing events, quantitative studies, and evaluation of the number of transcripts of each gene, identification of post-transcription revisions, and identification of single nucleotide differences, all of which provide a profound understanding of the genome and transcription (Wang *et al*., 2009; Vlk and Řepková, 2017).

Extensive studies have been performed on the physiological alterations in barley plants exposed to salinity stress (Ahmed *et al*., 2015; Ebrahim *et al*., 2020; Narimani *et al*., 2020), but only a few RNA-Seq studies have set out to characterize the transcriptome of barley plants in salinity stress conditions. Bahieldin *et al*. (2015) used RNA-Seq technique to investigate the transcriptome of wild barley under salinity stress of 500 mM. They found that transcripts that had a significant increase in expression were involved in various biological processes including electron transporters, flavonoid biosynthesis, reactive oxygen species, ethylene production, signaling networks, and protein folding. In a recent study by Yousefirad *et al*. (2020), transcriptome analysis using RNA-Seq in a salt-tolerant mutant line of barley showed that the families of WRKY, ERF, AP2/EREBP, NAC, CTR/DRE, AP2/ERF, MAD, MIKC, HSF, and bZIP, as the important transcription factors with specific gene expression, contribute to barley tolerance to 300 mM NaCl.

Although enormous attempts have been made to identify the genes in the signaling pathway of salinity stress, still a comprehensive understanding of molecular network(s) operational in triggering salinity tolerance is needed to be enlightened. Therefore, the present study aimed to evaluate differentially expressed mRNA in salinity- tolerant and salinity- susceptible genotypes and comparing the plant response to salinity and control conditions using RNA-Seq method in barley.

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MATERIALS AND METHODS

Plant Material and Cultivation Conditions

In this study, based on the obtained results of previous experiments, Afzal cultivar, as a salinity tolerant cultivar, and Yusef cultivar, as a salinity sensitive cultivar, were used (Narimani *et al*., 2020). Seeds were planted in plastic pots containing perlite after disinfection with 5% sodium hypochlorite. Then, the pots were irrigated with diluted Hoagland solution after germination (Hoagland and Arnon, 1950). Salinity stress of 200 mM NaCl was applied for 24 hours at 4–5 leaf stage with two replications. Control plants were not treated with NaCl. The root samples were harvested and freeze-dried in nitrogen and stored at -80°C.

RNA extraction, cDNA Library Construction and RNA Sequencing

According to the manufacturer's instructions, total RNA from the roots of the control and salinity-treated plants was prepared using the TRIzol (Invitrogene) kit, then, extracted RNA was treated with *Dnase*I enzyme. The quality of extracted RNA was checked using 1% agarose gel electrophoresis and optical absorption was evaluated by nanodrop device to determine RNA concentration and probable contamination. RNA samples containing RNA integrity number (RIN> 7) were used for constructing cDNA library and sequencing. Then, using the Illumina HiSeq 4500 platform with dual reading, sequencing was carried out in which the reading sequence was 150 pair base.

Bioinformatics and RNA-Seq Data Analysis

The Linux system and the Tuxedo pipeline were utilized to analyze the RNA-Seq data. For each sample, the quality of raw readings was individually controlled using fastQC software. The readings were then processed based on adapter sequence deletion, base quality, average reading quality, and reading length using Trimmomatic software (Bolger *et al*., 2014).

Aligning Reads with the Reference Genome

The spliced readings of samples under salinity stress and the control were individually matched on the reference genome of barley (*Hordeum vulgare* L.) using Bowtie2/Tophat2 software (Kim *et al*., 2013).

Analysis of the Differentially Expressed Genes (DEG)

Cufflinks, Cuffmerge and Cuffdiff software were employed to identify the differentially expressed genes with different expression. The Cufflinks software counts the number of readings aligned to the reference genome in each sample and aggregates them into a transcript file. The Cuffmerge software converts the aggregated files for samples by cufflinks into a single transcript file. The Cuffdiff software analyzes the differentially expressed genes in the form of either increased expression or reduced expression. R software was utilized to draw the Volcano and Heatmap.

