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Characterization of Heat-Responsive Transcription Factor under Heat Stress in Wheat Triticumaestivum L Using Bioinformatics Tools

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ABSTRACT:
Heat stress is one of the major problems in wheat growth and yield. It affects the growth and development of plant by causing denaturation of various enzymes, defunct pollen, and pseudo seed setting problem which ultimately affect the productivity. Wheat, being important food grain crop, has been neglected in terms of whole genome sequencing; very limited information is available on stress-associated genes and proteins. The mechanism of thermo tolerance has yet not been elucidated. Transcription factors are protein, which perform their role at transcriptional level by affecting the transcription process of SAGs. Here, we have characterized heat-responsive transcription factor by using bioinformatics tools. The nucleotide sequence of heat - responsive transcription factor was~ 1.1 kb.Clustal W analysis of heat-responsive transcription factor with other heat-responsive transcription factor sequence (reported from wheat) showed large variability in TFs nucleotide sequence. Based on phylogeny analysis, all HSF reported till date from wheat belongs to same origin as that of Triticumaestivum (Accession number KF208548.1).HSF gene showed different number of hits on wheat chromosomes. The image of pfam_Is: shows the presence of HSF_DNA- bind.HSF can be used as a suitable candidate gene for the breeding program or for manipulating the thermo tolerance of wheat using genetic engineering tool in order to develop climate-smart wheat crop.

INTRODUCTION:
In natural environment a plant faces number of biotic (pest infestation, disease, grazing, etc.) and abiotic stresses (high temperature, low temperature, drought, salinity etc.). Among abiotic stress, heat stress is one of the main stresses that severely affects the photosynthetic capacity, cellular and subcellular membrane components, and amount of protein in cell and also the activity of antioxidant enzyme; thus significantly limits crop yield(Kumar et al., 2012).Wheat (Triticumaestivum L.) is very sensitive to high temperature (Slafer and Satorre,1999) and trends in increasing growing season temperatures have already been reported for the major wheat-producing regions(Alexander et al., 2006; Hennessy et al., 2008)".Plant responses to high temperatures are mediated by both their inherent ability to survive and their ability to acquire tolerance to lethal temperatures. High temperature represents a significant constraint to the cultivation of important crops, such as wheat in large areas of the world (Kumar et al., 2013).Thermo tolerance capacity of genotypes depends on various genetic, physiological and biochemical parameters which are involved in modulating the defence mechanism under heat stress. It is the expression of stress-associated genes (SAGs) and stress-associated proteins (SAPs) which plays very important role in protecting the key enzymes from denaturation or aggregation under elevated temperatures (Kumar et al., 2013). Heat-responsive genes like transcription factors (TFs), heat-shock proteins (HSPs), signalling molecules, miRNAs etc. expression increases many fold in response to differential heat stress. An increase in the thermo tolerance capacity has been reported in different wheat germplasm with increase in the accumulation of the stress-associated proteins (Kumar et al., 2012) . Expression of heat shock proteins (HSPs) is the most studied molecular response under heat stress. HSPs saves protein from heat induced aggregation and thus during the recovery period, facilitate their

refolding (Maestri et al., 2002).HSFs alone can function in the maintenance of cellular homeostasis that include regulation of cell cycle, cell proliferation, redox homeostasis, cell death mechanisms etc. (Pirkkalaet al., 2001).HSFs are segmental transcription factors coded by a large gene. Families of eukaryotic transcription factor are helix turn helix proteins, zinc figure proteins, leucine zipper proteins, helix loop helix proteins. HSFs bind to heat shock elements (HSE) in a sequence definite and reversible method, leading to the activation of transcription (Morimoto et al., 1994). So, in the present study HSFs are characterised using bioinformatics tools.

MATERIALS AND METHODS:

Different bioinformatics tools were used for characterization of heat-responsive transcription factor. The chromosomal localization of cloned HSFa6e gene was analyzed by mapping the transcript Triticumaestivum chromosomes downloaded from Ensmbl Plants (http://plants.ensembl.org/Triticum_aestivum). THE nucleotide sequence of the cloned gene was analyzed for its restriction map by using NEB cutter from Biolab. The localization of the protein was predicted using CELLO v.2.5 sub Cellular Localization predictor software. Motif scan-my hit software was used for identification of motif scan in the heat responsive transcription factor sequence. Open Reading Frame of the HSF gene was characterized using ORF Finder (http://www.ncbi.nlm.nih.gov/projects/gorf/) from National Centre for Biotechnology Information (NCBI). Clustal Alignment tool (http://www.ebi.ac.uk/Tools/msa/clustalw2/). Neighbor joining trees were generated using clustalX with the default values of multiple alignment parameters.

