Effect of Heat Shock on Some Genes Involved in Heat Tolerance System in Barely

Shimaa M. Elshora[1], A. M. Elzoheiry[2], El-Sayed E. El-Shawy[3] and M. E. Eldenary[1]

1- Department of genetics, Faculty of Agriculture, Tanta University, Egypt

2- Department of genetics, Faculty of Agriculture, Zagazig University, Egypt

3- Barley Res. Dept., Field Crops Res. institute, Agricultural Res. Center, Egypt.

***Key words**: Barley. Abiotic stress. Heat shock Proteins . Gene Expression.*

Corresponding Author: Shimaa M. Elshora, shimaaelshora@gmail.com

Barley (*Hordeum vulgare*) is the most important cereal crop in the world after wheat, maize and rice (FAO-STAT, 2015). It is used mainly for animal and poultry feeding, as well as in the pharmaceutical industry and malt (Biel and Jacyno, 2013). Climate changes are one of the most challenging agricultural problems globally cultivation. Barley grain yield and quality are significantly affected by elevated temperature, Lobell *et al., (*2011). Heat stress is the most adverse abiotic constraint that significantly affects plant growth, physiology, yield, and productivity for most crops (Bilal *et al*., 2015 and Lobell *et al*., 2015). Heat stress causes many physiological effects i.e. membrane protein denaturation, enzyme inactivation, and changes in membrane permeability. These changes reduced ion flux, cause leakage of electrolytes and water content as well as cause the production of toxic compounds (*Mafakheri et al*., 2010). Heat shock proteins (HSPs) play a critical role in sensing and initiating heat shock response in plants during high temperature stress. Heat shock response is triggered by HSPs which are swiftly accumulated under temperature increments to reduce expected damage (Serrano *et al*., 2019). Plants induce different stress-responsive biomolecules as a part of their tolerance mechanisms. Molecular chaperones are of the most important biomolecules, which act to reduce the adverse effects of cells by stress. The heat shock response and the HSP are predicted to be evolutionary conserved. There is an intimate association between expressions of HSPs with that of resistance to high temperature stress (HTS) but in-depth mechanism through which HSPs work to increase thermo tolerance is yet to be fully understood (Singh *et al*., 2016).

Heat shock proteins bind to their substrate reversibly and an ATP-dependent in function manner to promote protein folding in a native state, and induce proteolysis and disaggregation of substrate proteins without forming part of

the final product. Among the five major Hsp families, a class of Hsp70 family proteins consists of a conserved N-terminal ATP-binding domain and C-terminal substrate-binding domain along with a C-terminal lid with a variable number of amino acids (Flaherty *et al*., 1990). Another class of molecular chaperone family Hsp90 proteins function in the form of a dimer; each promoter consists of an ATP-binding domain at the N-terminal, and linker M-domain and dimerization domain at the C-terminal (Pearl and Prodromal, 2006).

The Heat Shock Regulators (HSR) has a modular structure and is conserved among eukaryotes. Despite the variability in sequence and size, the mode of promoter recognition and their basic structure show high similarities (Bjork and Sistonen, (2010); Fujimoto and Nakai, (2010). Heat shock factors are in classes and groups, i.e., *Arabidopsis thaliana* has 21 HSFs in three classes (A, B and C), which include 14 different groups (A1 to A9, B1 to B4 and C1), Scharf *et al*., (2012). The roles of heat shock factor A1 (HSFA1) in response to the stress factors other than heat have not been determined. In response to high temperature, HSFA1 triggers the expression of different transcription regulators; Liu and Charng, (2012). HSFA2 is a heat-inducible transcription factor (Busch *et al*., 2005) and it is a secondary regulator under the control of at least one master regulato. Early and late heat shock gene expression can be mediated through this HSFA2 (Nishizawa, 2006).

In this investigation, the differential response to heat shock is studied by comparing heat tolerance with heat sensitive barley genotypes; in an attempt to clarify the correlation between some HSP regulatory and some biochemical indicators for heat tolerance.

MATERIALS AND METHODS

Barley Genotypes and Planting conditions

Four barley (*Hordeum vulgare*) genotypes as shown in Table (1) were obtained from Crop Research Institute, Sakha, Kafr-Elsheikh. Forty barley seeds of each genotype with three replicates were cultivated in plastic plates (20 x 9.5 x 7 cm) containing coco beet, perlite and clay soil. Germinated seeds were grown in a growth chamber at 18ºC and 5000 Lux light for 14/10 Light/Dark. Plants were irrigated with Hoagland solution (0.5) up to 23 days. Samples for heat stress (shock) treatment were collected as follow; control at 18ºC as well as 2h, 4h and 8h at 35°C. One and 48 h recovering treatments at 18ºC were carried out to compare the recovering response of plants.