Gene Ontology Analysis and KEGG Pathway of DEG

GO analysis was utilized to identify the DEG. It should be mentioned that AgriGO web application software is a useful software as it contains an extensive database of several biological steps as well as a variety of applications to understand the mechanisms involved in a gene set (Tian *et al*., 2017). KOBAS software was employed

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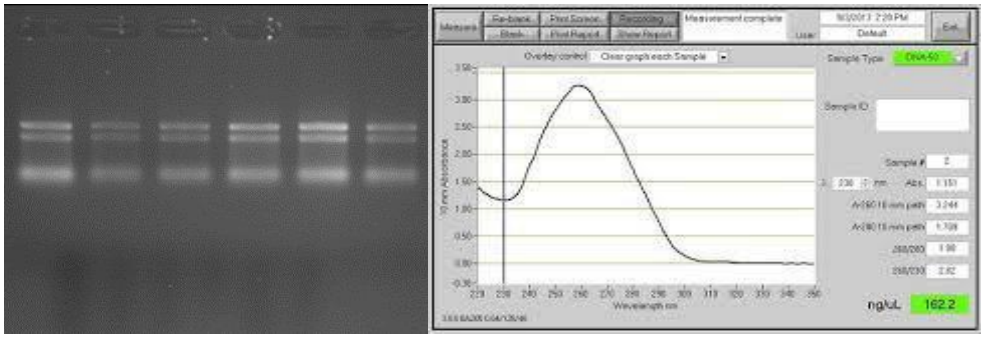

(a) (b)

Figure 1. RNA samples in agarose gel electrophoresis 1% (a), and Quantitative measurement of RNA samples using nanodrrap spectrophotometer (b).

for KEGG analysis (Corrected P-value< 0.05). Note that as there was no information on barley species at this site, a species close to barley, namely, rice was used (*Oryza sativa* L.) (Xie *et al*., 2011).

Plotting Interaction Networks of DEG and Analysis of Hubs

Cytoscape version 3.6.1 software was employed to plot the gene network. This software is bioinformatics software to visualize molecular cross-links, biological pathways, and integrating these networks with gene expression profiles or other data attributes. The String web application and Cyto-Hubba software were used to identify essential proteins/hubs.

RESULTS AND DISCUSSION

The results of the RNA quality control showed that the quality of the extracted RNA was optimal to produce the cDNA library for sequencing. Three bands of 28S, 18S, and 5S were observed indicating the good quality of extracted RNA. In analyzing the purity and quantity of RNA using a Nanodrop device, samples with absorption ratios of 260/280 and 230/260 close to 2±0.1 and the concentration above 30 μg μL^{-1} were selected and used for sequencing (Imbeaud *et al.*, 2005; Fleige and Pfaffl, 2006; Fleige *et al.*, 2006) (Figure 1). Another indicator used to evaluate the quality of extracted RNA was the RIN number, which is an indicator to evaluate the amount of decomposed RNA, ranging from 1 to 10. Samples sent with a RIN greater than 7 were selected for sequencing.

The number of reads available for each of the sequenced samples was about 28,000,000 reads with a length of 150 base pairs as paired-end. The results of the assessment of the readings quality with fastQC software showed that, in general, the reads had the necessary quality to perform downstream analyzes, however, the low quality reads were removed using Trimmomatic software to obtain the maximum quality. Clean data was obtained after applying the trimming. The results of matching the trimmed reads with the barley reference genome using the Bowtie2/Tophat2 software showed that about 88% of the reads in all samples aligned with the reference genome, indicating the good quality of the sequenced samples.

Treatment comparisons and genotypic comparisons were performed using Cuffdiff software with false discovery rate (FDR <0.01) FDR< 0.01 to identify the differentially expressed genes. The Volcano diagram was drawn separately for all treatments and genotypic comparisons to better understand the results of the Cuffdiff software. In Volcano diagram, Black dots