RESULTS AND DISCUSSION:

Sanger's di-deoxy method was used for sequencing of heat-responsive transcription factor which shows nucleotide sequence of ~ 1.1 kb (Fig.1). Number of amino acids coded by transcription factor gene was found to be 368 (Fig. 2).However, recent studies have uncovered structures of full length HSF from bacteria (Shiauet al., 2006), Composition of protein was determined by Expasy ProtParam tool was used to determine the molecular weight-88700.6, pI value of the protein was found to be 5.03 (Table-1). Identified HSF gene showed different number of hits on wheat chromosomes (Fig. 3). A motif scan showed the presence of different active sites like phosphorylation (cAMP and cGMP- dependent protein kinase phosphorylation, Casein kinase II phosphorylation), N-myristoylation, N-glycosylation site, Amidation site, Protein kinase C phosphorylation. The image of pfam_Is: shows the presence of HSF_DNA- bind (Fig. 4). Various internal restriction sites were identified with in the HSF sequence (Fig. 5). Clustal W analysis of heat-responsive transcription factor with other heat-responsive transcription factor sequence (reported from wheat) showed large variability in TFs nucleotide sequence (Fig. 6). Based on phylogeny analysis, all HSF reported till date from wheat belongs to same origin as that of Triticumaestivum (Accession number KF208548.1) (Fig. 7).Hsf proteins have been proposed to play a key role in regulating the expression of HSP genes, which have a significant impact on thermo tolerance (Kotaket al., 2007 ; Scharfet al., 2012)

ATGGACGCGATGCCGCCGGAGGGCATTGTGAAGGAGGAGGAGGAGGTGC
TGCTGCACGAGGAGCGCACGCACCCGGCGGCCGCGCCGCCGCAGCA
GCGGCAGGACGGGGCGCTGCCGCGGCCGATGGAGGGGCTGCACGAG
GCCGGGCCGCCGCCGTTCCTCACCAAGACGTACGACCTGGTGGAGGA
CCCGGCCACGGACCAGGTCGTGTCCTGGGGCCGCGCCGGCAACACCT
TCGTCGTCTGGGACCCGCACGTCTTCGCCGAGGCCCTGCTCCCGCGC
CTCTTCAAGCACTCCAACTTCTCCAGCTTCGTCCGCCAGCTCAACACC
TATGGATTCAGAAAGGTTGATCCTGACAGATGGGAGTTTGCAAATGAA
GGTTTCCTTAGGGGTCAGAGGCATCTTCTCAAGACGATCAAGAGAAG
GAAGCCGCCATCCAATGCGCCCTCATCACAGCAGCAGGCCCTCACAT
CTTGCCTGGAGGTTGGTGAGTTTGGATTTGAGGAAGAGATTGACAGG
CTCAAGCGCGACAAGAACCTCTTGATCACAGAGGTAGTGAAGCTAAG
GCAGGAGCAGCAAGCTACTAAGGATAATGTGCAAGCCATGGAAGGCA
GGCTACGAGCTGCTGAACAGAGACAGGCCCAGATGATGGGGTTCTTG
GCAAGAGCAATGCGTAACCCACACTTCTTCCAGCAATTAGTCCAGAA
GCAAGATAAGAGGAAGGAGCTTGAAGATGCCATCTCGAAGAAAAGA
AGGCGGCCTATAGACAATGCTCCATTTTATGGTTCGGGGGCAACAACA
AGTCAGAGCGAGCAACTTGATTCACAGTTCCTGTTTGATTCTGGTGTC
CTCAGTGAACCTGGAATGAACGGGATGGAGAATTTAGCGCAGAACAT
TCAGGAGCTTGGGCAGGGCAAAACAGACGAGGAGAAGAAGGATGAA
GCTAATGGGCAGCTGGACATCAACAGCGATTTCTGGGCAGAGCTATTT
TCTGATGATTTTGGAGACGAAGACGGGTCTGGGCTGTCAGAGTTGGA
GGGAAGGAGACCTGAAGATATCGATGAACTGGGTCAGCAGTTGGGGT
ATCTGAGTTCTACTAGCCCGCAGTAG

Figure 1- Nucleotide sequence of HSF; Sanger's di-deoxy method was used for sequencing of nucleotide