Electrolyte leakages

Electrolyte leakage (EL) was measured as an indicator for quantification of plant cell membrane damage and cell death. Individual seedlings (0.5 gm. of each) in three replicates were used to measure the electric conductivity (EC meter Adwa-AD32). Seedling parts were placed in a test tube containing 10 ml of

sterile distilled water. The conductivity of the solution was measured three times i.e., immediately after rinsing, after one hour and after one hour of boiling (then cooled to room temperature). Leakage rate of electrolytes (expressed in μS·cm−1.FW·h−1) was calculated as the net conductivity of the solution with seeds immersed for 1 hr., divided by the total conductivity after boiling according to Lutts *et al*., (1996) with some modification.

Electrolyte leakages (EL) = (LEC1) – (LEC0) / (LEC2) – (LEC0).

Where: LEC0 = Measure immediately after soaking the samples in distilled- water, LEC1= Measure after soaking the samples in distilled-water for one hour and LEC2= Measure after boiling the samples for an hour.

Lipid Peroxidation Evaluation

Lipid peroxidation was evaluated as the concentration of 2-thiobarbituricacid (TBA) reactive products, equated with malondialdehyde (MDA), as described by Anjum *et al*., (2012) with slight modifications according to (Hendry and Grime,1993). Plant tissue (0.5 gm.) was homogenized in 5 ml (5% W/V) trichloroacetic acid (TCA), and centrifuged at 4000 rpm at 5ºC for 10 min. The chromogenicity was formed by mixing 2 ml of supernatant with 3 ml of reaction mixture (20% TCA and 0.5% TBA). The mixture was heated at 100ºC for 15 min., and then stopped by rapid cooling in an ice-water bath. The reaction was centrifuged at 4000 rpm at 5ºC for 10 min. The absorbance was then read at 532 nm and correction for unspecific turbidity was done by subtracting the absorbance of the same at 450 and 600 nm. The TBA-reactive products (MDA) were expressed as (nmol. g-1) DW and calculated as flow:

[(Abs 532 – Abs 600) - 0.0571 * (Abs 450 – Abs 600)] / 0.155.

Samples were collected and immediately frozen using liquid nitrogen and kept at –80ºC until use for further biochemical and molecular analysis.

Total soluble protein extraction

One half gram of plant tissue was ground to a fine powder then added to liquid nitrogen. Ground powder was homogenized in ice cold mortar and pestle in 1.0 ml of extraction buffer containing 20% of sucrose, 50mM of Tris, 50mM of NaCl and Protease inhibitors(Sigma-Aldrich) according to (Eldenary and Elshawy, 2014)with some modifications. Concentration of extracted proteins was determined according to Bradford, (1976).

Antioxidant enzymes assays

Changes in isozyme activities for antioxidant enzymes were studied using native PAGE (under non-reduced, non-denatured conditions) at 5ºC according to the suggested method by weydert and Cullen (2010). Native- PAGE was carried out for SuperOxide Dismutase (SOD) and Catalase (CAT).

Native PAGE Analysis of Antioxidant Enzymes

Native PAGE was performed according to (Laemmlie, 1970) without SDS. An equal amount of protein was separated on the native gel which was then rinsed in the detection reaction buffer according to the type of enzyme.

For SODs activity was detected by nitroblue tetrazolium (NBT) reduction by superoxide radicals that were photochemically generated; according to Beauchamp and Fridovich, (1971). After electrophoresis, the gels were covered with a solution containing 0.25 mg/mL−1 of NBT and 0.1 $mg \cdot mL^{-1}$ of riboflavin, and then exposed to a light. The two types of SOD (Mn-SOD and Cu/Zn-SOD) were identified using inhibitors. Mn-SOD was diagnosed by its sensitivity to a 5 mM of H_2O_2 and 1 mM of KCN, while Cu/Zn-SOD was identified by its sensitivity to 1 mM of KCN (Navari-Izzo *et al*., 1998).

CAT activity in native PAGE gels was determined using the methodology According to Woodbury *et al*., (1971).

Total RNA Extraction

Total RNA was extracted from barley seedlings of control and heat treated plants for the different genotypes using EZ-10 Spin column Plant RNA Mini-Preps Kit (BIO BASIC CANADAINC) according to the attached protocol. RNA quantity and purity was determined using Nano drop spectrophotometer (Bio Drop µLITE.UK). RNA samples with 260/280 nm ratio more than 1.9 were considered as acceptable for RT-qPCR reactions. RNA quality and integrity were confirmed via electrophoresis on a 1.5% agarose gel. A two µg of total RNA were used for c-DNA synthesis in a 20-µl of reaction mix using oligo (dT) primer and the HiSenScript™ RH cDNA synthesis kit (iNtRON Biotechnology).

Determination of Gene expression

Five primer pairs (Table 2) were designed using the NCBI Primer-BLAST program (http://www.ncbi.nlm.nih.gov/tools/primer-blast). Real-time quantitation of gene expression (RT-qPCR) analysis was carried out to confirm the induced changes in the gene expression. RT-qPCR reactions were conducted using 5X HOT FIREPol R EvaGreen R q-PCR Mix Plus (ROX) (enzynomics- Korea) in a 20 µL of reaction volume. The reactions were run (Applied Biosystem™ Step One Plus™ Real Time PCR system) using alpha tubulin *Hordeum vulgare* gene as an internal control (Accession number U40042.1). All tested samples were conducted in two biological replicates.

