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GENE EXPRESSION AND SINGLE NUCLEOTIDE POLYMORPHISMS (SNP) MARKER OF HEAT SHOCK PROTEIN (HSP) GENE IN WHEAT (*TRITICUM AESTIVUM* L.)

Heba A. Mahfouze, Walaa A. Ramadan and Sherin A. Mahfouze*

National Research Centre, Genetic Engineering and Biotechnology Research Division, Genetics and Cytology Department, Dokki, 12622, Egypt

Abstract

Heat stress is considered more challenging of the wheat yield loss during grain filling in Egypt and worldwide. To overcome the high-temperature effects on wheat by developing heat-tolerant wheat genotypes. The aim of the work was to examine the effect of heat stress on the gene expression of eight bread wheat genotypes differed in heat susceptibility, compared with the control. In addition, detection of heat-tolerant and heat-susceptible bread wheat genotypes by a Single nucleotide polymorphisms (SNP) marker designed from heat-shock protein (HSP16.9) gene was determined. SDS-PAGE showed that all the heat-sensitive and heat-tolerant genotypes were recorded an increase in the gene expression except genotype IG 138786 heat shocked at 37°C for 2 h, compared with the control (25°C/24 h). Moreover, the proteomic analysis under the heat stress conditions showed expression of three different kinds of HSPs (30, 35 and 100) were appeared to be upregulated in thermotolerant Gemiza 9, while downregulated in the heat-susceptible genotypes. These results indicate that the expression of HSPs is genotype-specific. On the other hand, Single nucleotide polymorphism (SNP) found in HSP16.9 gene differentiated between heat-tolerant and heat-susceptible bread wheat genotypes. Thus, seven the heat-sensitive genotypes scored one amplified fragment of 197 bp. However, the heat-tolerant genotype failed to give any amplicon. These genotypes could be used to improve tolerance to high-heat in wheat breeding programs by recurrent parents the heat-tolerant wheat line and susceptible high-quality one as donor parents.

Key words **:** Heat stress, SDS-PAGE, heat-tolerant genotypes, heat-susceptible genotypes, PCR, cluster analysis.

Introduction

Global climate change is becoming a severe problem for plants. Among abiotic stress, heat stress has a negative effect on the productivity and the plant's growth (Eitzinger *et al*., 2010; IPCC 2012; Xu *et al*., 2012; Zhao *et al*., 2018). The exposure of the wheat growing areas of high temperature (>35C) at late growth stages in the temperate environments causes 40% losses. Particularly, during the grain filling duration and hence adversely affecting the growth of plants, grain quality and harvest. High heat reduces the grain fill period and decreases the time to apoptosis and yield ripening (Altenbach *et al*., 2003). All living organisms can be stimulated to generate heat-shock proteins (HSPs) in reaction to temperature stress (Park and Seo, 2015). However, HSPs in plants are more complex than HSPs in other organisms. A heat shock response in plants is a ubiquitous phenomenon resulting in changing gene expression and protein translation. Plant HSPs can be divided into five main classes depending on the similarity and their cellular localization: HSP60, HSP70, HSP90, HSP100, and small heat shock proteins (sHSPs) (15 to 30 kDa) (Wang *et al*., 2004; Yildiz and Terzi, 2008; Chen *et al*., 2018). Three classes (CI, CII, and CIII) are existent in the cytosol or in the nucleus and the other three are found in the plastid, endoplasmic reticulum and mitochondria, respectively (Sato *et al*., 2008). Tolerant of heat stress is a complex phenomenon and controlled by multiple genes due to biochemical and physiological alterations and no single trait elucidates the heat tolerance mechanism. The illustration of the molecular and genetic principle of heat tolerant to identify molecular markers will increase the efficiency of wheat breeding programs aimed to develop varieties tolerant of the heat (Garg *et al*., 2012). In plant genetic researches, marker-assisted selection (MAS) allows a strategy for accelerating the

***Author for correspondence :** E-mail : Sherinmahfouze@yahoo.com

wheat breeding programs. Single nucleotide polymorphisms (SNP) reveal in practically unlimited numbers and some variations in nucleotide sequences between populations have a possible link with phenotypes which can be transformed into genetic markers for high-throughput genotyping (Rafalski, 2002).