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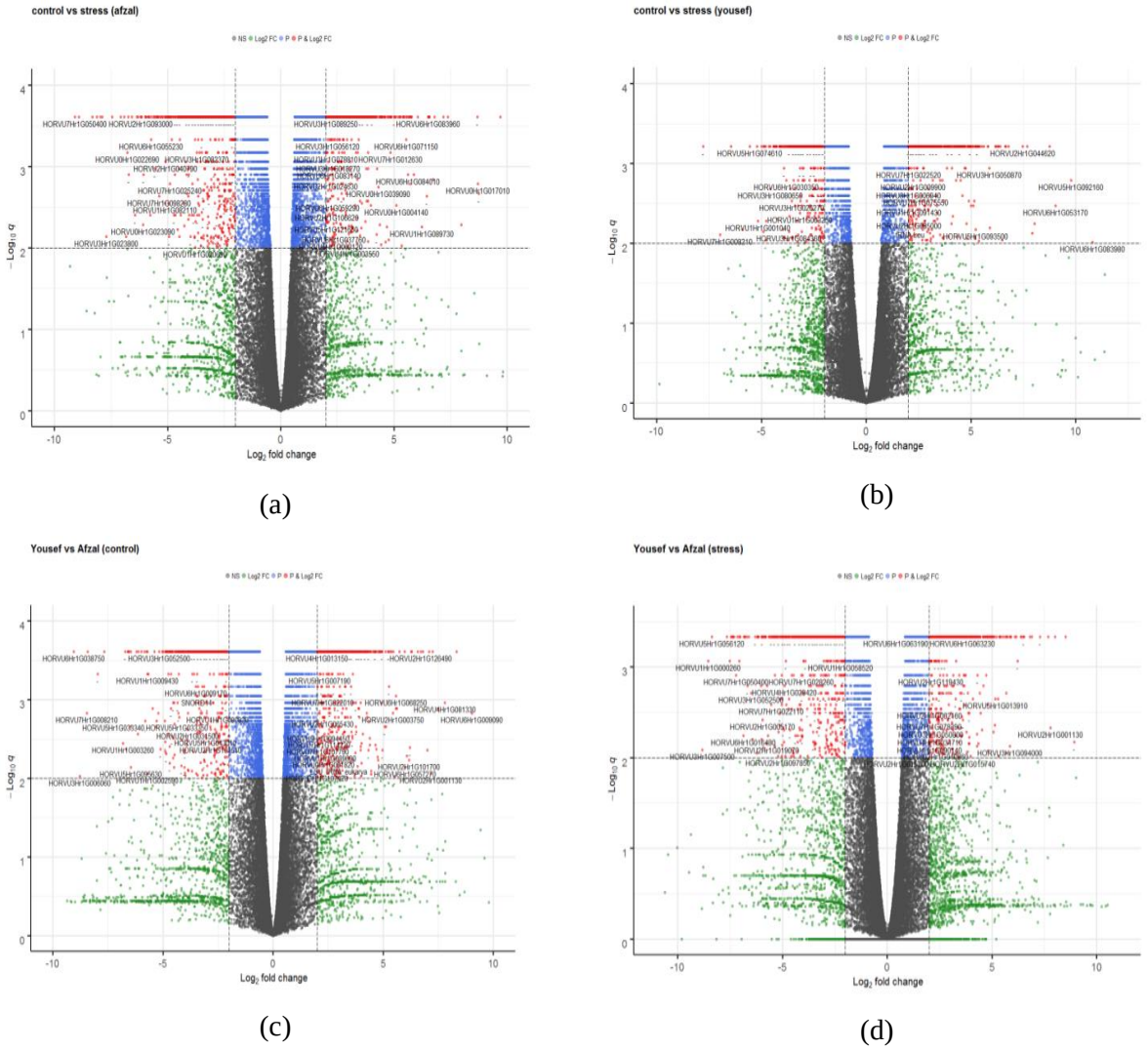

Figure 2. Volcano diagram for treatment and genotypic comparisons: (a) Control *vs* stress (Afzal), (b) Control *vs* stress (Yusef), (c) Yusef *vs* Afzal (control), and (d) Yusef *vs* Afzal (stress).

are related to genes with non-significant expression change with log fold change (FC) between +2 and -2, green dots are related to genes with expression changes with log FC more than +2 and less than -2, blue dots are related to genes with significant expression changes at a probability level of 0.01 with log FC between +2 and -2, and red dots are related to genes with significant expression changes at a probability level of 0.01 with log FC more than +2 and less than -2 (Figure 2).

