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A putative heat-responsive transcription factor (TaHD97) and its targets in wheat (*Triticum aestivum*) providing thermotolerance

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Transcription factors (TFs) are protein, which perform their role at transcriptional level by affecting the expression of various genes associated with metabolic pathways, growth and stress-associated genes (SAGs) at different developmental stages. Here, we identified 38 novel heat-responsive transcription factor genes from wheat *cv.* HD2985 by mining the *de novo* transcriptome data derived from heat shock (HS) treated wheat. Based on digital gene expression (DGE), a putative transcript (*TaHD97*) of ~1.1 kb was amplified and cloned from wheat *cv.* HD2985. The presence of heat stress transcription factor (HSF) DNA binding domain was observed in the amino acid sequence. Differential expression of *TaHD97* was observed in HD2985 (thermotolerant) and HD2329 (thermosensitive) under heat stress. Tissue specific expression analysis showed up-regulation of *TaHD97* in leaves, stem and endospermic tissues and down-regulation in root under HS. A positive correlation was established between the expression of *TaHD97* and its target gene (*HSP17* and *HSP90*) in wheat under heat stress. HSP17 transcripts were observed more in leaves of HD2985, as compared to HD2329. Thermotolerance related biochemical enzymes (SOD, CAT, GPX and TBARS) were observed higher in wheat *cv.* HD2985 showing maximum expression of *TaHD97* under heat stress. There is a need for the functional validation of the gene *TaHD97* in order to use it for the regulation of sHSP (catalytic chaperone) - a novel approach towards augmenting thermotolerance in wheat under heat stress.

Keywords: Heat stress, thermotolerance, wheat, *TaHD97*, antioxidant enzymes

Introduction

Heat stress induces the expression of a number of stress-associated genes (SAGs) and proteins (SAPs) associated with key metabolic processes[1]. Transcription factors (TFs) are key regulators of abiotic stress responses in plants. Recently, advanced genetic models revealed that heat shock TFs (HSFs) have both specific and cooperative function and are not limited to heat shock response only. HSFs also regulate the expression of novel target genes in response to distinct stimuli[2]. These TFs probably initiate the indirect late phase of responses by binding to *cis*-acting elements in the promoters of specific target genes[3]. These *cis*-acting elements consist of the palindromic nucleotide sequence (5'-AGAANNTTCT-3') that serves as recognition as well as binding site for HSFs[4]. The HSFs network in plants is controlled at the transcriptional level by cooperation of distinct HSF members and by interaction with chaperones.

Bioinformatics analyses identified 56 *Triticum aestivum* HSF (TaHSF) members in wheat. The transcript levels of A2 and A6 members of HSFs significantly increase under HS, suggesting their regulatory role. Based on structural analysis, *Arabidopsis* HSFs have been classified into three major classes - class A, B and C and 14 groups (A1-9, B1-4 and C1). Recently, a systematic analysis of the HSF family in rice using a phylogenomics-based approach has been reported[5]. Members of the *HSFA1* group in *Arabidopsis* collectively function as the master regulators of heat shock response[6]. The rice HSF family has representatives in most of the groups[7,8].

HSFs play an important role in stress response, during growth and development[9]. Kumar *et al*[1] (2013) reported increase in the expression of HSPs (*HSP70* and *HSP17*) with the increase in expression of HSFs (*HSF3* and *HSFA4a*) in wheat under HS of 42°C for 2 h.

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The HSP70 and HSP60 proteins are among the most highly conserved proteins in nature, consistent with a fundamental role in response to heat stress[10]. Different HSFs have also been shown to act synergistically. Thus, plants appear to have a distinct ability to control the expression of heat-responsive genes through the heat shock factors (HSFs).

Very limited information is available on HSF genes in wheat and their role in thermotolerance. In present investigation, we conducted genome-wide survey of HSFs in wheat and cloned a candidate HSF gene for a study of its correlation with the functional catalytic chaperones.

Materials and Methods

Plant Sample and Heat Stress Treatment

Two popular wheat cultivars HD2985 (thermo-tolerant) and HD2329 (thermo-sensitive) were used in the present study. Pre-soaked seeds (Bavistin @ 0.5 %) were sown in thirty six pots (eighteen for each variety) having equal quantity of perlite to farm yard manure (FYM) mixture. The plants were raised in a growth chamber with controlled conditions (22 ± 3°C, relative humidity of 75 %, and 8 h light with intensity of 250 μmol m^{-1} s^{-1} PAR) in the National Phytotron Facility, IARI, New Delhi. Plants (three pots from each variety) were exposed to heat stress (42°C for 2 h) at vegetative, pollination and grain-filling stages; a control in triplicate was kept at 22 ± 3°C for each stage and the samples were collected based on the Feekes scale[11]. The heat stress was given as mentioned in by Kumar *et al*[12] (2019). Samples (root, stem, flag leaf,) were collected in triplicates from both the cultivars and stored at −80°C for down-processing. For the validation of *TaHD97* TF gene, 15 days old seedlings of wheat *cvs*. HD2985 and HD2329 under the control (22 ± 3ºC) and HS-treated (42°C for 2 h) conditions were used in triplicates.

Total RNA Isolation and cDNA Synthesis

Total RNA was extracted from collected plant tissues, using Trizol method (Invitrogen, UK). To estimate the quantity and quality of isolated RNA, spectrophotometry was performed and the data was analysed using software N. D. (V.3.3.0). Complementary DNA (cDNA) synthesis was carried out by using Revert Aid H Minus First stand cDNA synthesis kit (Fermentas, Thermo Fisher Scientific, USA) as per the information given by the manufacturer.

De novo Transcriptome Analysis

An experiment was executed in our laboratory for the identification of novel heat-responsive genes and transcription factor in wheat using *de novo* assembly (NCBI BioProject Database: PRJNA171754). Differentially expressed genes were functionally annotated by different database as NCBI non-redundant, TriFLDB.