The aim of the study was to examine the effect of exposure to high temperature on gene expression among eight bread wheat genotypes differing in response to heat stress. In addition, we identify on heat-tolerant and heat-susceptible bread wheat genotypes by the SNP marker designed from HSP16.9 gene.

Materials and Methods

Plant Materials

A total of eight wheat genotypes, including commercial cultivars and accessions, were used in this research. Six wheat accessions were provided by The International Center for Agricultural Research in the Dry Areas, Syria (http://www.icarda.org). In addition, two commercial cultivars were obtained from The Agricultural Research Center, Giza, Egypt (Table 1).

Table 1: The eight genotypes used in this study.

No.	Genotype	Origin
1	*Triticum aestivum* cv. Gemiza 10	Egypt
2	*T. aestivum* IG 92811	Algeria
3	*T. aestivum* IG 139010	Iran
4	*T. aestivum* IG 115798	Jordan
5	*T. aestivum* IG 43251	Pakistan
6	*T. aestivum* IG 138786	Syria
7	*T. aestivum* IG 94631	Tunisia
8	*T. aestivum* cv. Gemiza 9	Egypt

High-Temperature Stress Treatments

Ninety wheat grains of each genotype were surface-sterilized with 1% sodium hypochlorite for 30 min, then rinsed with dsH_2O three times and soaked in the dark overnight at room temperature. The grains of eight genotypes were germinated in Petri dishes on two layers of filter paper in an incubator maintained at 25°C (16 h day/8 h night, 80% humidity). Ten-day the seedlings were set into three groups in three replicates.

The first group: Ten plants were placed at 25°C for 24 h (the control).

The second group: Ten plants were heat shocked at 37°C for 2 h.

The third group: Ten plants were heat shocked at 40°C for 2 h.

Each group was under a constant light/dark regime with 16 h light and 8 h darkness and watered with tap water.

Protein Expression

The first leaf tissues (500 mg) of seedlings of the control and heat stressed b read wheat genotypes were sampled for electrophoresis by Sodium dodecyl sulphate-polyacrylamide gel electrophoresis (SDS-PAGE) technique, using 15% polyacrylamide gels according to the method of Laemmli, (1970) as modified by Studier, (1973).

DNA Extraction and Single Nucleotide Polymorphisms (SNP) Marker

Genomic DNA was extracted from ten-day-old seedling leaves of all the genotypes by the CTAB method (Doyle and Doyle, 1990). Two Primer sets HSP16.9 and SNP marker designed from HSP16.9 gene of bread wheat to identify heat tolerant and sensitive genotypes as shown in Table 2 (Garg *et al*., 2012). For marker analysis, a Polymerase chain reaction (PCR) was performed in a 25 µl volume containing 100 ng of genomic DNA, 2.5 µl of 10X PCR buffer, 200 µM of each dNTPs, 0.2 µM of each primer and 1.0 unit of *Taq* DNA polymerase. The thermocycling program consisted of an initial denaturation at 94°C/4 min, followed by 30 cycles of 94°C/45 sec, annealing temperature Table 2/45 sec, 72°C/60 sec and a final cycle of 72°C/5 min at in S 1000 Thermal Cycler (Biometra Inc., Germany). Amplification products were separated on a 1.5% agarose gel containing 1X TBE buffer (89 mM Tris-HCl, 89 mM boric acid, 2.5 mM EDTA, pH 8.3) at 90 V. The genomic DNA was stained with RedSafe Nucleic Acid Staining Solution (1/20,000) (iNtRON Biotechnology, Inc. Kr).

Data Analysis

A matrix for SDS-PAGE was generated by scoring reproducible bands as 1 for their presence and as 0 for their absence across the genotype. Jaccard's similarity coefficients were computed according to (Nei and Li, 1979). The data were subsequently used to construct a dendrogram using the un-weighted pair group method of

Table 2: The primers used in this study.