RESULTS AND DISCUSSION

Physiological and biochemical analysis

Electrolyte leakage (EL) is one of the physiological parameters used for estimation of cell membrane stability due to its sensitivity to heat stress (Rehman *et al*., 2016). The electrolyte leakage was measured as an indicator for the injury of the membranes their stability. Figure (1)

showed the measured EL as Electric Conductivity (EC) of seedling leaves of the tested four barley genotypes; according to Faralli *et al*., (2015).

The estimated oxidation rates (SOD) under HS stress comparing with the control is shown in Fig. (2). The reaction on the gel indicated that in the studied genotypes did not show significant increase in the Cu/Zn-SOD isozyme activity. However, Mn-SOD isozyme activity (high molecular weight band) was obtained only in the moderate (adaptive) genotype G2000 which showed the presence of two types of isozyme. In spite of catalase (CAT) all studied genotypes showed only one similar isoform of CAT enzyme under heat shock stress as well as the control plants, Fig. (8). Comparing there results (on shoots) with Kuralay *et al*., (2021) who exposed barley seedlings to combined of drought as well as high temperature stresses and showed considerably lower CAT and SOD activities in the shoots and this may confirm these results. While in the same barley seedlings; SOD and CAT activities in the roots were drastically increased under high temperature stresses and they detected two new SOD isoforms in the roots.

Lipid peroxidation is an indicator for the oxidative effect of the abiotic stress especially in the sensitive genotypes that might have not enough antioxidant content (enzymatic/non-enzymatic). The concentration of 2-thiobarbituricacid (TBA) reactive products, equated with Malondialdehyde (MDA) were evaluated. Although, there were no significant differences in SOD and CAT activities with HS treatment, the MDA showed clear differences between sensitive and tolerant genotypes. Figure 3 showed that G129 genotype (sensitive) appeared high lipid peroxidation compared with the G134 (tolerant) genotype. G134 (tolerant) genotype appeared negative values even with increased HS exposure times. The other sensitive genotype (G135) showed low lipid peroxidation (negative value) in the control and 2h at 35ºC, while the lipid peroxidation was increased with HS times increasing. Interestingly, the G2000 genotype showed high lipid peroxidation but it was reduced when the plants were transferred to 18ºC after 48 h. Yingyan *et al*., (2013) reported that MDA was significantly increased in barley seedlings with the rising of temperature, and the clearest values were at the range of 35°C- 40°C. They also concluded that the tolerant genotypes for HS stress appeared lower in MDA than the sensitive genotypes.

The interpretation for this behavior is that the tolerant genotype G134 may have non enzymatic antioxidant which reduced the harmful oxidative effect.

Differential HSPs gene expression

The four tested genotypes (exposed to HS at 35°C for three different times) were evaluated for gene expression. The selected 4 genes related to heat shock tolerance i.e. HSP70, HSP90, HSFA1 and HSFA2 genes were analyzed using qRT-PCR technique. Two of them (HSFA1&2)

are transcription factors. Expression level was standardized on the α-tubulin gene as the internal control gene. Relative expression (RQ) of the studied genes is shown in Figs. (4, 5, 6 and 7).

Figure (4) illustrated that HSP 70 gene was significantly increased about 15 fold in the sensitive genotype G-135 (2 h at 35°C) compared with the control, while the increased value was only about 4 fold in the tolerant genotype G134 with the same treatment compared with the control. On the other hand, the moderate genotype G2000 and the sensitive one (G129) did not show a significant increase in the expression of HSP70 gene under HS treatments.

The other studied gene of HSP90 (Fig. 5) showed up regulation (70 fold) in the sensitive genotype (G129) under HS (2h at 35ºC condition, but the tolerant genotype (G134) showed a quite increase (3 fold) with the same treatment in comparison with the control plants. This result was agreed with, Faralli *et al*., (2015) who exposed barley seedlings to heat shock stress and found that the expressions for HSP18 and HSP90 genes on qRT-PCR were significantly increased. Moreover, they mentioned that HSP70 gene was transcribed in the control and shocked seedlings, but its expression was not significantly like HSP90 gene .Other investigation by Sadura *et al*., (2020) who concluded that it may rely on the ability of the membranes to continuously accumulation for HSP70 gene proteins; as a result they did not need to perform over expression, but the opposite in HSP90 and HSP18 genes were occurred.

The regulator HSFA1 transcription factor for HSP70 gene showed higher expression levels under HS 8h at35ºC (more than 3 fold) in the tolerant genotype G134 (Fig. 6) compared with the control plants under normal condition (at 18ºC),while the sensitive genotype G129 under all conditions were remain around the control value. Heerklotz *et al*., (2001), and Mishra *et al*., (2002); reported that, in plants, HSFA1 is constitutively expressed and has a unique function as "a master regulator" of heat shock response.