Amplification of Heat-Responsive Transcription Factor

Primers Designing, Amplification and Cloning

Domain based data mining of wheat showed the presence of several putative HSFs. The retrieved transcript sequences were subjected to ClustalW alignment and conserved region was used for the designing oligo's. Forward and reverse primers were designed using Genefisher2 primer designing software (http://bibiserv.techfak.uni-bielefeld.de/genefisher2/), and quality was checked using Oligo Analyser (Integrated DNA Technologies, USA) (Table 1). RT-PCR amplification followed by cloning, screening and Sanger's sequencing were carried out as mentioned in our earlier publication.

In-silico Characterization of Cloned TaHD97 Gene

The nucleotide sequence was used for the identification of open reading frame (ORF) using ORF finder (http:// www.ncbi.nlm. nih.gov/gorf/gorf.html). The sequence of amino acid was used for the identification of the conserved domain using the tool of NCBI conserved domain search (http://www.ncbi.nlm. nih.gov/Structure/cdd/wrpsb.cgi). Further, the identification of phosphorylation sites and kinase specific phosphorylation sites were predicted using the NetPhos and NetPhosK (http://www. cbs.dtu.dk/ services/NetPhosK/). The amino acid sequence was subjected to motif scan using the databases of PeroxiBase profiles (perox), HAMAP profiles (hamap), (http:// myhits.isb-sib.ch/cgi-bin/ motif_scan#pfam_ls:HSP70).

Expression Analysis Using Quantitative RT-PCR

TaHD97 (Acc. no. KP259293.1), *HSP90* (Acc. no. JN052206), and *HSP17* (Acc. no. JN572711) were subjected to qRT-PCR for the expression analysis using KAPA SYBR Green qPCR master mix on the CFX96 real-time PCR platform (BioRad, UK). β-actin gene (Acc. no. AF282634) was used as endogenous control for normalizing the C_t value. Relative expression of genes were quantified using the $2^{-\Delta\Delta Ct}$ method[13] (Table 2).

Estimation of Biochemical Parameters Associated with Thermotolerance

Superoxide Dismutase Activity Assay

Superoxide dismutase (SOD) activity was determined by measuring the inhibition in photoreduction of

Table 1 — Mining of *de novo* transcriptome data of wheat for the identification of heat-responsive transcription factor (TF) genes

Transcript IDs	Chromosomal localization	Notation
HD2329 (Thermosusceptible)		
HD2329_Stress_transcript_5774	dna:scaffold scaffold:IWGSP1:IWGSC_CSS_2AL_scaff_6370272:1:4961:1	heat shock protein binding
HD2329_Stress_transcript_7214	dna:scaffold scaffold:IWGSP1:IWGSC_CSS_6BL_scaff_4321631:1:11419:1	dnaj heat shock n-terminal domain-containing protein
HD2329_Stress_transcript_7756	dna:scaffold scaffold:IWGSP1:IWGSC_CSS_4BL_scaff_6990050:1:3750:1	heat stress HD2329_Stress_transcription factor a-1b
HD2329_Stress_transcript_8502	dna:scaffold scaffold:IWGSP1:IWGSC_CSS_2BL_scaff_8050210:1:14690:1	HD2329_Stress_transcription factor rf2b-like
HD2329_Stress_transcript_10450	dna:scaffold scaffold:IWGSP1:IWGSC_CSS_3B_scaff_10766520:1:24405:1	heat- and acid-stable phosphoprotein
HD2329_Stress_transcript_16627	dna:scaffold scaffold:IWGSP1:IWGSC_CSS_2BL_scaff_8037467:1:10372:1	heat shock factor protein 4
HD2329_Stress_transcript_17684	dna:scaffold scaffold:IWGSP1:IWGSC_CSS_5BL_scaff_10879329:1:23525:1	kda class iv heat shock protein
HD2329_Stress_transcript_18200	dna:scaffold scaffold:IWGSP1:IW GSC_CSS_5DL_scaff_4598213:1:13044:1	heat shock factor
HD2329_Stress_transcript_22436	dna:scaffold scaffold:IWGSP1:IWGSC_CSS_2DL_scaff_9898261:1:4179:1	heat shock factor protein 4
HD2329_Control_transcript_12353	dna:scaffold scaffold:IWGSP1:IWGSC_CSS_4DL_scaff_4DL_14373906:1:4475:1	heat stress HD2329_Control_transcription factor a-1b
HD2329_Control_transcript_16520	dna:scaffold scaffold:IWGSP1:IWGSC_CSS_3DL_scaff_6953547:1:18439:1	heat- and acid-stable phosphoprotein
HD2985 (Thermotolerant)		
HD2985_Stress_transcript_9649	dna:scaffold scaffold:IWGSP1:IWGSC_CSS_4DL_scaff_4DL_14373906:1:4475:1	heat stress HD2985_Stress_transcription factor a-1b
HD2985_Control_transcript_12551	dna:scaffold scaffold:IWGSP1:IWGSC_CSS_2AL_scaff_6370272:1:4961:1	heat shock protein binding
HD2985_Stress_transcript_11669	dna:scaffold scaffold:IWGSP1:IWGSC_CSS_3B_scaff_10766520:1:24405:1	heat- and acid-stable phosphoprotein
HD2985_Stress_transcript_11669	dna:scaffold scaffold:IWGSP1:IWGSC_CSS_3B_scaff_10766520:1:24405:1	heat- and acid-stable phosphoprotein
HD2985_Stress_transcript_11823	dna:scaffold scaffold:IWGSP1:IWGSC_CSS_3DL_scaff_6953547:1:18439:1	heat- and acid-stable phosphoprotein
HD2985_Stress_transcript_12081	dna:scaffold scaffold:IWGSP1:IWGSC_CSS_3AL_scaff_4289028:1:3379:1	heat- and acid-stable phosphoprotein
HD2985_Stress_transcript_12230	dna:scaffold scaffold:IWGSP1:IWGSC_CSS_2AL_scaff_6439261:1:7602:1	heat shock factor protein 4
HD2985_Stress_transcript_16474	dna:scaffold scaffold:IWGSP1:IWGSC_CSS_4AS_scaff_5934783:1:12253:1	hsf-type dna-binding domain containing expressed
HD2985_Stress_transcript_16876	dna:scaffold scaffold:IWGSP1:IWGSC_CSS_5DL_scaff_4598213:1:13044:1	heat shock factor protein 4
HD2985_Stress_transcript_461	dna:scaffold scaffold:IWGSP1:IWGSC_CSS_1BL_scaff_3852546:1:4535:1	dnaj heat shock family protein
HD2985_Stress_transcript_54816	dna:scaffold scaffold:IWGSP1:IWGSC_CSS_4AL_scaff_4AL_7173264:1:10888:1	alpha-crystallin domain of heat shock protein-containing protein
HD2985_Stress_transcript_575	dna:scaffold scaffold:IWGSP1:IWGSC_CSS_1BL_scaff_3852546:1:4535:1	dnaj heat shock family protein
HD2985_Stress_transcript_692	dna:scaffold scaffold:IWGSP1:IWGSC_CSS_1BL_scaff_3852546:1:4535:1	dnaj heat shock family protein
HD2985_Stress_transcript_7291	dna:scaffold scaffold:IWGSP1:IWGSC_CSS_1AL_scaff_3922902:1:6366:1	dnaj heat shock protein
HD2985_Stress_transcript_733	dna:scaffold scaffold:IWGSP1:IWGSC_CSS_1BL_scaff_3852546:1:4535:1	dnaj heat shock family protein
HD2985_Stress_transcript_7730	dna:scaffold scaffold:IWGSP1:IWGSC_CSS_4DL_scaff_4DL_14373906:1:4475:1	heat stress HD2985_Stress_transcription factor a-1b
HD2985_Stress_transcript_9315	dna:scaffold scaffold:IWGSP1:IWGSC_CSS_4DL_scaff_4DL_14373906:1:4475:1	heat stress HD2985_Stress_transcription factor a-1b
HD2985_Stress_transcript_9632	dna:scaffold scaffold:IWGSP1:IWGSC_CSS_4AS_scaff_5936883:1:19007:1	heat stress HD2985_Stress_transcription factor a-1b