Primer	Single nucleotide sequence (5'—3')	Molecular sizes (bp)	Annealing temperature (AT)°C
HSP16.9 F	52 -CAGCAATCAACACCACGATG-32	307	60
HSP16.9 R	52 -TGCCACTTGTCGTTCTTGTC-32		
SNP F	52 -GAGGCGGACGAACGTGTTC(A8G)-32	197	55
SNP R	52 -TGACCTCCTCCTTCTTCACG-32		

arithmetic averages (UPGMA) (Sneath and Sokal, 1973) employing sequential, agglomerative hierarchic and non-overlapping clustering (SAHN). All the computations were carried out using the software NTSYS0PC (Numerical Taxonomy and Multivariate Analysis System), version 2.02 (Rohlf, 2000). Correlation coefficients were calculated using similarity coefficients obtained from SDS-PAGE analysis.

Results and Discussion

Effect of Temperature on Gene Expression by SDS-PAGE

SDS-PAGE recorded the differences in the content of proteins depending on a number of bands among eight bread wheat genotypes, when heat shocked either at 37 or 40°C for 2 h, compared with the control plants at 25°C/ 24 h as shown in Fig. 1 and Table 3. The electrophoresis was estimated based on the molecular weight (MW) (kDa). A number total of 16 bands were found ranging from 1.5 to 300 kDa. Seven out of these were monomorphic (43.75%), while nine were polymorphic (56.25% polymorphism). The highest expression was found in the temperature-susceptible genotypes such as Gemiza 10, IG 139010 and IG 115798 exposed to heat stress (37 and 40°C for 2 h), IG 43251 (37°C/2 h), IG 138786 (40°C/2 h) and the temperature-tolerant Gemiza 9 variety (37°C/2 h) (14 polypeptides), followed by Gemiza 10 (the control, 25°C/24 h), IG 92811 (37 and 40°C for 2 h), IG 43251 (25°C/24 h and 40°C/2 h) and IG 94631 (37°C/2 h) and the heat-tolerant Gemiza 9 variety (the control, 25°C/24 h) (13 subunits). However, the lowest expression was observed in the temperature-sensitive genotype IG 138786 (37°C/2 h) (eight bands) (Fig. 1 and Table 3). On the other hand, it was observed synthesis of heat shock protein (HSP) of 100 kDa in the heat-tolerant Gemiza 9, also in heat-sensitive genotypes *i.e.*, Gemiza 10, IG 92811, IG 139010, IG 138786 and IG

Table 3: SDS-PAGE analysis of eight bread wheat genotypes exposed to different heat stress.

Band No.	MW (kDa)	Genotypes																					
		Gemiza 10			IG 92811			IG 139010			IG 115798			IG 43251			IG 138786			IG 94631			Gemiza 9	
		25°C	37°C	40°C	25°C	37°C	40°C	25°C	37°C	40°C	25°C	37°C	40°C	25°C	37°C	40°C	25°C	37°C	40°C	25°C	37°C	40°C	37°C	40°C
1	300	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1
2	200	1	1	1	1	1	1	1	1	1	1	1	1	1	1	0	1	0	1	1	1	1	1	1
3	165	1	1	1	1	1	1	0	1	1	0	1	1	0	1	1	0	0	1	0	1	1	1	0
4	100	0	1	1	0	1	1	0	1	1	1	1	1	1	1	1	0	1	1	0	1	1	0	1
5	80	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1
6	57	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1
7	42	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1
8	39	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1
9	35	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	1	1
10	31	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1
11	30	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	1	1
12	27	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1
13	24	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	0	1	0	1	0	0	0
14	18	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	0	1	0	1	0	0	0
15	3	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	0	1	1	1	1	1	1
16	1.5	1	1	1	0	0	0	1	1	1	0	1	1	1	1	1	1	0	1	0	0	0	1	0
Bands number = 16		13	14	14	12	13	13	12	14	14	12	14	14	13	14	13	12	8	14	9	13	11	13	14	12

1 = Presence of band , 0 = absence of band, Gemiza 10 (susceptible); IG 92811 (susceptible); IG 139010 (susceptible); IG 115798 (susceptible); IG 43251 (susceptible); IG 138786 (susceptible); IG 94631 (susceptible) and Gemiza 9 (tolerant).

Table 4: Genetic similarity and genetic distance statistics to eight bread wheat genotypes subjected to different temperature stress.