Figure (7) Showed the expression level of HSFA2 transcription factor that was higher (10 fold) for 2h at 35ºC in the sensitive genotype (G135), while moderate high expression (6 fold) in the tolerant genotype (G134) under HS condition was occurred at the same treatment. The adaptive genotype G2000 showed only 2 fold expression at HS for the exposure time 4 h at 35ºC. Scharf *et al*., (1998) pointed out that HSFA1 factor during normal condition is distributed in the cytoplasm and upon activation by heat stress, nuclear localization of HsfA1 starts which then leads to the expression of HSFA2 and HSFB1 and the formation of hetero oligomer (termed super activator complexes) between HSFA1 and HSFA2 transcription factors.

SUMMARY

Sever climatic changes, especially high temperature, is one of the most

important abiotic factors defining the yield potential of temperate cereal crops such as barley. In this work, 4 barley genotypes were used to study the differential response to heat shock. Physiological data pointed that the sensitive genotype showed high leakage and lower electric conductivity. The sensitive genotype (G129) showed high lipid peroxidation compared with G134, the tolerant one. The quantitative PCR analysis for the studied heat shock proteins and transcription factors showed that the level of gene expression of HSP70 was significantly increased after a short time under HS in the sensitive genotype, while a slight increase was observed in the tolerant genotype. The HSP90 showed up regulation in the sensitive genotype G129 under HS condition, but the tolerant genotype G134 showed quite an increase in comparison with control plants. The regulator HSFA1 showed higher expression level in the tolerant genotype G134 comparing with G129. The expression level of HSFA2 was higher in the sensitive genotype (G135), while moderate high expression in the tolerant genotype G134.

REFERENCES

Anjum S. A., Saleem M. F., Wang L., Bilal M. F., Saeed A., (2012). Protective role of glycinebetaine in maize against drought-induced lipid peroxidation by enhancing capacity of antioxidative system. AJCS, 6(4):576-583.

Beauchamp C. and Fridovich, I., (1971). Superoxide dismutase: Improved assays and an assay applicable to acrylamide gels. Analytical Biochemistry, 44:276-287.

Biel W. and Jacyno E., (2013). Chemical composition and nutritive value of spring hulled barley varieties. Bulg. J. Agric. Sci., 19: 721-727.

Bilal M., Rashid R.M., Rehman S. U., Iqbal,F., Ahmed J., Abid M. A., Ahmed Z. and Hayat A., (2015). Evaluation of wheat genotypes for drought tolerance J. Green Physiol. Genet. Genom. 1:11-21.

Bjork J. K. and Sistonen L., (2010). Regulation of the members of the mammalian heat shock factor family. FEBS J.; 277:4126-4139.

Bradford M. M., (1976). A rapid and sensitive method for the quantitation of microgram quantities of protein utilizing the principle of protein-dye binding. Analytical biochemistry, 72: 248-254.

Busch W., Wunderlich M. and Schoffl F., (2005). Identification of novel heat shock factor-dependent genes and biochemical pathways in *Arabidopsis thaliana*. Plant J. 41:1-14.

El Denary M. E. and El-Shawy E. E., (2014). Molecular and Field Analysis of Some Barley Genotypes for Water Stress Tolerance. Egypt. J. Genet. And Cytol., 43: 187-198.

FAOSTAT, (2015). Food and Agriculture Organization of the United Nations. Statistical Database.

Faralli M., Lektemur C., Rosellini D. and Gürel, F., (2015), Effects of heat shock and salinity on barley growth and stress-related gene transcription. Biologia Plantarum, 59: 537-546.

Flaherty K. M., DeLuca F. C. and Mckay D.B., (1990). Three-dimensional structure of the ATPase fragment of a 70k heat-shock cognate protein. Nature, 346:623-628.

Fujimoto M. and Nakai A., (2010). The heat shock factor family and adaptation to phototoxic stress. FEBS J., 277:4112-4125.

Heerklotz D., Döring P., Bonzelius F., Winkelhaus S. and Nover L., (2001). The balance of nuclear import and export determines the intracellular distribution and function of tomato heat stress transcription factor HsfA2. Mol. Cell Biol. 21: 1759-1768.

Hendry G. A. and Grime J. P., (1993). Methods in Comparative Plant Ecology: A laboratory manual. Published in 1993 by Chapman & Hall, London ISBN 0 412 46230 3.

Kuralay Z., Assylay K., Bakhytgul G., Roza Y., Nurgul I., Ulbike A., Assemgul B., Zhanerke T., Rustem O. and Zhaksylyk M., (2021). ROS status and antioxidant enzyme activities in response to combined temperature and drought stresses in barley. Acta Physiologiae Plantarum .43:114.

Laemmli U.K., (1970). Cleavage of structural proteins during the assembly of the head of bacteriophage T4. Nature, 227(5259):680-685.

Liu H. C. and Charng Y. Y., (2012). Common and distinct functions of *Arabidopsis* class A1 and A2 heat shock factors in diverse abiotic stress responses and development. Plant Physiol., 163:276-290.

