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In-Silico Characterization of Heat Responsive Transcription Factor under High Temperature Stress in Wheat (Triticumaestivum L.)

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ABSTRACT:
Transcription factor plays an important role in modulating the thermotoleranceby modulating the expression of heat shock protein under heat stress. In the present study *In-silicocharacterization* of cloned HSFa6e shows the presence of HSF_DNA- bind domain which starts at residue 57 and end at 150 consisting of total 97 amino acids. BlastN search showed maximum homology with clone NIASHv2013N08 (acc. no. AK363263.1) reported from *Hordeumvulgare*. ORF finder showed presence of 368 amino acids. There is a need to exploit the expression profiling of heat-responsive transcription factor in order to enhance the thermo tolerance capacity of wheat.

KEYWORDS: Wheat, Heat stress, Transcription factor, *In-silico*

INTRODUCTION:

Heat shock factors (HSFs) are transcription factors are found to be expressed in all eukaryotic organisms. In plants, HSF proteins form a large family, with 21 members in *Arabidopsis thaliana*, 25 members in rice (*Oryza sativa L*), and at least 56 members in wheat (Scharf et al., 2012; Xue et al., 2014). Transcription factors are the protein which interacts with specific DNA sequence, regulating the rate of transcription (Latchman 1997). Transcription factor include one or more DNA Binding Domain (DBDs) which interact with specific DNA sequence which are closely present to the genes that they regulate (Ptashne 1997). HSFs also help in regulation of novel targets genes in response to distinct stimuli. Heat stress causes the enhancement in the expression of numerous heat stress genes. Under normal condition HSPs are present in inactive form but as soon as plant undergoes stress these HSFs gets activated by oligomerisation and bind to heat shock elements which are present in the promoter of heat stress responsive genes. HSFs under normal state form cytoplasmic complex with HSP90/HSP70 chaperon (Hahn et al., 2011). Under heat stress conditions HSFs are released from chaperon complex and binds to HSE of target gene. HSEs are mainly characterized by multiple inverted repeats of AGAAn sequences and at least three HSE motifs are required for efficient HSF oligomer binding in eukaryotic organism (Xiao et al., 1991). HSF proteins in plants are divided into three classes: HSFA, HSFB, and HSFC (Scharf et al., 2012). Several HSFA subclasses (A1, A2, A3, A4, and A9) have been shown to serve as transcriptional activators for HSP genes (Mishra et al., 2002; Xue etal., 2014), whereas HSFA5 acts as a specific repressor for HsfA4 (Baniwal et al., 2007). In particular, constitutively expressed subclassHSFA1 genes serve as master regulators for triggering heat response (Mishra et al., 2002; Liu et al., 2011;Liu and Charng, 2012). In wheat, most HSFA genes are expressed at moderately high levels under normal conditions (Xue et al., 2014). In the present study in-silico characterization is done to identify the structure heat-responsive transcription factor in wheat.

MATERIALS AND METHOD:

The nucleotide sequence was characterized for homology search using the BLASTn tool of the National Center for Biotechnology Information (NCBI; http://blast.ncbi.nlm. nih.gov/). The nucleotide sequence was

used for the identification of open reading frame using ORF finder (http:// www.ncbi.nlm.nih.gov/gorf/gorf.html). The amino acid sequence was derived using the Expasy translation tool (http://web.expasy.org/translate/). 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/).

RESULTS AND DISCUSSION:

BlastN search showed maximum homology with HSFa6e (accession no.KF208548.1) reported in *Triticumaestivum* followed by HSFa2c (accession no.HM446025.1) reported in *Hordeumvulgare*(Table 1).Number of amino acids coded by this transcription factor gene were found to be 368 (Fig. 1).Conserved domain search tool showed the presence of single HSF_DNA- bind domain which starts at residue 57 and end at 150 consisting of total 97 amino acid (Fig. 2). Further, the identification of phosphorylation sites and kinase specific phosphorylation sites were predicted using the NetPhosK. The amino acid sequence was used for the identification of the phosphorylation site and we observed serine (8 sites) threonine (3 sites) and tyrosine (1 site) above threshold (Fig. 3), which might help the HSF in its dual action of protein folding and chaperonic activity. Hydropathy analysis was used to find position of helices in the primary structure of the cloned gene protein, and results showed that at 170 to 200 positions there is strong tendency to form a helix (Fig. 4). The cloned gene was then subjected for its transmembrane topology prediction and we found that between 170 to 200 positions in the amino acid sequence transmembrane tendency is maximum (Fig. 5).The protein was predicted to be chloroplastic in nature. 3D structure of the cloned transcription factor was determined using Phyre[2] software (Fig. 6). Structure of the transcription factor showed that this gene has structure similar to heat shock factor DNA binding domain.