Table 2 — List of primers used for the expression profiling of TaHD97 TF gene along with their target genes (*HSP17*, *HSP90)* in wheat under heat stress

Genes	Primer sequences (5'-3')	Tm (°C)
qTaHD97-F	CCAACTTCTCCAGCTTCGTC	60
qTaHD97-R	TGCTGCTGATGTCTTCATCC	59.9
qHSP17(F)	AGT GGGTAG CGAGTT TCCTGTGAT	65.2
qHSP17(R)	CAAACAACCACCAGTACG CACGAA	65.3
qHSP90(F)	TGATGATGGGTGGACTGCCAACAT	62.7
qHSP90(R)	TCTCGAAGAGCAGCATCACAAGGT	62.7
β-Act(F)	GCGGTCGAACAACTGGTATT	63.7
β-Act(R)	GGTCCAAACGAAGGATAGCA	63.8

*TaHD97 (Acc. no. KP259293.1), HSP90 (Acc. no. JN052206), HSP17 (Acc. no. JN572711), β-actin gene (Acc. no. AF282634).

nitroblue tetrazolium (NBT) by SOD enzyme[14]. One unit (U) of SOD activity was defined as the amount of enzyme causing 50% inhibition of photochemical reduction of NBT.

Guaiacol Peroxidase (GPX) Activity Assay

The activity of guaiacol peroxidase (GPX) was determined with slight modification[15]. The oxidation of guaiacol into tetraguaiacol was estimated by measuring the absorbance at 470 nm against the reagent blank, using extinction coefficient of 26.6 mM^{-1} cm^{-1}.

Catalase* (*CAT*) *Activity Assay

CAT activity was determined by following the consumption of hydrogen peroxide (H_2O_2) (extinction coefficient, 39.4 mM^{-1} cm^{-1}) at 240 nm over a 3 min interval[16]. The tissue extracts were used for the quantification of soluble protein content by using Bradford method[17].

Estimation of Lipid Peroxidation

The level of lipid peroxidation was measured in terms of thiobutyric acid reactive substance (TBARS) content[18]. The TBARS content was calculated according to its extinction coefficient (155 mM^{-1} cm^{-1}).

Results

Identification of Differentially Expressed Heat-Responsive Transcription Factors

Whole transcriptome analysis of control (22 ± 3ºC) and HS-treated (42ºC, 2 h) samples of wheat *cvs*. HD2985 and HD2329 were performed on Illumina HiSeq 2000 platform. We observed ~3000 differentially expressed genes under HS which were further mined for the identification of heat-responsive TF genes. We identified 38 putative unigenes having a domain sequence showing homology with HSF. Based on the digital gene expression (DGE), we selected transcript_16876 for cloning and characterization.

Cloning and *in silico* Characterization of Transcription Factor

RT-PCR amplification using transcript specific forward and reverse primers gave an amplicon of ~1.1 kb. The cloned gene was sequenced by Sanger's dideoxy method and the nucleotide sequence was submitted to National Centre for Biotechnology Information (NCBI) GenBank with Accession no. KP259293.1.

TaHD97 gene was 1108 nt long carrying a 5' UTR region of 34 bp and 3' UTR of 249 bp. The gene ORF encoded a protein with 275 aa. Physiochemical analysis of protein showed the molecular weight of 30 kDa, pI value of 8.4, and has 40 positively and 38 negatively charged amino acids in the sequence. The presence of single HSF_DNA-bind domain was observed in the sequence which confirms the nature of TaHD97 gene as transcription factor (Fig. 1a).