Genotypes	Genotypes																							
	Gemiza 10			IG 92811			IG 139010			IG 115798			IG 43251			IG 138786			IG 94631			Gemiza 9		
	25°C	37°C	40°C	25°C	37°C	40°C	25°C	37°C	40°C	25°C	37°C	40°C	25°C	37°C	40°C	25°C	37°C	40°C	25°C	37°C	40°C	25°C	37°C	40°C
Gemiza 10 (25°C)	1.00																							
Gemiza 10 (37°C)	0.86	1.00																						
Gemiza 10 (40°C)	0.79	0.93	1.00																					
IG 92811 (25°C)	0.77	0.79	0.71	1.00																				
IG 92811 (37°C)	0.67	0.69	0.63	0.85	1.00																			
IG 92811 (40°C)	0.75	0.64	0.69	0.82	0.69	1.00																		
IG 139010 (25°C)	0.63	0.75	0.69	0.79	0.93	0.64	1.00																	
IG 139010 (37°C)	0.67	0.57	0.50	0.73	0.62	0.70	0.57	1.00																
IG 139010 (40°C)	0.60	0.63	0.67	0.64	0.79	0.75	0.86	0.57	1.00															
IG 115798 (25°C)	0.56	0.69	0.63	0.71	0.86	0.57	0.93	0.62	0.79	1.00														
IG 115798 (37°C)	0.63	0.75	0.69	0.79	0.93	0.64	1.00	0.57	0.86	0.93	1.00													
IG 115798 (40°C)	0.67	0.69	0.63	0.71	0.86	0.69	0.93	0.62	0.92	0.86	0.93	1.00												
IG 43251 (25°C)	0.63	0.75	0.69	0.79	0.93	0.64	1.00	0.57	0.86	0.93	1.00	0.93	1.00											
IG 43251 (37°C)	0.63	0.75	0.69	0.79	0.93	0.64	1.00	0.57	0.86	0.93	1.00	0.93	1.00	1.00										
IG 43251 (40°C)	0.71	0.63	0.56	0.77	0.92	0.75	0.86	0.67	0.85	0.79	0.86	0.92	0.86	0.86	1.00									
IG 138786 (25°C)	0.63	0.75	0.69	0.79	0.93	0.64	1.00	0.57	0.86	0.93	1.00	0.93	1.00	1.00	0.86	1.00								
IG 138786 (37°C)	0.63	0.75	0.69	0.79	0.93	0.64	1.00	0.57	0.86	0.93	1.00	0.93	1.00	1.00	0.86	1.00	1.00							
IG 138786 (40°C)	0.60	0.63	0.67	0.64	0.79	0.75	0.86	0.54	1.00	0.79	0.86	0.92	0.86	0.86	0.85	0.86	0.86	1.00						
IG 94631 (25°C)	0.67	0.69	0.63	0.85	1.00	0.69	0.93	0.62	0.79	0.86	0.93	0.86	0.93	0.93	0.92	0.93	0.93	0.79	1.00					
IG 94631 (37°C)	0.67	0.69	0.63	0.85	1.00	0.69	0.93	0.62	0.79	0.86	0.93	0.86	0.93	0.93	0.92	0.93	0.93	0.79	1.00	1.00				
IG 94631 (40°C)	0.60	0.63	0.67	0.77	0.92	0.75	0.86	0.54	0.85	0.79	0.86	0.79	0.86	0.86	0.85	0.86	0.86	0.85	0.92	0.92	1.00			
Gemiza 9 (25°C)	0.63	0.75	0.69	0.79	0.93	0.64	1.00	0.57	0.86	0.93	1.00	0.93	1.00	1.00	0.86	1.00	1.00	0.86	0.93	0.93	0.86	1.00		
Gemiza 9 (37°C)	0.63	0.75	0.69	0.79	0.93	0.64	1.00	0.57	0.86	0.93	1.00	0.93	1.00	1.00	0.86	1.00	1.00	0.86	0.93	0.93	0.86	1.00	1.00	
Gemiza 9 (40°C)	0.56	0.69	0.73	0.71	0.86	0.69	0.93	0.50	0.92	0.86	0.93	0.86	0.93	0.93	0.79	0.93	0.93	0.92	0.86	0.86	0.92	0.93	0.93	1.00