Lobell D. B., Schlenker W. and Costa-Roberts J., (2011). Climate trends and global crop production since 1980. Science, 333: 616-620.

Lobell D. B., Hammer G. L., Chenu K., Zheng B., McLean G. and Chapman S. C., (2015). The shifting influence of drought and heat stress for crops in northeast Australia Glob. Biol., 21: 4115-4127.

Lutts S., Kinet, J. M. and Bouharmont J. (1996). NaCl-induced senescence in leaves of rice (*Oryza sativa* L.) cultivars differing in salinity resistance. Ann. Bot. 78: 389-398.

Mafakheri A., Siosemardeh A., Bahramnejad B.,Struik P. C. and Sohrabi Y., (2010). Effect of drought stress on yield, proline and chlorophyll contents in three chickpea cultivars. Australian Journal, 4(8):580-585.

Mishra S. K., Tripp J., Winkelhaus S., Tschiersch B., Theres K., Nover L.

and Scharf K. D., (2002). In the complex family of heat stress transcription factors, HsfA1 has a unique role as master regulator of thermo tolerance in tomato. Genes & Development, 16: 1555-1567.

Navari-Izzo N. F., Quartacci, M. F., Pinzino C., and Vecchia, F. D., (1998).Thylakoid-bound and stromal antioxidative enzymes in wheat treated with excess copper. Physiologia Plantarum, 104(4):630-638.

Nishizawa, (2006). The role of class A1 heat shock factors (HSFA1s) in response to heat and other stresses in *Arabidopsis*. Plant J.,l48 (4):535-47.

Pearl L. H. and Prodromou C., (2006). Structure and mechanism of the Hsp90 molecular chaperone machinery. Annu. Rev. Biochem., 75:271-294.

Rehman S. U., Bilal M., Rana R. M., Tahir M. N., Shah M. K. N., Ayalew H. and Yan G., (2016). Cell membrane stability and chlorophyll content variation in wheat (*Triticum aestivum*) genotypes under conditions of heat and drought Crop Pasture Sci., 67:712-718.

Sadura I., Libik-Konieczny M., Jurczyk B., Gruszka D., Janeczko A., (2020). HSP Transcript and Protein Accumulation in Brassinosteroid Barley Mutants Acclimated to Low and High Temperatures. International Journal of Molecular Science, 10 21(5):1889.

Scharf K. D., Berberich T., Ebersberger I. and Nover L., (2012) .The plant heat stress transcription factor (Hsf) family: structure, function and evolution. Biochim. Biophys. Acta, 1819: 104-119.

Serrano N., Ling Y., Bahieldin A. and Mahfouz M. M., (2019). Thermo priming reprograms metabolic homeostasis to confer heat tolerance. Scientific Reports, vol. 9, no. 1, pp. 181.

Singh R. K., Jaishankar J., Muthamilarasan M., Shweta S., Dangi A. and Prasad, M., (2016). Genome-wide analysis of heat shock proteins in C4 model, foxtail millet identifies potential candidates for crop improvement under abiotic stress. Scientific Reports, 32641. DOI:10.1038/srep 32641

Weydert, C. and Cullen J., (2010). Measurement of superoxide dismutase, catalase and glutathione peroxidase in cultured cells and tissue. Nat. Protoc., (1):51-66.

Woodbury W., Spencer A. K., Stahman M. A., (1971). An improved procedure using ferricyanide for detecting catalase isozymes. Analytical Biochemical Nov, 44(1):301-5.

Yingyan H., Shuangxi F., Qiao Z. and Yanan W., (2013). Effect of heat stress on the MDA, proline and soluble sugar content in leaf lettuce seedlings. Agricultural Sciences, 4:112-115.

Table (1): Tested barley genotypes; pedigree and heat stress tolerancy.

Genotype	Pedigree	Heat stress tolerancy
G-129	Deir Alla106/Cel//As 46/Aths *2	Sensitive
G-135	ZARZA/BERMEJO/4/DS4931//GLORIA	Sensitive
G-134	Alanda-01/4/WI2291/3/Api/CM67//L2966-69	Tolerant
G-2000	Giza117/Bahteem52//Giza118/FAO86/3/Baladi16/Gem	Moderate

Sensitivity, Tolerance or Moderation for the Genotypes identification was obtained according to Barley Res. Dept., Field Crops Res. institute, Agricultural Res. Center, Egypt.

Table (2): Gene name, primer sequence and accession number.

Gene	Forward Primer 5'→3'	Reverse Primer 5' →3 '	Accession number
α-Tubulin	AATGCTGTTGGAGGTGGAAC	GAGTGGGTGGACAGGACACT	U40042.1
Hsp70	AAGGACAAGCTTGCGGACAA	ACTAGCTCAGCATACAGGCAC	L32165.1
HSP90	CGTCGTTGGATGGTTTTGGC	GCAGATGAAAGCAATAAGCAGGG	AY325266.1
HSFA1	ATGATGGCCTGAACCCTGAA	TTCCGGGTTGATGAAGAGCT	HM446022.1
HSFA2	AGATGATGGGGTTCTTGGCA	GCTCACTCTGGCTTGTTGTC	HM446025.1

α-Tubulin: Alpha tubulin, Hsp70: Heat shock protein70, HSP90: Heat shock protein 90. HSFA1: Heat shock factor A1, HSFA2: Heat shock factor A2.