Number of amino acids	368/1107
Molecular weight	88700.6
Theoretical pI	5.03

Table 1-Compostion of amino acid of heat-responsive transcription factor

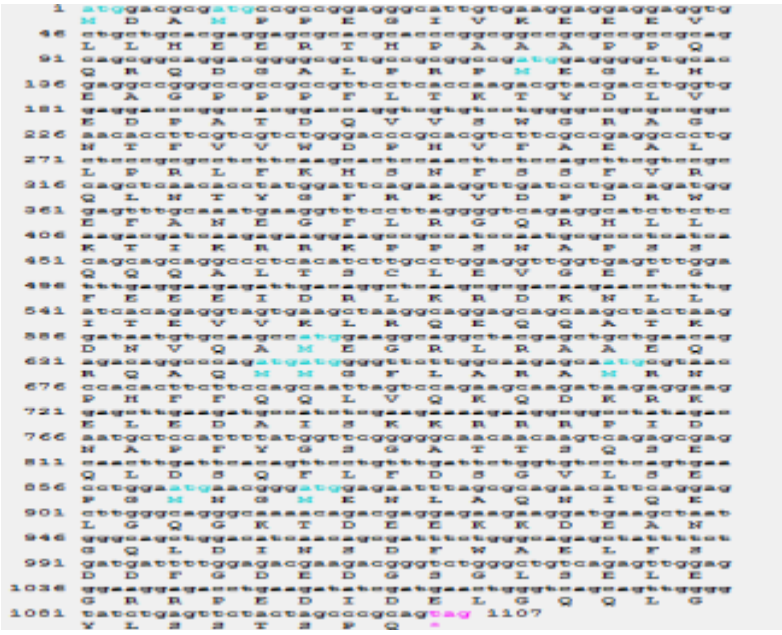

Figure 2-Open reading frame of HSF; ORF finder tool from NCBI was used for identifying the protein coding region present in the sequence

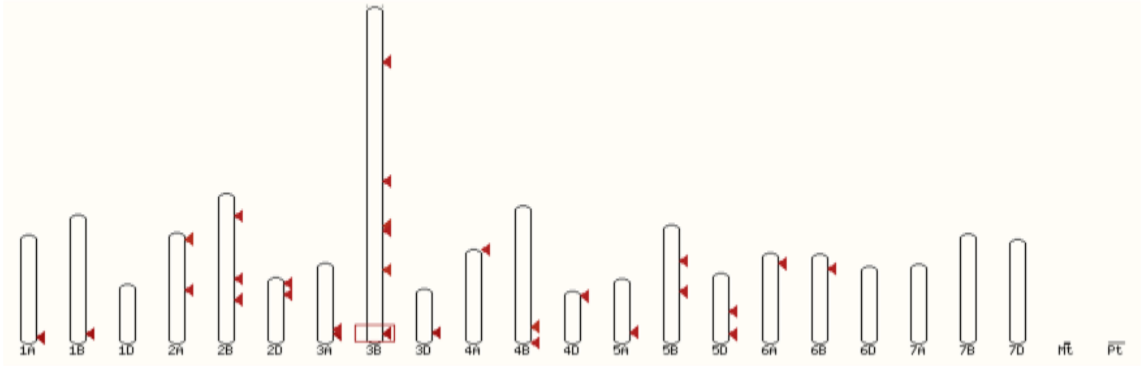

Figure 3- Chromosomal localisation of HSF;by mapping on Triticumaestivum chromosomes downloaded from Ensmbl Plants