We observed serine (8 sites), threonine (5 sites), and tyrosine (1 site) in the sequence above threshold, which might facilitate the TaHD97 TF in its dual action of protein folding and chaperonic activity under elevated temperature (Fig. 1b). The hydropathy analysis of TaHD97 TF showed the presence of more hydrophobic amino acid in the sequence compared to hydrophilic (Fig. 1c). The presence of hydrophobic and hydrophilic amino acid decides the structure of the protein. TaHD97 TF protein is basically hydrophobic in nature. Three dimensional (3D) structure of the TaHD97 protein showed the presence of alpha-helixes and beta pleated-sheets along with helix bundle and beta barrel fold to form globular structure (Fig. 1d).

BLASTn homology search showed maximum similarities with the TF genes reported from *Triticum* species and *Hordeum vulgare*. Multiple ClustalW alignment showed the presence of heat shock binding domain in the sequence showing homology with the

HSF domain present in other heat-responsive TFs reported from closely related species. Motif scan analysis showed the presence of different motifs like phosphorylation (cAMP and cGMP-dependent protein kinase phosphorylation, casein kinase II phosphorylation), N-myristoylation site, N-glycosylation site, amidation site, protein kinase C phosphorylation, etc. (Fig. 1e). The TaHD97 protein was predicted to be chloroplastic in nature based on the presence of signal peptide sequence identified using ChloroP.

Validation of Heat-Responsive Nature of TaHD97 in Wheat Seedlings

The relative expression of *TaHD97* was high (1.77-fold) in HD2985 (thermotolerant), as compared

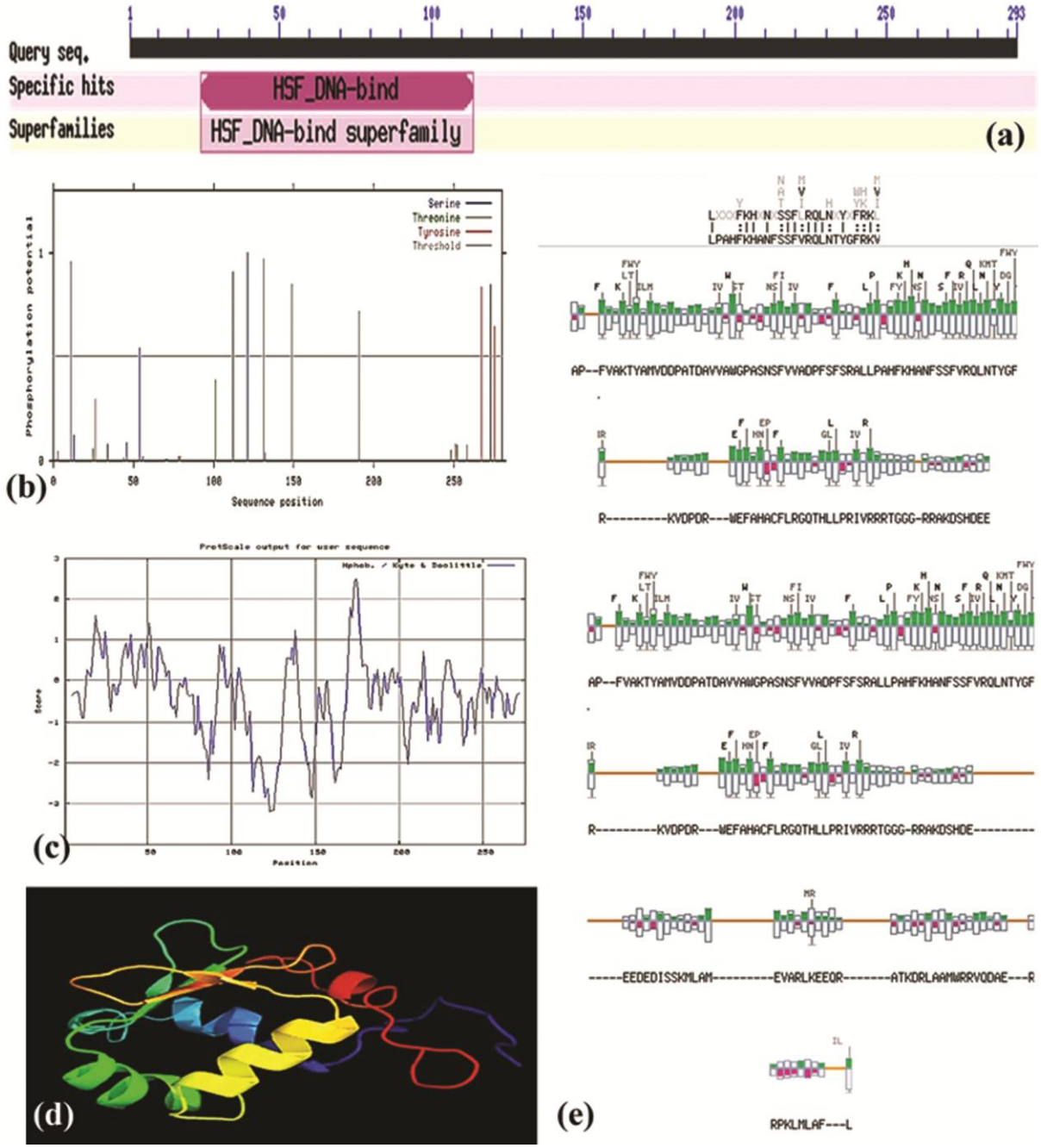

Fig. 1 — *In silico* characterization of *TaHD97* TF gene identified and cloned from wheat. (a) Conserved domain analysis, (b) phosphorylation site prediction, (c) Hydropathy analysis, (d) 3D structure of the TaHD97 protein, (e) Motif scan analysis. Bioinformatic tools available online were used for the characterization.

to HD2329 (thermosensitive) (Fig. 2a). Similarly, down-regulation of *TaHD97* gene was observed in the root tissues of both the cultivars in response to HS; down-regulation was more acute in HD2985 (0.20-fold), relative to that in HD2329 (Fig. 2b). The variation in the expression of *TaHD97* under HS in two cultivars confirms that the gene may be involved in the heat-response.