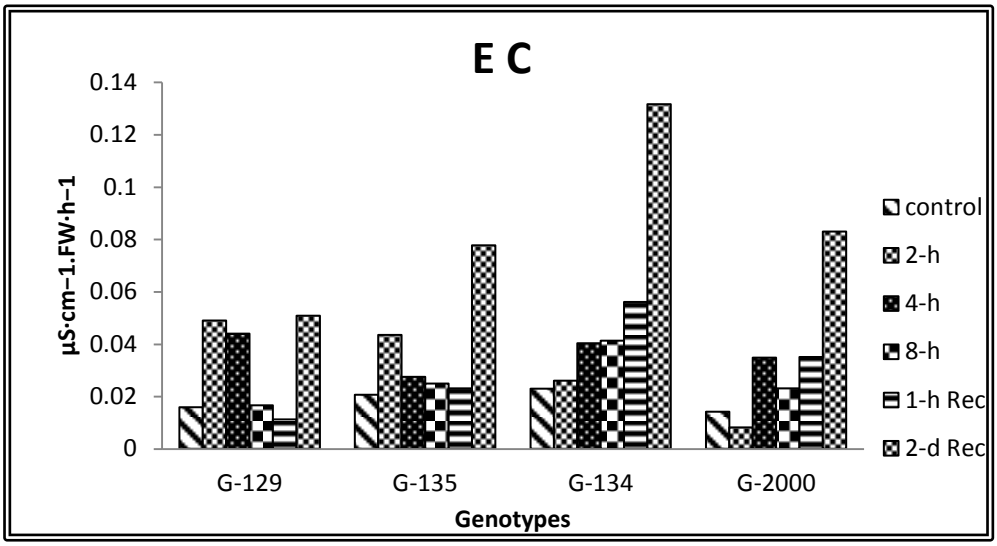

Fig. (1): EC for the tested barley genotypes under heat shock treatment.

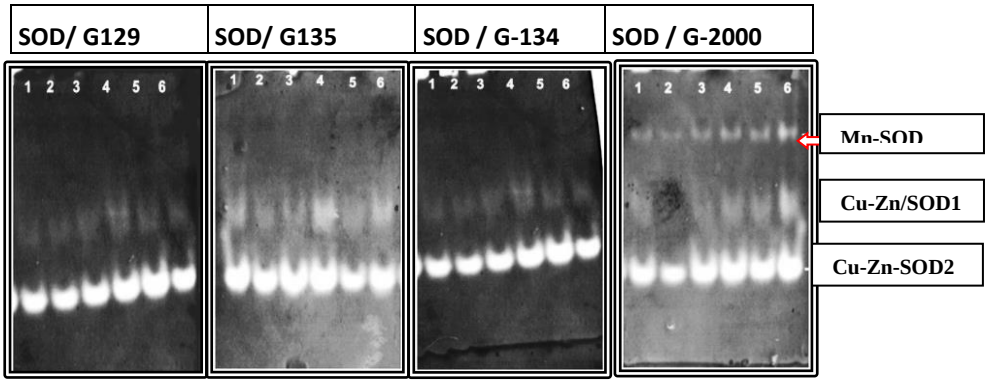

Fig. (2): ative gel showed SOD activities in control and heat shock treated seedlings. Lane 1 was control and lanes 2, 3 and 4 were exposed to 35ºC for 2 h, 4h and 8h, respectively. While lanes 5 and 6 were recovering at 18°C for 1h and 48 h, respectively.

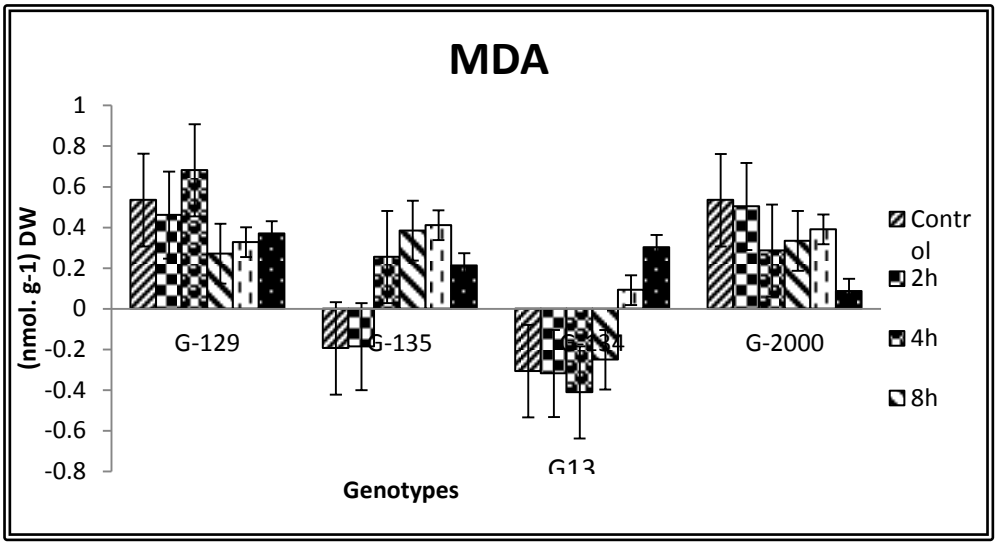

Fig. (3): Showed MDA for the tested barley genotypes under heat shock treatment.