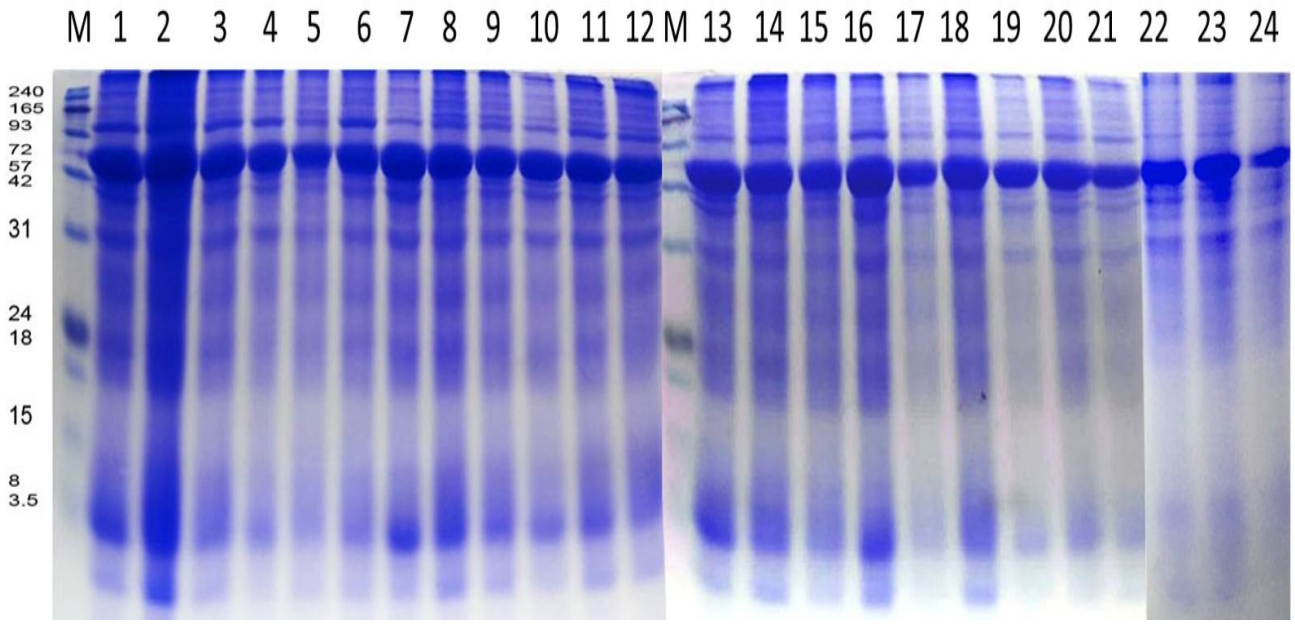
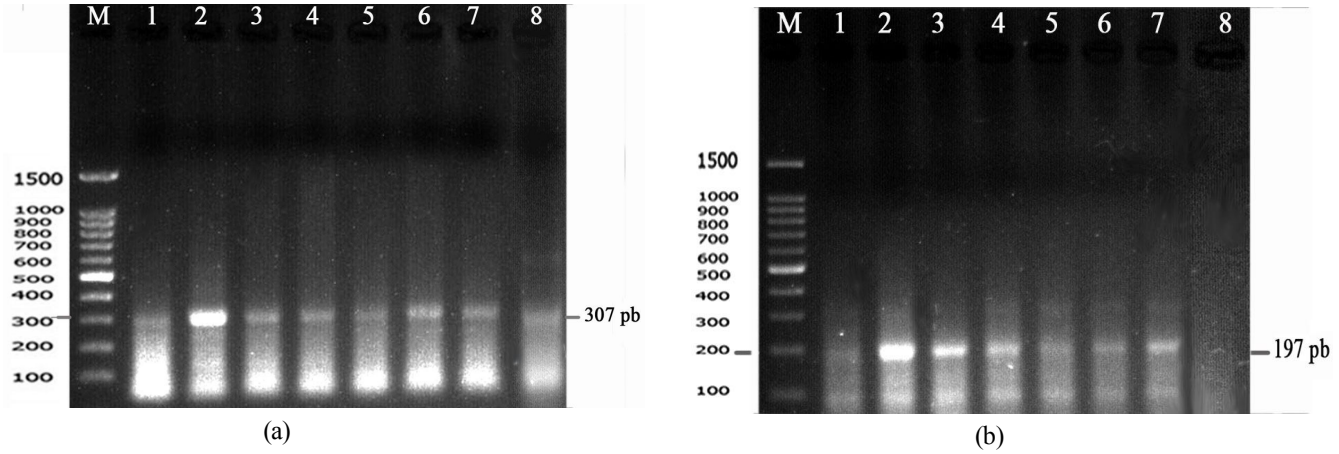