The results obtained from Cuffdiff were filtered based on the significance properties at the probability level of FDR= 0.01, Log FC> 1.5. It was observed that in the treatment comparison of the control with stress in tolerant cultivar of Afzal, 2953 genes had significant expression changes, 1143 genes of which had an expression increase between 1.5 and 9.7 fold, and 1,810 genes of which had an expression decrease between 1.5 and 9 fold. Genotypic comparison of Yusef with Afzal under control conditions showed that 2,360 genes had significant expression changes, 1,456 genes of which had an expression increase between 1.5 to 11 fold, and 904 genes of

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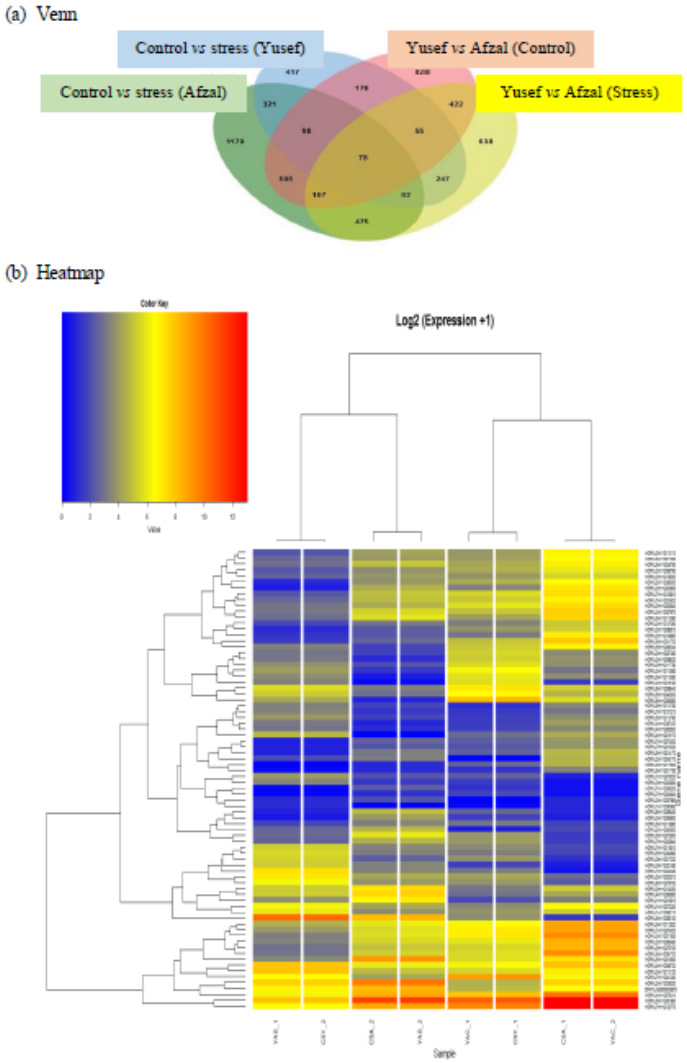

Figure 3. (a) Venn and (b) Heatmap related to the rate of common DEGs in treatment and genotypic comparisons.

which had an expression decrease between 1.5 to 9 fold. The total number of genes expressed in genotypic comparison of Yusuf with Afzal under salinity was 2,206 genes, 1,169 genes of which had an expression increase between 1.5 to 8 fold and 1,037 genes had an expression decrease between 1.5 and 8.8 fold. In treatment comparison of the control with salinity for the sensitive cultivar of Yusef, the total number of expression-changed genes was 1,552, 699 genes of which had an expression increase between 1.5 to 10.8 fold and 853 genes had an expression decrease between 1.5 to 6.9 fold. The maximum number of genes with significant expression changes was related to comparison of the control with salinity stress in tolerant Afzal. The minimum number of genes with significant expression changes was related to comparison of the control with salinity sensitive cultivar Yusef.

The rate of common gene expressions in four treatments and genotypic comparisons was displayed using the Venn. Then, based on the information obtained from the Venn, the Heatmap for 78 common genes was drawn to show the difference in gene expression (Figure 3). Gene ontology

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analysis was performed using the AgriGO website and option of Singular Enrichment Analysis and Fisher test for common expression-changed genes with FDR< 0.05. In general, the results were presented in three main groups, Biological Processes (BP), Molecular Functions (MF) and Cellular Components (CC).