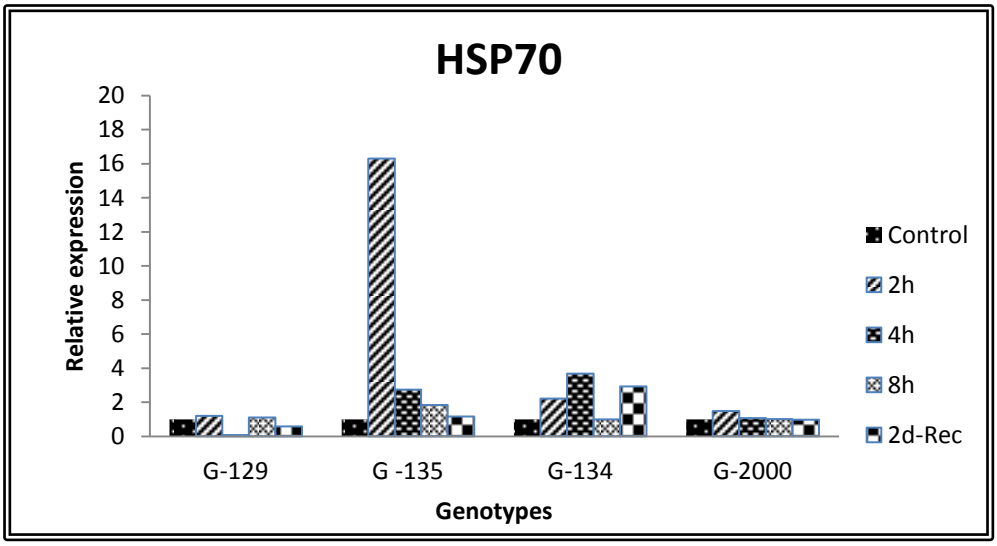

Fig. (4): levels of HSP70 gene expression for the tested barley genotypes under heat shock treatments.

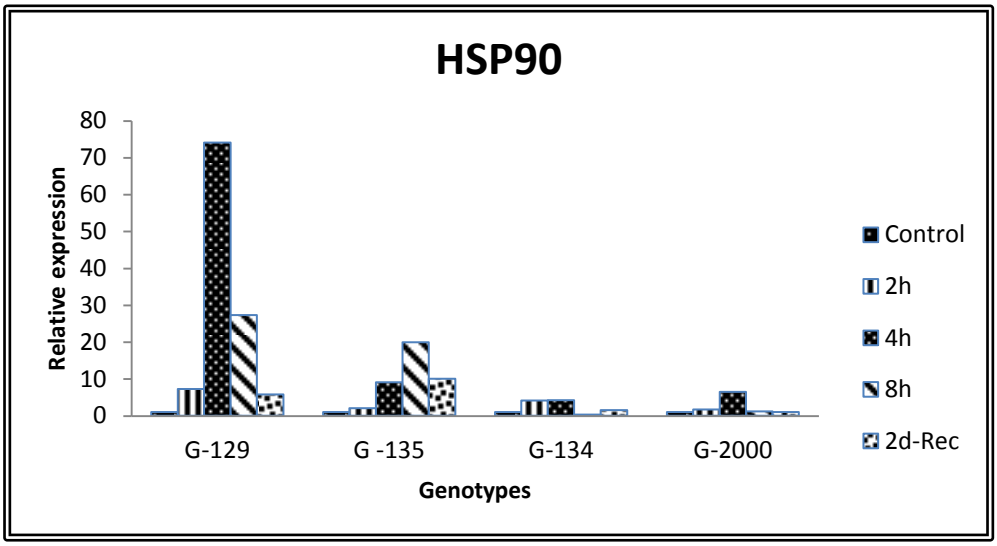

Fig. (5): levels of HSP90 gene expression for the tested barley genotypes under heat shock treatments.