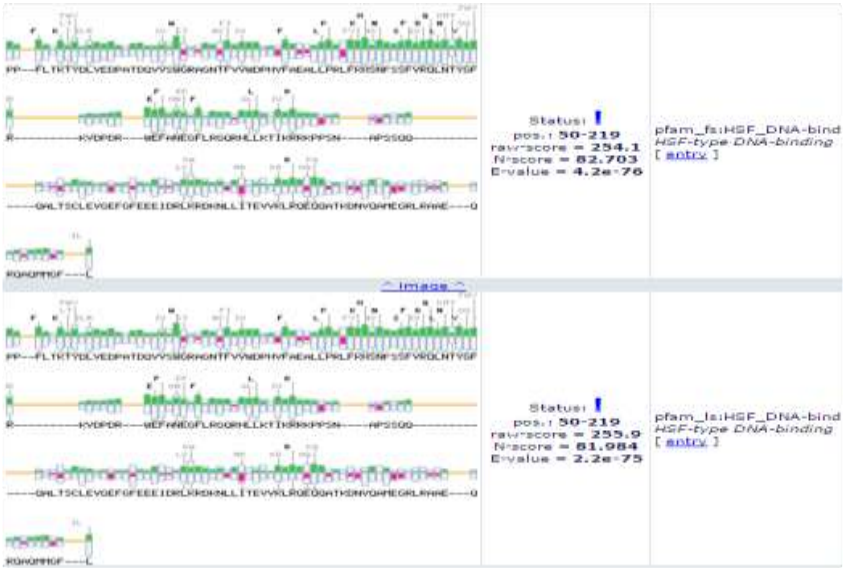

Figure 4- Motif scan search for the HSF; Motif scan-my hit software was used for identification of motif sacan; pfam image shows the presence of HSF_DNA-bind in the protein

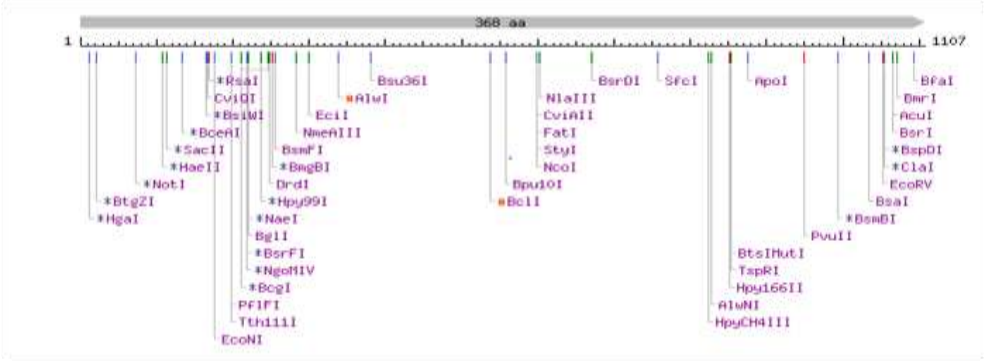

Figure 5- Restriction digestion map of HSF; NEB cutter from Biolabs was used for identification of enzymes which has internal site within the gene

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HSFa2c      CCGATGGAGGGGCTGCACGAGGCCGGGCCGCCGCCGTTCCTCACCAAGACGTACGACCTG
Niash       CCGATGGAGGGGCTGCACGAGGCCGGGCCGCCGCCGTTCCTCACCAAGACGTACGACCTG
HSF         CCGATGGAGGGGCTGCACGAGGCCGGGCCGCCGCCGTTCCTCACCAAGACGTACGACCTG
HSFa6e      CCGATGGAGGGGCTGCACGAGGCCGGGCCGCCGCCGTTTCTCACCAAGACGTACGACCTG
            ************************************ ********************

HSFa2c      GTGGAGGACCCGGCCACGGACCAGGTCGTGTCCTGGAGCCGCGCCGGCAACAGCTTCGTC
Niash       GTGGAGGACCCGGCCACGGACCAGGTCGTGTCCTGGAGCCGCGCCGGCAACAGCTTCGTC
HSF         GTGGAGGACCCGGCCACGGACCAGGTCGTGTCCTGGGGCCGCGCCGGCAACACCTTCGTC
HSFa6e      GTGGAGGACCCGGCCACGGACCAGGTCGTGTCCTGGGGCCGCGCCGGCAACACCTTCGTC
            ************************************ *************** *******

HSFa2c      GTCTGGGACCCGCACGTCTTCGCCGACGCGCTGCTCCCGCGCCTCTTCAAGCACTCCAAC
Niash       GTCTGGGACCCGCACGTCTTCGCCGACGCGCTGCTCCCGCGCCTCTTCAAGCACTCCAAC
HSF         GTCTGGGACCCGCACGTCTTCGCCGAGGCCCTGCTCCCGCGCCTCTTCAAGCACTCCAAC
HSFa6e      GTCTGGGACCCGCACGTCTTCGCCGAGGCCCTGCTCCCGCGCCTCTTCAAGCACTCCAAC
            ************************** ** ******************************

HSFa2c      TTCTCCAGCTTCGTCCGGCAGCTCAACACCTATGGATTCAGAAAGGTTGATCCTGACAGA
Niash       TTCTCCAGCTTCGTCCGGCAGCTCAACACCTATGGATTCAGAAAGGTTGATCCTGACAGA
HSF         TTCTCCAGCTTCGTCCGCCAGCTCAACACCTATGGATTCAGAAAGGTTGATCCTGACAGA
HSFa6e      TTCTCCAGCTTCGTCCGCCAGCTCAACACCTATGGATTCAGAAAGGTTGATCCTGACAGA
            ***************** ******************************************