The study of GOs related to common genes with different expressions in comparisons of control with stress showed that up-regulated genes related to BP group including response to stresses and non-biological stimuli (such as HORVU7Hr1G020960 with a 3 fold increase in expression in Peroxidase activity, heme binding and oxidative stress response), metabolic amino acid processes of the glutamine family with gene ontology (GO): 0009064 and FDR= 0.0029 (including HORVU3Hr1G085760 with a 2.46 fold increase in expression of proline biosynthesis), reaction to oxygen-containing compounds, response to chemicals (such as HORVU7Hr1G096780 with a 2 fold increase in expression in response to auxin), organization of plant cell wall and cell growth, metabolic processes of carbohydrates such as cellulose biosynthesis, trehalose biosynthesis, the metabolic process of fructose 6-phosphate, gluconeogenesis activity, activity of phosphoenolpyruvate carboxykinase and cell glucose biosynthesis, and CC group with GO: 0005576 and significance of FDR= 0.0021.

In comparison to the control, the down-regulated genes were divided into two groups of biological process and molecular function. Subgroups of the biological process included the regulation of metabolic processes, the regulation of the biosynthesis processes of cellular macromolecules, the regulation of gene expression, and the regulation of RNA biosynthesis processes. The main subgroups of molecular function were hydrolase pathways, binding processes, and antioxidants.

In the GO analysis of genes with common expression changes in comparisons of Yusef with Afzal, it was observed that genes with significant expression increase are related to BP, MF and CC groups. The BP group includes response to stimuli and stress such as HORVU5Hr1G076690 with a 3.69 fold increase in expression of response to auxin, HORVU5Hr1G091820 with a 2 fold increase in expression of antiporter activity and membrane transporter, HORVU2Hr1G013740 with a 2.3 fold increase in expression of peroxidase activity, reaction to oxidative stress, oxidation-reduction processes and heme binding, HORVU7Hr1G092530 with an increase in expression of D-acylglycerol kinase activity and kinase protein activator C, HORVU5Hr1G081620 with a 3 fold increase in expression of f phosphorylation signal transfer and protein binding. The MF group includes endopeptidase inhibitor activity, ADP binding, enzyme activity regulation, oxidoreductase, tetrapyrrole binding such as HORVU2Hr1G060480 with a 2.58 fold increase in expression of heme binding, oxidation-reduction process, chlorophyll binding, photosystem I and photosynthesis. The CC group includes the organization of the cell wall and catabolic lignin.

In the study of GO of the common down-regulated genes in the comparison of Yusef with Afzal, it was observed that these genes are in the MF group whose main subgroups include binding processes with a significance level of FDR= 0.355 and catalytic activity with a significance level of FDR= 0.037. There is difference in the number of genes involved in GO groups and some processes in all three groups.

Oxidative stress is one of the side effects of salinity stress on plants caused by high levels of osmosis and the toxicity of sodium ions, followed by the accumulation of active oxygen molecules such as H_2O_2, hydroxide radicals and superoxide in the cell that cause damage to membrane lipids, proteins and nucleic acids. ROS is refined by the activity of antioxidant enzymes such as superoxide dismutase, peroxidase, catalase and glutathione transferase (Qiu *et al*., 2002; Pan *et al*., 2006).. In this study, the group of

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genes that were affected by salinity stress were related to antioxidants (GO: 0016209) with FDR= 0.0014, including HORVU2Hr1G013740 with a 2.3-time increase in the expression of peroxidase activity in the genotype of salt tolerant Afzal.

The results of this study showed that sodium ion transporters genes have a significant increase in the expression. For example, HORVU5Hr1G091820 with a 2 fold increase in the expression of antiporter activity and membrane transporter and HORVU2Hr1G018300 with a 4 fold increase in the expression of protein phosphorylation and signal transfer. *HKT*, *NHX* and *SOS* genes are crucial contributors in modulating root and shoot ion homeostasis in plant cells under salinity stress conditions (Arzani and Ashraf, 2016). SOS1, a plasma membrane Na^+/H^+ exchanger, is one of the most important gene in excluding Na^+ from cytosol (Arabbeigi *et al*., 2018). Activation of this pathway eventually leads to the activation of some transporters. Environmental stresses induce calcium ions to enter the cytoplasm through the apoplast pathway or other pores that these calcium entry channels are a type of sensor for understanding the stress. As a result, the ionic salinity stress path of the calcium-dependent SOS path is activated. The purpose of this type of pathway is to transfer ions, which control the homeostasis of ions under salinity stress. Rivandi *et al*. (2011) reported that SOS2 and SOS3 kinases interact with each other and are directly and sequentially phosphorylated. Activation of SOS2 by phosphorylation activates the SOS1 transponder, this gene activates the Na^+/H^+ antiporter on the vacuole membrane to allow sodium ions to enter the vacuole and is also responsible for the release of sodium ions through the plasma membrane. Higher gene expression of the vacuolar Na^+/H^+ antiporter *AtNHX1* led to increased salinity tolerance in transgenic rapeseed (Yamaguchi and Blumwald, 2005) and wheat (Xu *et al*., 2008).