Tissue-Specific Expression Profiling of TaHD97 in Wheat During Grain-Filling Stage

Leaves of wheat *cvs.* HD2985 and HD2329 showed 1.88-fold and 1.62-fold increase in the expression of *TaHD97* under HS (42ºC, 2 h), as compared to control (22 ± 3ºC; Fig. 2c). Transcript profiling of *TaHD97* in stem showed 1.5 and 2.5-fold increase in expression in HS-treated HD2329 and HD2985,

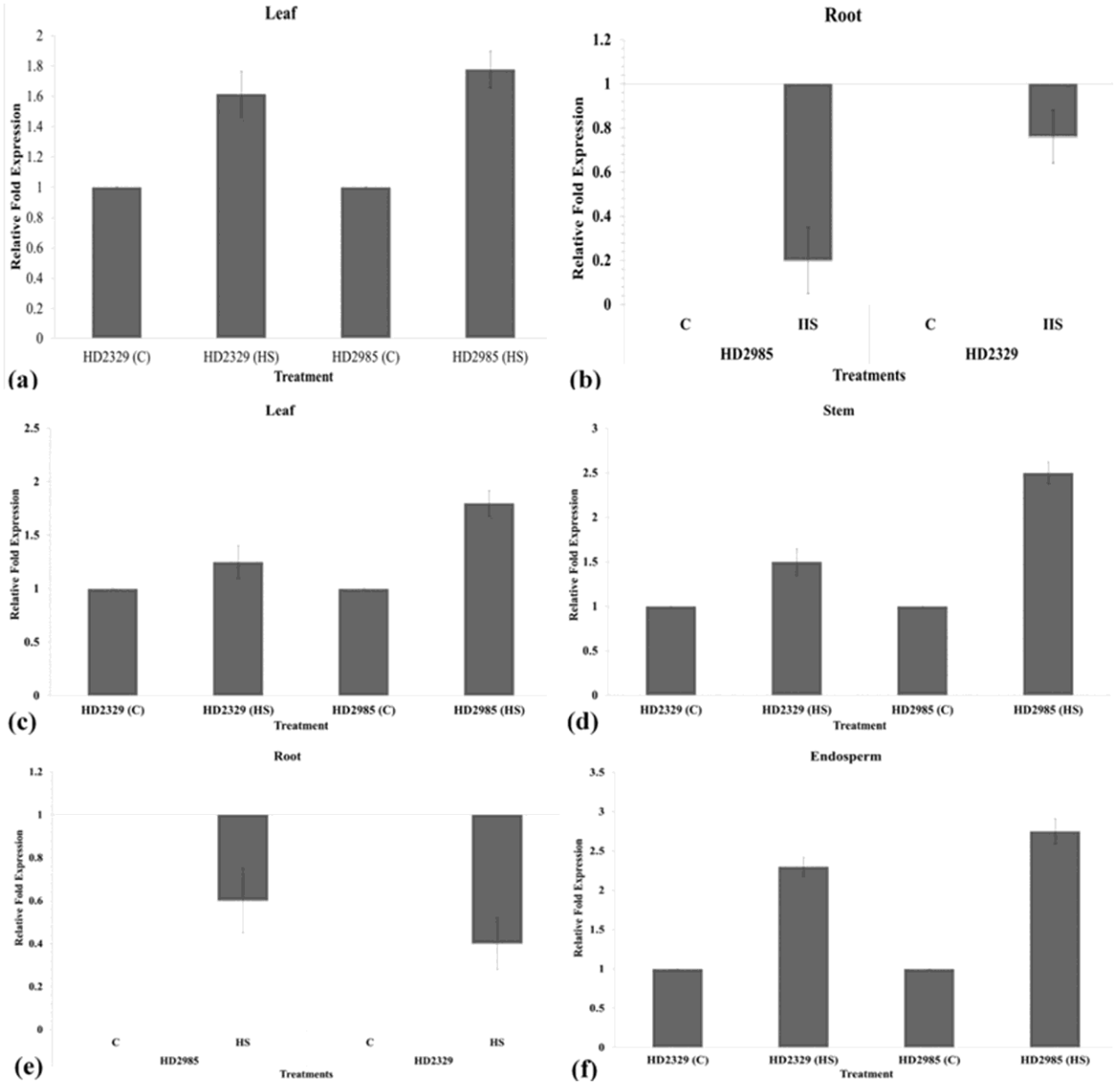

Fig. 2 — Validation of cloned putative *TaHD97* TF gene in wheat seedling under HS. (a) Expression analysis of *TaHD97* TF gene in leaf of wheat seedling, (b) Expression analysis of *TaHD97* in roots of wheat seedling under control and HS- treated conditions; Expression analysis of *TaHD97* TF gene in (c) leaf, (d) stem, (e) root, (f) endospermic tissues of wheat under control and HS-treated conditions during grain-filling stage; Two wheat *cvs*. HD2985 (thermotolerant) and HD2329 (thermosensitive) were used for the characterization; C- 22 ± 3ºC, HS - 42ºC, 2 h; β- actin gene was used as endogenous control for normalizing the Ct value; Pfaffl method was used for the relative fold calculation; Error bar denotes *SEM* (n = 3).

relative to control (Fig. 2d). Expression analysis of *TaHD97* showed down-regulation in roots and up-regulation in endospermic tissue under control and HS-treated condition (Fig. 2 & f).

Temporal Transcript Profiling of Target Genes Under Heat Stress

In silico analysis suggested that *HSP17* and *HSP90* are most probable targets of TaHD97 TF. Transcript profiling of *HSP17* during vegetative stage showed 22-fold and 14-fold increase in the expression in HS-treated (42ºC, 2 h) HD2985 and HD2329, relative to control (22 ± 3ºC) (Fig. 3a). Expression analysis during pollination and grain-filling showed significant increase in the expression of *HSP17* in both the cultivars under HS (Fig. 3b & c); maximum expression was observed during grain-filling stage in HD2985 (32-fold), whereas 18-fold was observed in case of HD2329.