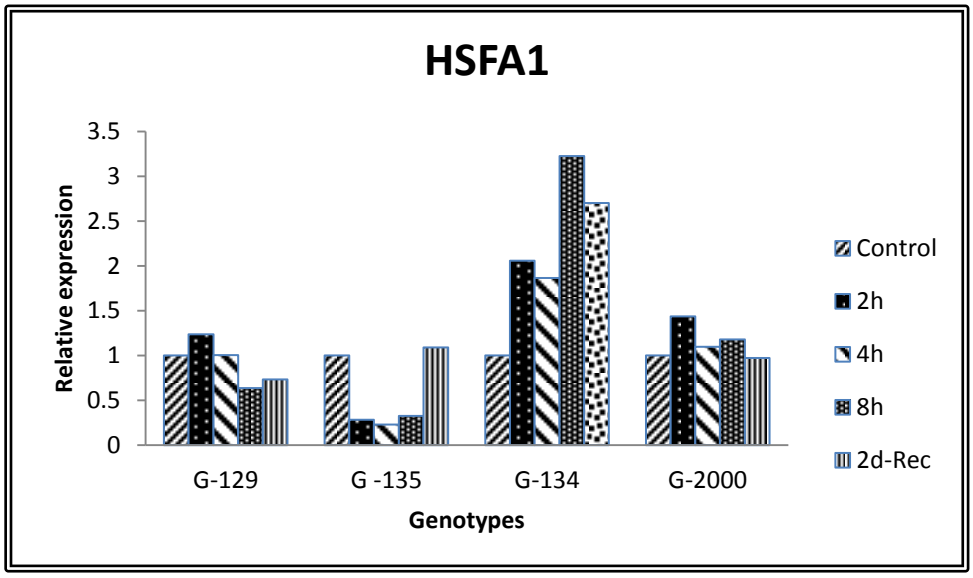

Fig. (6): Levels of HSFA1 gene expression for the tested barley genotypes under heat shock treatments.

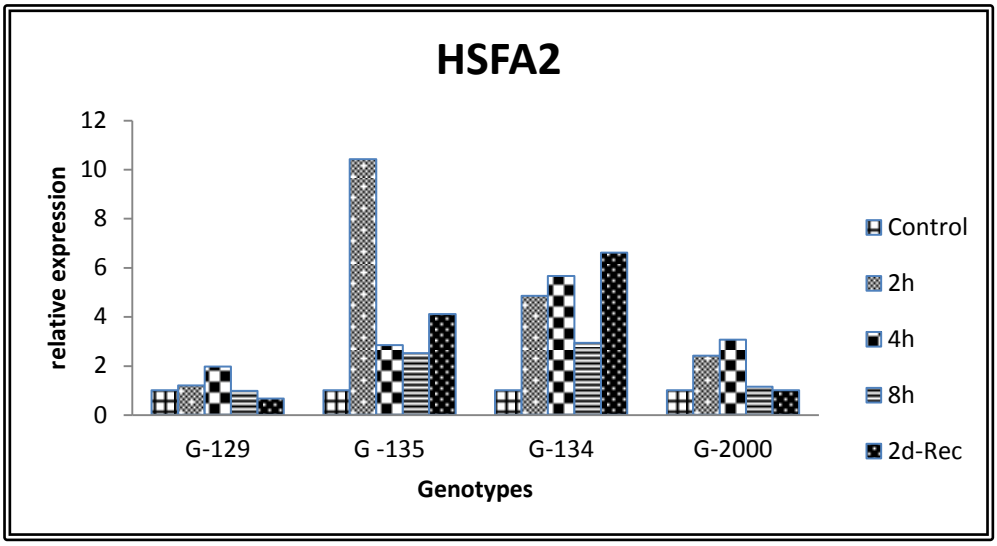

Fig. (7): Levels of HSFA2 gene expression for the tested barley genotypes under heat shock treatments.

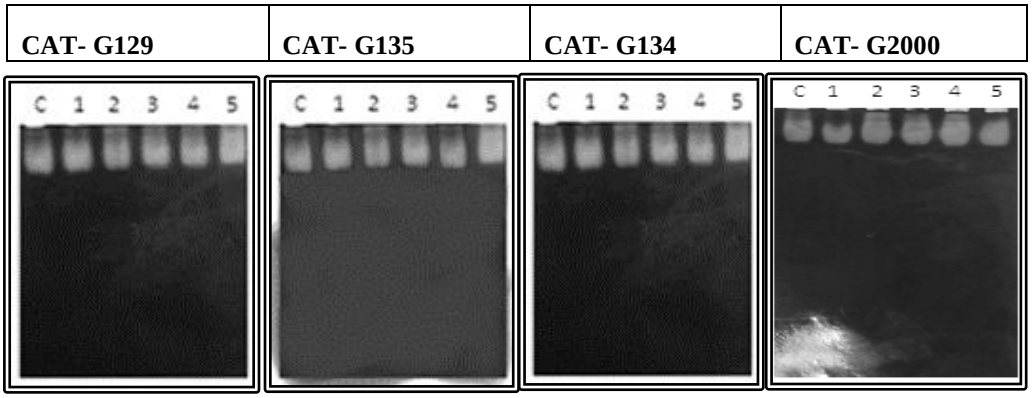

Fig. (8): Native gel showed Catalase activities in control and heat shock treated seedlings. Lane C was control and lanes 1, 2 and 3 were exposed to 35ºC for 2 h, 4h and 8h, respectively. While lanes 4 and 5 were recovering at 18ºC for 1h and 48 h, respectively.