The results of this study showed that HORVU1Hr1G014580 with a 3-time increase in expression under salinity conditions compared to the control conditions plays an important role in the phospholipase C activity, metabolic lipid processes and conduction of intracellular signal, and HORVU7Hr1G092530 with a significant increase in expression plays an important role in the D-acylglycerol kinase activity of Afzal tolerant cultivar. Membrane lipids may be directly involved in responding to stress through membrane fluidity and other physicochemical properties. An important function of lipid components is in the production of secondary signaling molecules.

Biological synthesis of lipids and their decomposing enzymes play many roles, directly or indirectly, by affecting stress signaling and stress tolerance. Phospholipids are the main components of the cell membrane and act as precursors for the production of secondary messengers in response to non-biological stresses. Enzymes that break down lipid include phospholipases A2, C, and D, and a special type of phosphoinositide called phospholipase C has been studied more. The high activity of this enzyme causes the hydrolysis of phosphatidylinositol 4, 5-bisphosphate and the production of two important molecules of D-acylglycerol and inositol triphosphate. These two molecules are secondary messengers that can activate the kinase protein and cause the release of calcium, respectively (Tester and Davenport, 2003).

Another biochemical response of plants to salinity stress is the accumulation of compatible soluble substances such as carbohydrates and proline. These substances in the cell not only play a role in the osmotic regulation in the cell, but also prevent the production of free radicals, sweeping the ROS, protecting the integrity of the membrane, and stabilizing proteins. In addition, a number of osmolytes protect cellular compounds from damage caused by water loss (Ashraf and Foolad, 2013). An

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Table 1. KEGG pathway of DEG in treatment comparisons (control vs stress).

Term	ID	Number of gene	*P*-value
Starch and sucrose metabolism	osa00500	10	0.0007
Inositol phosphate metabolism	osa00562	5	0.0019
Nitrogen metabolism	osa00910	4	0.0021
Galactose metabolism	osa00052	5	0.0031
Plant hormone signal transduction	osa04075	9	0.0064
Ether lipid metabolism	osa00565	3	0.0087
Fructose and mannose metabolism	osa00051	4	0.0219
Glycerophospholipid metabolism	osa00564	4	0.0402
Carbon fixation in photosynthetic organisms	osa00710	4	0.0436
Alanine, aspartate and glutamate metabolism	osa00250	3	0.0444
Metabolic pathways	osa01100	37	0.0453
Biosynthesis of secondary metabolites	osa01110	22	0.0475

Table 2. KEGG pathway of DEG in genotypic comparisons (Yusef *vs* Afzal).

Term	ID	Number of gene	*P*-value
Phenylpropanoid biosynthesis	osa00940	9	0.0012
Carbon fixation in photosynthetic organisms	osa00710	5	0.0177
Biosynthesis of secondary metabolites	osa01110	26	0.0211
Metabolic pathways	osa01100	43	0.0223
Phenylalanine metabolism	osa00360	3	0.0368
Proteasome	osa03050	4	0.0408
Porphyrin and chlorophyll metabolism	osa00860	3	0.0419
Tyrosine metabolism	osa00350	3	0.0446
Histidine metabolism	osa00340	2	0.0465

increase in cellular proline accumulation is the most common response to drought and salinity in plant species (Arabbeigi *et al*., 2019). In barley, 300 mM NaCl stress caused a huge increment of proline content relative to the control (Ebrahim *et al*., 2020). Environmental stresses, especially salinity, lead to increase in soluble sugars such as sucrose, glucose and fructose (Sotiropoulos, 2007). This study also showed that genes of carbohydrate biosynthesis, especially fructose, as well as genes of proline biosynthesis from glutamate had a significant increase in expression in Afzal under salinity stress conditions as compared to non- stress conditions.