Fig. 1: SDS-PAGE banding patterns of total soluble proteins extracted from eight bread wheat genotypes subjected to different temperatures. Lane M: protein marker. Lane 1: Gemiza 10 (control, 25°C/24h); lane 2: Gemiza 10 (37°C/2h); lane 3: Gemiza 10 (40°C /2h); lane 4: IG 92811 (control, 25°C/24h); lane 5: 92811 (37°C/2h); lane 6: 92811 (40°C/2h); lane 7: IG 139010 (control, 25°C/24h); lane 8: IG 139010 (37°C/2h); lane 9: IG 139010 (40°C/2h); lane 10: IG 115798 (control, 25°C/24h); lane 11: IG115798 (37°C/2h); lane 12: IG 115798 (40°C/2h); lane 13: IG 43251 (control, 25°C/24h); lane 14: IG 43251 (37°C/2h); lane 15: IG 43251 (40°C/2h); lane 16: IG 138786 (control, 25°C/24h); lane 17: IG 138786 (37°C/2h); lane 18: IG 138786 (40°C/2h); lane 19: IG 94631 (control, 25°C/24h); lane 20: IG 94631 (37°C/2h); lane 21: IG 94631 (40°C/2h); lane 22: Gemiza 9 (control, 25°C/24h); lane 23: Gemiza 9 (37°C/2h) and lane 24: Gemiza 9 (40°C /2h).



(a) (b)

Fig. 2: PCR products of eight the heat-tolerant and heat-susceptible bread wheat genotypes using primer HSP16.9 (a) and SNP marker (b). Lane M: 100 bp DNA ladder.

Lane 1: Gemiza 10 (susceptible); lane 2: IG 92811 (susceptible); lane 3: IG 139010 (susceptible); lane 4: IG 115798 (susceptible); lane 5: IG 43251 (susceptible); lane 6: IG 138786 (susceptible); lane 7: IG 94631 (susceptible) and lane 8: Gemiza 9 (tolerant).

94631 when exposed to heat stress 37 and 40°C for 2 h Table 3. On the other hand, two polypeptides of 30 and 35 kDa scored in the heat tolerant Gemiza 9 variety and absent in the sensitive genotypes. On the contrary, two subunits of 18 and 24 kDa were found in all the sensitive genotypes and disappeared in the thermotolerant Gemiza 9 variety Table 3. In the current study, the composition of the soluble protein fractions of heat-tolerant and heat-susceptible eight bread wheat genotypes, grown under different temperature conditions resulted in an increase of some proteins may be involved in a defense mechanism or decrease due to heat stress. Depending on the proteomic analysis under the heat stress conditions, showed expression of three different kinds of HSPs (30, 35 and 100) were appeared to be upregulated in thermotolerant Gemiza 9, while downregulated in the