The relative fold expression of *HSP90* at vegetative stage was 1.26-fold in HS-treated HD2329 and 1.45–fold in HS-treated HD2985, as compared to control (Fig. 3d). Transcript profiling during pollination stage showed 1.4-fold and 4.09-fold increase in the expression of *HSP90* in HS-treated HD2329 and HD2985, as compared to control (Fig. 3e). Similar pattern of expression was observed during grain-filling stage in both the cultivars (Fig. 3f).

TaHD97 TF Regulate Different Biochemical Parameters Associated with Thermotolerance

SOD activity was observed maximum in HS-treated wheat *cv*. HD2985 (10.4 U/mg protein) followed by HS-treated HD2329 (7.2 U/mg protein) during pollination stage (Fig. 4a). Similar pattern of CAT activity was observed during milky and mealy-ripe sub-stages of grain-filling. Similarly, CAT activity was

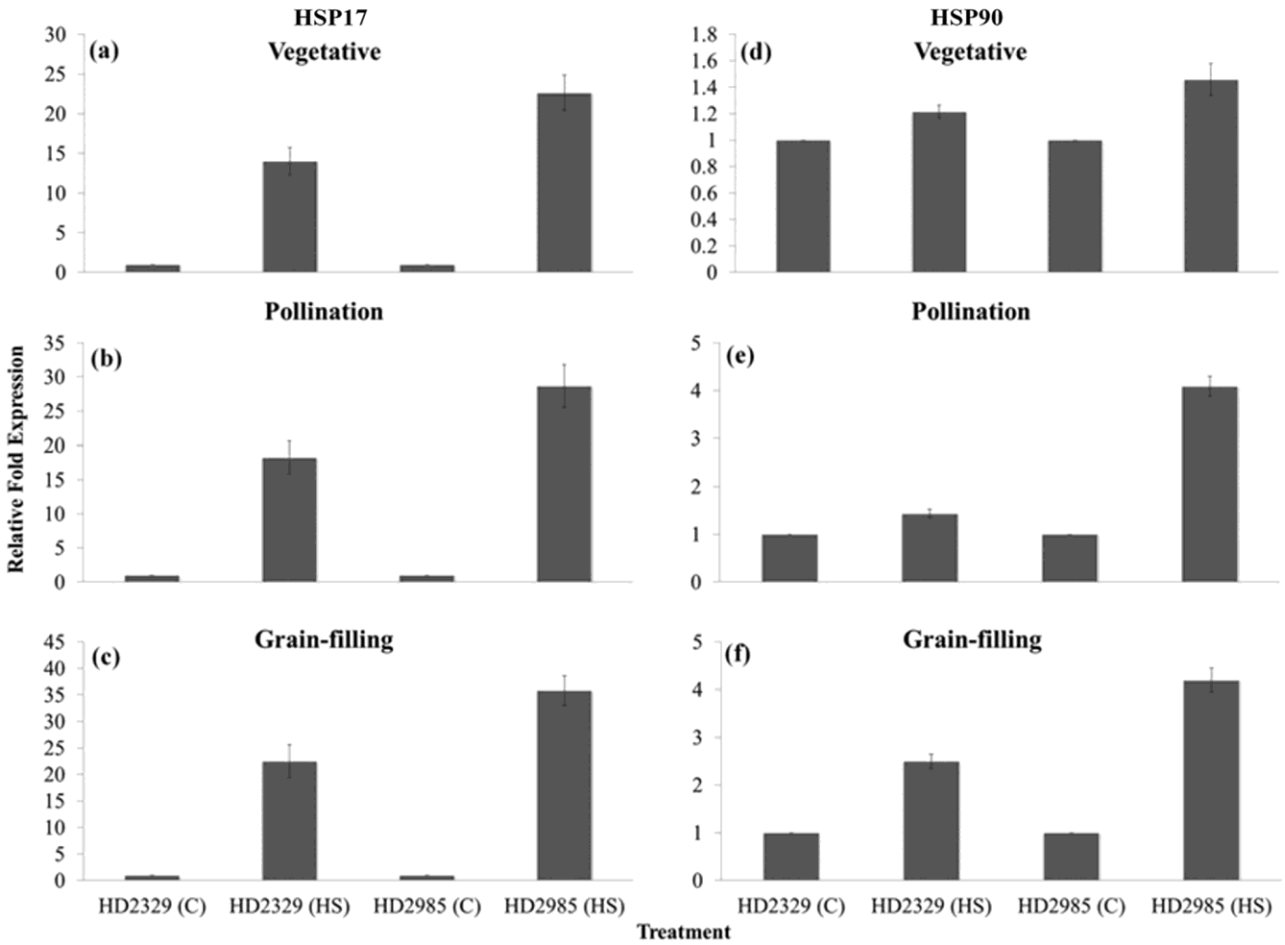

Fig. 3 — Transcript profiling of targets of *TaHD97* TF gene in wheat at different stages of growth and development. Expression analysis of *HSP17* gene during (a) vegetative, (b) pollination, and (c) grain-filling stages, expression analysis of HSP90 during (d) vegetative, (e) pollination, and (f) grain-filling stages under control and HS- treated conditions; Two wheat *cvs*. HD2985 (thermotolerant) and HD2329 (thermosensitive) were used for the characterization; C- 22 ± 3ºC, HS - 42ºC, 2 h; β- actin gene was used as endogenous control for normalizing the Ct value; Pfaffl method was used for the relative fold calculation; Error bar denotes *SEM* (n = 3).

observed maximum in HS-treated wheat *cv*. HD2985 during mealy-ripe stage (18.2 U/mg protein) followed by milky-ripe stage (17.8 U/mg protein), whereas minimum activity was observed in control sample of wheat *cv*. HD2329 during pollination stage (3.2 U/mg protein) (Fig. 4b).