The results of the study of the KEGG pathway relating to the expression-changed genes in the treatment comparisons and genotypic comparisons are shown in Tables 1 and 2.

Drawing a gene network for common Differentially Expressed Genes (DEG) using the Cytoscape version 3.6.1 software and the String site showed that 12 genes were selected as hub genes in the network. These genes showed expression change under salinity conditions and the tolerant genotype of Afzal compared to the control conditions and the sensitive genotype of Yusef (Figure 4). PR1 and PR4 proteins are produced by various stresses, including biological stresses such as microbial contamination and their stimuli, and non-biological stresses such as salinity and drought, and mechanical wounds in plants. OSM34 is a protein similar to osmotin and belongs to the Thaumatin family. AT3G01190 is a peroxidase superfamily protein. CDC2 is a

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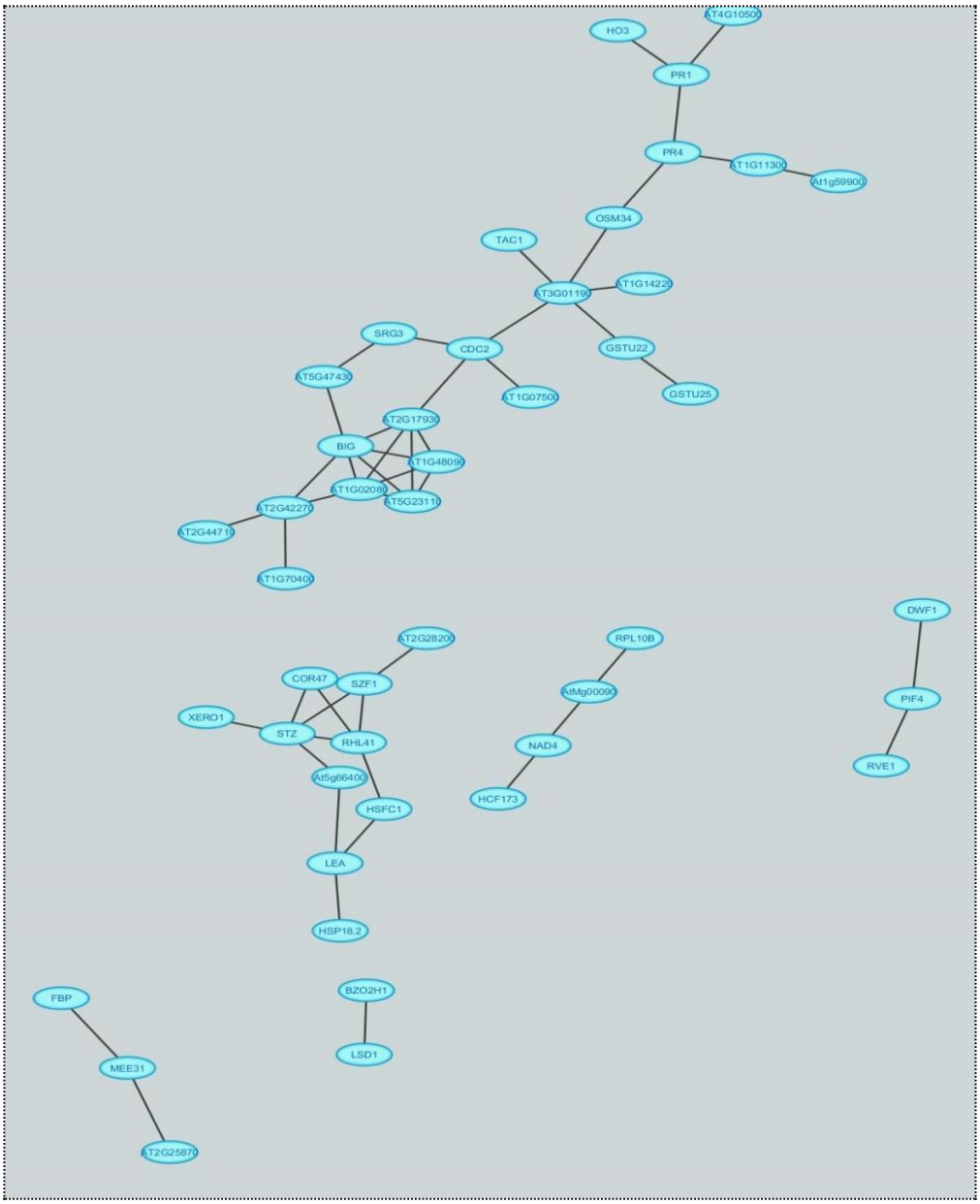