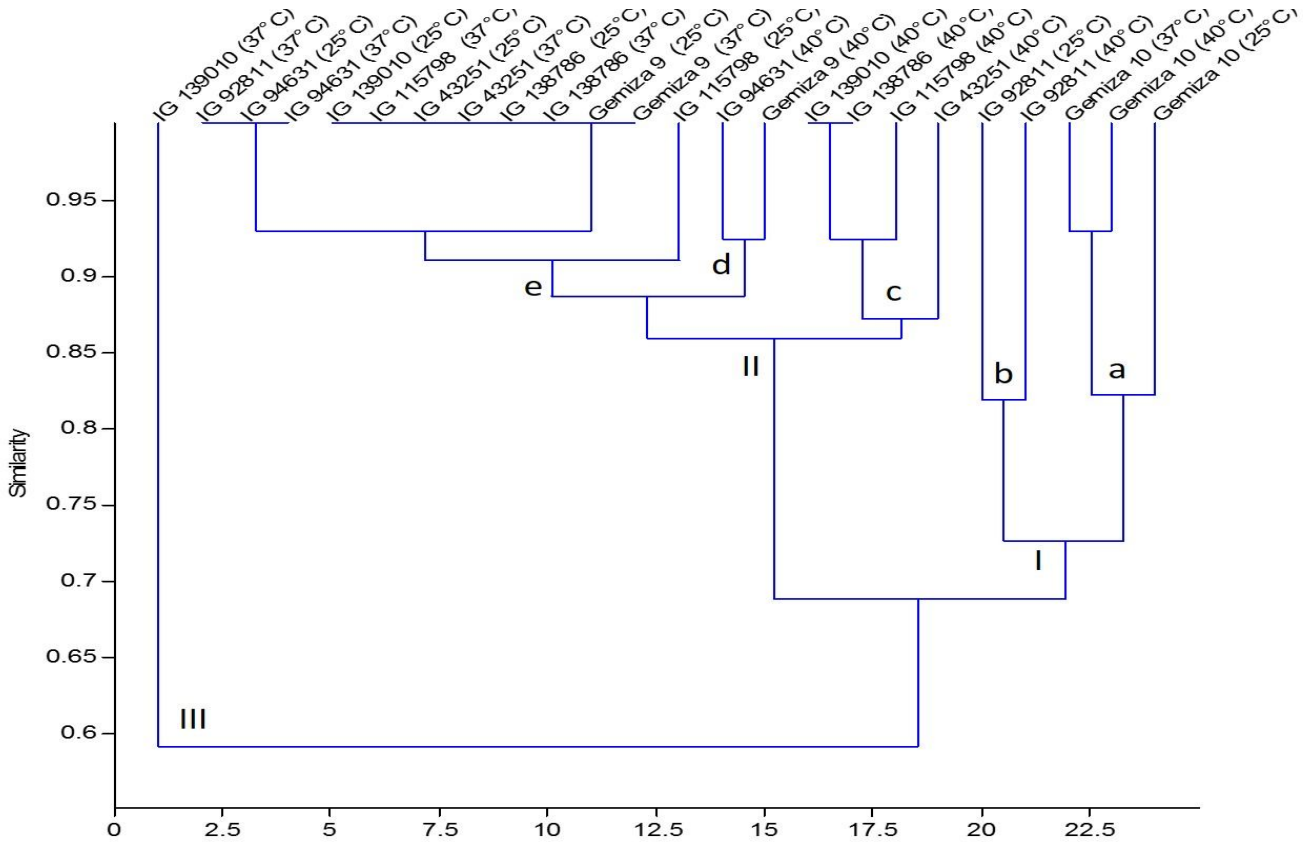


Fig. 3: UPGMA dendrogram of eight bread wheat genotypes subjected to different heat stress depend on Jaccard s similarity coefficient.

heat-susceptible genotypes. These results indicate that the expression of HSPs is genotype-specific. These results were confirmed by other authors Vierling and Nguyen, (1992); Treglia *et al*., (1999) recorded synthesis of HSPs in both bread and durum wheat under thermal stress conditions during the ripening stage by activation of the heat shock genes. El-Enany *et al*., (2013) detected synthesis of new proteins in *L. albus* and *L. luteus* plants exposed to temperature stress and their absence of the control plants due to activation of genes linked to heat stress. Also, De Rocher *et al*., (1991); Hsieh *et al*., (1992); Yildiz and Terzi, (2008); Mishra *et al*., (2017); Chen *et al*., (2018) found that synthesis of HSPs proteins in many plants during heat stress resulted from accumulating over 1.0% of total leaf protein under the heat conditions. Biamonti and Caceres, (2009) mentioned that all stresses stimulate gene expression and HSPs synthesis in the cells. Nevertheless, stressing agents led to the block of all metabolic operations, involving DNA replication, transcription, mRNA export and translation. In this finding, it was observed synthesis of HSP100 in some the heat-sensitive genotypes such as Gemiza 10, IG 92811, IG 139010, IG 138786, and IG 94631. These results were agreement with Krishnan *et al*., (1989) mentioned that the temperature-susceptible genotypes synthesized HSP earlier than the themotolerant ones. Yildiz and Terzi, (2008) observed an increase HSPs in heat-tolerant cultivars compared to heat-susceptible ones. In addition, HSPs showed at high-temperature treatment (37→50°C) are responsible for the heat-tolerant. In this study, it was found variations among genotypes which have the same genome for HSP synthesis, which is positively correlated with the photosynthesis process and tolerance genotype of the heat (Knight and Ackerly, 2001). However, O'Connell, (1994) found that there was no correlation between HSP aggregation and heat tolerance.