HSFa2c      TGGGAGTTTGCAAATGAAGGTTTCCTTAGGGGTCAAAGGCATCTTCTGAAGATGATCAAG
Niash       TGGGAGTTTGCAAATGAAGGTTTCCTTAGGGGTCAAAGGCATCTTCTGAAGATGATCAAG
HSF         TGGGAGTTTGCAAATGAAGGTTTCCTTAGGGGTCAGAGGCATCTTCTCAAGACGATCAAG
HSFa6e      TGGGAGTTTGCAAATGAAGGTTTCCTTAGGGGTCAGAGGCATCTTCTCAAGATGATCAAG
            ************************************ *********** **** *******
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Figure 6- Clustal W alignment of the identified Heat-responsive transcription factorwith that of other HSF genes reported from plant

Figure 7- Phylogeny analysis of heat responsive transcription factor gene with that of other HSF genes reported from wheat

CONCLUSION:

Heat-responsive modulates the thermo tolerance capacity of plant by protecting the denaturation or aggregation of heat shock proteins under elevated temperatures. In wheat, the information on heat-responsive genes and proteins is limited and the mechanism associated with the thermo tolerance has not been fully characterized. Here, we have identified and characterized heat shock transcription factor by using bioinformatics tools which can be further utilized to enhance the thermo tolerance in wheat and to develop a thermo tolerant smart wheat crop..

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REFERENCES:

1. Kumar, S., Singh, R., Grover, M., & Singh, A. K. (2012). Terminal heat—an emerging problem for wheat production. Biotechnol Today, 2(2), 7-9.
2. Satorre, E. H., &Slafer, G. A. (1999).Wheat: Ecology and physiology of yield determination. CRC Press.
3. Alexander, L. V., Zhang, X., Peterson, T. C., Caesar, J., Gleason, B., Klein Tank, A. M. G., &Tagipour, A. (2006). Global observed changes in daily climate extremes of temperature and precipitation. Journal of Geophysical Research: Atmospheres, 111(D5).
4. Hennessy, K., Fawcett, R., Kirono, D., Mpelasoka, F., Jones, D., Bathols, J.,Whetton, P., Stafford Smith, M., Howden, M., Mitchell, C., &Plummer, N.(2008). An assessment of the impact of climate change on the nature and frequency of exceptional climatic events.CSIRO and Bureau of Me-teorology, http://www.daff.gov.au/data/assets/pdffile/0007/721285.
5. Kumar, R. R., Sharma, S. K., Goswami, S., Singh, G. P., Singh, R., Singh, K., &Rai, R. D. (2013).Characterization of differentially expressed stress-associated proteins in starch granule development under heat stress in wheat (Triticumaestivum L.). Indian J BiochemBiophys, 50(2), 126-138.
6. Maestri, E., Klueva, N., Perrotta, C., Gulli, M., Nguyen, H. T., &Marmiroli, N. (2002). Molecular genetics of heat tolerance and heat shock proteins in cereals. Plant Molecular Biology, 48(5-6), 667-681.
7. Morimoto, R. I. (1998). Regulation of the heat shock transcriptional response: cross talk between a family of heat shock factors, molecular chaperones, and negative regulators. Genes & development, 12(24), 3788-3796.
8. Pirkkala, L., Nykänen, P., &Sistonen, L. E. A. (2001). Roles of the heat shock transcription factors in regulation of the heat shock response and beyond. The FASEB Journal, 15(7), 1118-1131
9. Ali, M.M., Roe, S.M., Vaughan, C.K., Meyer, P., Panaretou, B., Piper, P.W., Prodromou, C., &Pearl, L.H. (2006). Crystal structure ofan HSF-nucleotide-p23/Sba1 closed chaperone. Nature 440,1013-1017
10. Scharf, K. D., Berberich, T., Ebersberger, I., and Nover, L. (2012). The plant heat stress transcription factor (Hsf) family: structure, function and evolution.Biochimica et BiophysicaActa (BBA)-Gene Regulatory Mechanisms, 1819(2), 104-119.
11. Kotak, S., Larkindale, J., Lee, U., von Koskull-Doring, P., Vierling, E., &Scharf, K. D. (2007a). Complexity of the heat stress response in plants. Current opinion in plant biology, 10(3), 310-316.