Guaiacol peroxidase (GPX) activity assay showed maximum activity in HS-treated wheat *cv*. HD2985 during mealy-ripe (130 U/mg protein) followed by milky-ripe stages (122 U/mg protein) (Fig. 4c). The GPX activity was observed minimum in control sample of wheat *cv*. HD2329 during pollination stage (62.0 U/mg proteins). The lipid peroxidation of the collected samples was estimated in terms of TBARS. The TBARS was observed maximum in HS-treated wheat *cv*. HD2329 (58 mmol/g FW) during mealy-ripe followed by milky-ripe (50 mmol/g FW), whereas minimum TBARS were observed in control sample of wheat *cv*. HD2985 during pollination stage (26 mmol/g FW) (Fig. 4d).

Cluster Plot analysis

Cluster plot analysis of *TaHD97* TF gene and *HSP17* was carried out in order to know the correlation between the expression of TF and their target gene under HS at different stages of growth and development (Fig. 5). A positive correlation ($R^2 = 0.981$) was established between the expression of *TaHD97* TF gene and *HSP17* in wheat *cv*. HD2985 under HS. We observed significant increase in the

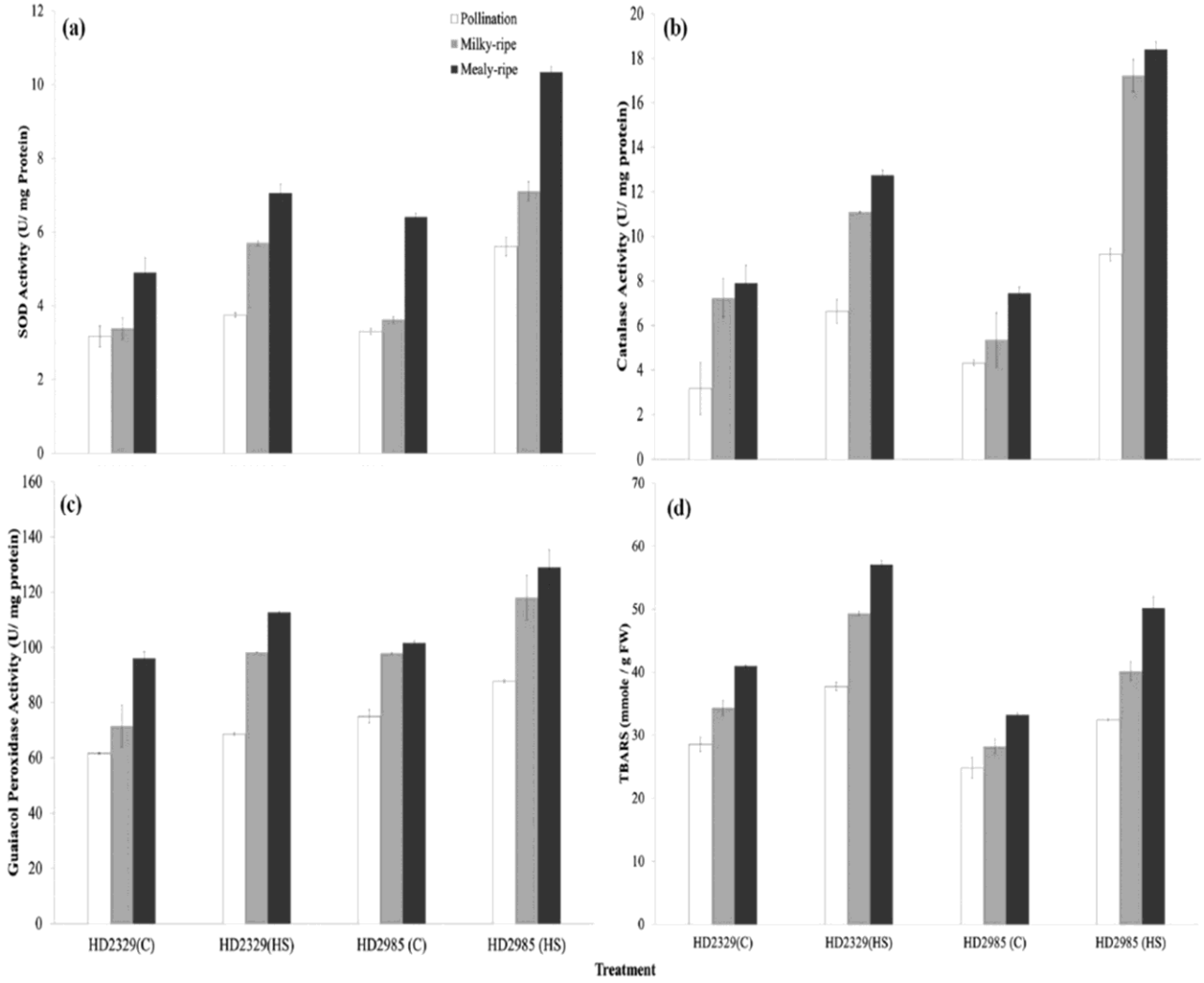

Fig. 4 — Estimation of biochemical parameters associated with thermotolerance in contrasting wheat *cvs*. under heat stress. (a) SOD activity assay, (b) Catalase activity assay, (c) GPX activity assay, (d) Lipid peroxidation in thermotolerant (HD2985 and thermosensitive (HD2329) *cvs*. of wheat at vegetative, pollination and grain-filling stage under control and HS-treated conditions; C- 22 ± 3ºC, HS - 42ºC, 2 h; Error bar denotes *SEM* (n = 3).