SNP Marker

A primer set HSP 16.9 scored one amplified fragment with molecular size 307 bp with all the heat-susceptible bread wheat genotypes e.g., Gemiza 10, IG 92811, IG 139010, IG 115798, IG 43251, IG 138786 and IG 94631 and the heat-tolerant Gemiza 9 variety (Fig. 2). On the other hand, The SNP marker was used to differentiate between heat-tolerant and heat-sensitive bread wheat genotypes. Seven the heat-sensitive genotypes e.g., Gemiza 10, IG 92811, IG 139010, IG 115798, IG 43251, IG 138786 and IG 94631 gave one band of 197 bp. On the contrary, heat-tolerant Gemiza 9 has not recorded

any amplicon (Fig. 2). These results were agreed with those obtained by Garg *et al*., (2012); Mahmud *et al*., (2018) identified SNP marker to screen the heat-tolerant and heat-sensitive genotypes of *Triticum aestivum* L., using HSP16.9 gene. DNA amplified fragment covering a partial nucleotide sequence of wheat *HSP16.9* was amplified from heat-sensitive genotype (K7903) and heat-tolerant genotype (RAJ4014) and then analyzed for the existence of the SNP marker. One SNP marker was scored between these genotypes and the amino acid sequence analysis, found that the base transition (A/G) located at 31 amino acid resulted in point mutation from Aspartic acid to Aspargine residue. Then, designed allele-specific primers depend on the SNP marker can use to select the heat-tolerant and heat-sensitive wheat genotypes. Efeoðlu, (2009) mentioned that heat shock proteins are not controlled by one gene, but by different genes group depending on the growth stages and tissue species.

Cluster Analysis

The genetic identity between eight bread wheat genotypes exposed to different heat felled into the range of 0.50 to 0.93 as shown in the UPGMA tree (Table 4 and Fig. 3). Three major clusters were obtained (I, II and III). The first cluster (I) included two groups: The first group (a): (similarity range of 0.79 to 0.93) involved into Gemiza 10 (the control at 25°C/24 h, 37 and 40°C for 2 h). The second group (b): (similarity range of 0.64 to 0.82) composed of IG 92811 subjected to 25°C/24 h and 40°C/2 h. The second cluster (II): The first group (c): (similarity range of 0.54 to 0.93) composed of IG 43251, IG 115798, IG138786 and IG 139010 stressed with the heat at 40°C/2 h. The second group (d): (similarity range of 0.50 to 0.93) contained on Gemiza 9 and IG 94631 stressed with temperature 40°C/2 h. The third group (e): (similarity range of 0.56 to 0.93) consisted of IG 115798 and IG 139010 (the control, 25°C/24 h), IG 115798 and IG 92811 (37°C/2 h), IG 94631, IG 43251, IG138786 and Gemiza 9 (25°C/24 h and 37°C/2 h). The Cluster (III): (similarity range of 0.50 to 0.73) included on IG 139010 exposed to the temperature stress 37°C for 2 h, this cluster referred to be most distinct but joined with the clusters I and II (Fig. 3). It should be taken into consideration in the breeding programs using as recurrent parents the heat-tolerant wheat genotype and susceptible high-quality one as donor parents.

Conclusion

An increase in the earth heat has far-reaching impact and any raise on the optimum temperature led to adverse effects on the growth of plants and crop productivity. Heat shock proteins (HSPs) play important role in relation to heat stress tolerant. Effect of temperature on the gene expression was investigated by SDS-PAGE. Our results showed the synthesis of three different types of HSPs with molecular weights 30, 35 and 100 kDa in the heat-tolerant Gemiza 9. Furthermore, SNP marker presented in *HSPs* differentiated between the heat-susceptible and the heat-tolerant bread wheat genotypes. However, these genotypes could be used in the future wheat breeding programs.

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