Figure 4. Gene network for Differentially Expressed Genes (DEG).

type of cyclin-dependent kinase protein. AT2G17930 is a protein of the phosphatidylinositol 3-kinase family. BIG is an auxin carrier protein. AT2G42270 is a small nuclear ribonucleoprotein helicase protein. STZ refers to Cys2/His2-type zinc fingers proteins. RHL4 is a zinc finger protein. MME3 belongs to the mannose-6-phosphate isomerase type I family. PIF4 is a negative transcription factor in the phytochrome B signaling pathway (Liu and Xue, 2006; Vogt *et al*., 2010; Kumar *et al*., 2013; Sigdel *et al*., 2015; Liu *et al*., 2019).

CONCLUSIONS

Overall, the results of the study showed that when barley is under salinity stress, various mechanisms are activated involving

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multiple genes with different functions, which contribute to homeostasis survival of the plant cells. Identification and modeling of the abovementioned gene expression regulatory network in response to stresses will help the researcher in developing strategies to improve tolerance to salt stress in barley.

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آنالیز ترانسکریپتوم پاسخ دهنده به تنش شوری در ریشه جو (*Hordeum vulgare* L.)

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چکیده

تنش شوری یکی از مهم‌ترین تنش‌های محیطی است که منجر به کاهش رشد و عملکرد گیاهان زراعی می‌شود. جو یک محصول مهم زراعی است که به عنوان گیاهی متحمل به شوری در بین غلات شناخته می‌شود. در این مطالعه، ترانسکریپتوم پاسخ دهنده به تنش شوری در ریشه ارقام متحمل (افضل) و حساس (یوسف) مورد بررسی قرار گرفت. توالی‌یابی ترانسکریپت‌های mRNA (اصطلاحاً RNA-Seq) با استفاده از پلت فرم ایلومینا HiSeq، بعد از فیلتر شدن برای RNA با دم ۳´ پلی آدنیله که فقط شامل mRNA باشد، اجرا شد. جهت شناسایی ترانسکریپت‌های تغییر بیان یافته از پایپلاین Tuxedo استفاده گردید. نتایج توالی یابی نشان داد که پس از تریمینگ اولیه خوانش‌ها، بالای ۲۰ میلیون خوانش (۹۲ درصد) برای تمامی نمونه‌ها باقی ماند و از این تعداد خوانش، حدود ۸۸ درصد با ژنوم جو هم ردیف شدند. آنالیزهای بیوانفورماتیکی نشان داد که ژن‌های تغییر بیان یافته در فرآیندهای مختلفی همچون فعالیت آنتی‌پورتر و ترانسپورتر غشایی، آنتی اکسیدان، طیف وسیعی از آبشارهای کینازی و فسفاتازی، ترارسانی، متابولیک کربوهیدرات‌ها، اسیدهای آمینه و لیپیدها، فرآیندهای اتصال، پاسخ به هورمون‌های گیاهی، فعالیت کاتالیتیک و سازماندهی دیواره سلولی نقش دارند. در تجزیه شبکه ژنی مشاهده شد که ژنهای کلیدی از جمله پروتئین‌های درگیر در ایجاد مقاومت اکتسابی سیستمیک، پروتئین‌های خانواده پراکسیداز، پروتئین کیناز وابسته به سایکلین، فسفاتیدیل اینوزیتول کیناز، پروتئین-های حامل اکسین، مانوز ۶ فسفات ایزومراز، هلیکازها و فاکتورهای رونویسی در ایجاد تحمل به شوری

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نقش مهمی ایفا می‌کنند. این اطلاعات می توانند به عنوان یک منبع ارزشمند در مطالعات آینده برای دستورزی‌های ژنتیکی گیاه جو و توسعه رقم متحمل به شوری استفاده شوند.

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