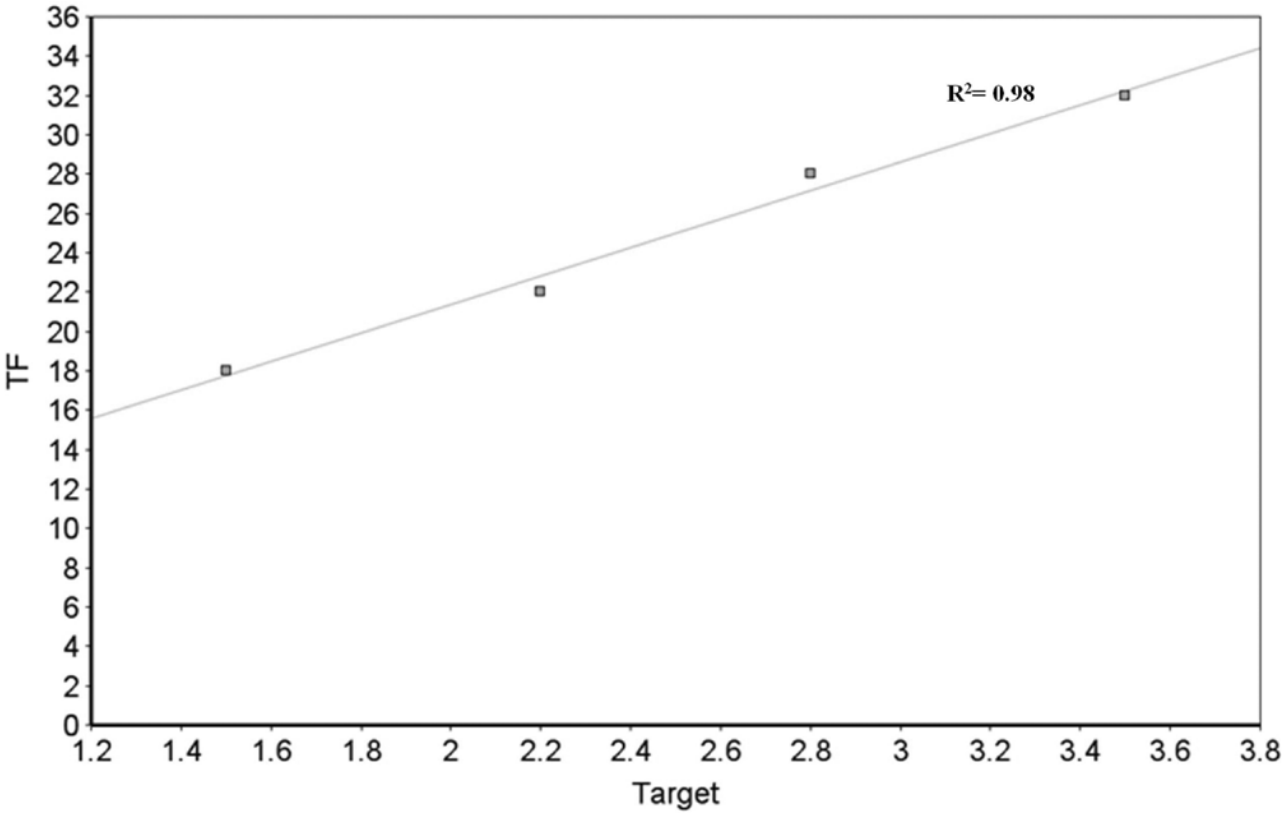

Fig. 5 — Cluster map analysis to analyse the correlation between the *TaHD97* TF and its target gene (HSP17) in wheat under heat stress.

expression of target gene (*HSP17*) with the increase in the expression of cloned *TaHD97* TF gene under HS in both the cultivars.

Discussion

In plants, the activity of genes of HSF family is quite diverse regulating the expression of SAGs involved in heat stress-tolerance[19]. HSF genes have been extensively analysed in the model plants like *Arabidopsis*, rice, etc. Transcription factors (TFs) represent the key regulators of plant growth and development, through regulation of the target genes expression at transcription level. HSFs bind to heat shock elements (HSE) in a sequence specific and reversible manner, leading to the activation of transcription[20]. HSFs regulating the expression of genes involved in stress response have been reported in *Arabidopsis*[21]. Here, we cloned a heat-responsive TF gene from wheat *cv*. HD2985. Different heat-responsive TFs have been reported in other plant species like *Arabidopsis* (21 TFs) and rice (25 TFs)[22]. Similarly, 25 full length HSFs genes were identified by Kilian *et al* (2012), from *Malus domestica* through conserved domain (CD) based homology search. Although, the wheat genome is hexaploid and the HSF family structure is expected to be more complicated than that in diploid plant species, there might be many more unknown or novel HSFs that need to be characterised. Our result showed that TaHD97 TF gene has conserved HSF-DNA binding domain, with hydrophobic amino acid residues - a characteristic feature of all the heat-responsive TF protein. HSFs are characterized by the presence of HSF–DNA binding domain, having central helix turn helix motif and a bipartite oligomerization domain, which is made up of hydrophobic heptad repeats.

We observed increase in the expression of *TaHD97* TF gene in thermotolerant (HD2985) and thermosensitive (HD2329) wheat *cvs*. under the HS during different stages of growth suggesting its important regulatory role in augmenting the thermotolerance. Tissue specific expression analysis showed down-regulation of *TaHD97* in roots of both the cultivars (tolerant and sensitive). The reason being the location of root which does not come in direct contact with the HS because of the presence of soil

loaded with moisture all-round the surface. Similar results were observed in case of stem and endosperm, where expression of *TaHD97* TF gene was more in HD2985 than in HD2329. Xue *et al* (2014) reported very high expression of *HSFA2* and *HSFA6* in wheat endosperm under HS.

Transcript profiling showed positive correlation between the expression of *HSP17* and *TaHD97* under HS. Our findings are in conformity with the observation of Kumar *et al* (2012). Similar pattern of expression was observed for the *HSP90* in wheat under HS. HSF3 has been reported to act as inducer of many heat inducible genes and involved in enhancing the thermotolerance of *Arabidopsis*[23]. Some of the functional HSEs present in the promoter region of *HSP17*, *HSP26*, *HSP70* and *HSP90* genes were characterised to be used as binding sites TaHSFA2b, which further functions as transcriptional activator for the above mentioned genes. Manipulation of *TaHD97* TF through genetic engineering approach will help to modulate the expression of HSPs-the key members of defence network pathway for the development of 'climate-smart' crop.

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