TABLE 1: NCBI BLASTN SEQUENCE HOMOLOGY:

Description	Max score	Total Score	Query cover	E value	Ident	Accession
Triticumaestivum heat shock factor A6e (HsfA6e) mRNA, partial cds	1735	1735	89%	0.0	98%	KF208548.1
Hordeumvulgare subsp. vulgare heat shock factor A2c (HsfA2c) mRNA, complete cds	1615	1615	99%	0.0	93%	HM446025.1
Hordeumvulgare subsp. vulgare mRNA for predicted protein, complete cds, clone: NIASHv2013N08	1615	1615	99%	0.0	93%	AK363263.1
Hordeumvulgare subsp. vulgare mRNA for predicted protein, complete cds, clone: NIASHv1077C17	1615	1615	99%	0.0	93%	AK358460.1
PREDICTED: Brachypodiumdistachyon heat stress transcription factor A-2c-like (LOC100828307), transcript variant X3, mRNA	1127	1127	90%	0.0	87%	XM_010236438.1

PREDICTED: Brachypodiumdistachyon heat stress transcription factor A-2c-like (LOC100828307), transcript variant X2, mRNA	1127	1127	90%	0.0	87%	XM_010236437.1
PREDICTED: Brachypodiumdistachyon heat stress transcription factor A-2c-like (LOC100828307), transcript variant X1, mRNA	1127	1127	90%	0.0	87%	XM_010236436.1
Sorghum bicolor hypothetical protein, mRNA	857	857	90%	0.0	82%	XM_002467170.1
PREDICTED: Oryzabrachyantha heat stress transcription factor A-2c-like (LOC102713670), mRNA	809	809	86%	0.0	82%	XM_006662297.1
Hordeumvulgare subsp. vulgare mRNA for predicted protein, complete cds, clone: NIASHv2060N1	558	558	46%	6e-155	86%	AK367706.1
Loliumperenne HSF-type DNA-binding domain containing protein mRNA, partial cds	800	800	69%	0.0	85%	JF747476.
PREDICTED: Zea mays heat shock factor protein HSF30 (LOC100280736), transcript variant X5, mRNA	756	756	89%	0.0	81%	XM_008675461.1
PREDICTED: Zea mays heat shock factor protein HSF30 (LOC100280736), transcript variant X4, mRNA	756	756	89%	0.0	81%	XM_008675458.1
PREDICTED: Zea mays heat shock factor protein HSF30 (LOC100280736), transcript variant X3, mRNA	756	756	89%	0.0	81%	XM_008675450.1

Met D A **Met** P P E G I V K E E E V L L H E E R T H P A A A P P Q Q R Q D G A L P R P **Met** E G L H E A
G P P P F L T K T Y D L V E D P A T D Q V V S W G R A G N T F V V W D P H V F A E A L L P R L F K H
S N F S S F V R Q L N T Y G F R K V D P D R W E F A N E G F L R G Q R H L L K T I K R R K P P S N A
P S S Q Q Q A L T S C L E V G E F G F E E E I D R L K R D K N L L I T E V V K L R Q E Q Q A T K D N V
Q A **Met** E G R L R A A E Q R Q A Q **Met Met** G F L A R A **Met** R N P H F F Q Q L V Q K Q D K R K E L E
D A I S K K R R R P I D N A P F Y G S G A T T S Q S E Q L D S Q F L F D S G V L S E P G **Met** N G **Met** E
N L A Q N I Q E L G Q Q G K T D E E K K D E A N G Q L D I N S D F W A E L F S D D F G D E D G S G L S
E L E G R R P E D I D E L G Q Q L G Y L S S T S P Q

Figure 1-Amino acid sequence of heat-responsive transcription factor using Expasy tool from Swiss Institute of Bioinformatics (SIB) was used for translation of protein sequence

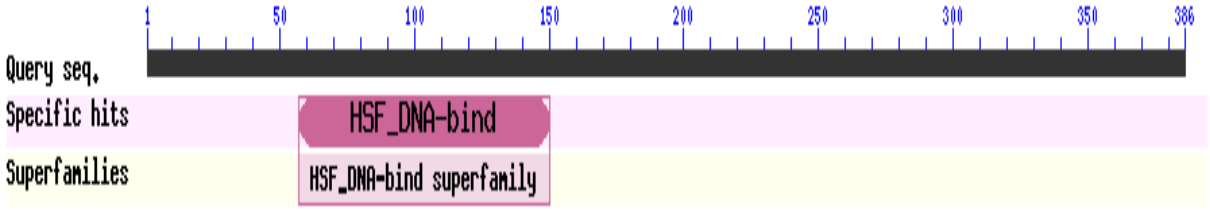

Figure 2- Conserved Domain identified in the heat-responsive transcription factor using Conserved Domain search tool of NCBI was used for domain analysis

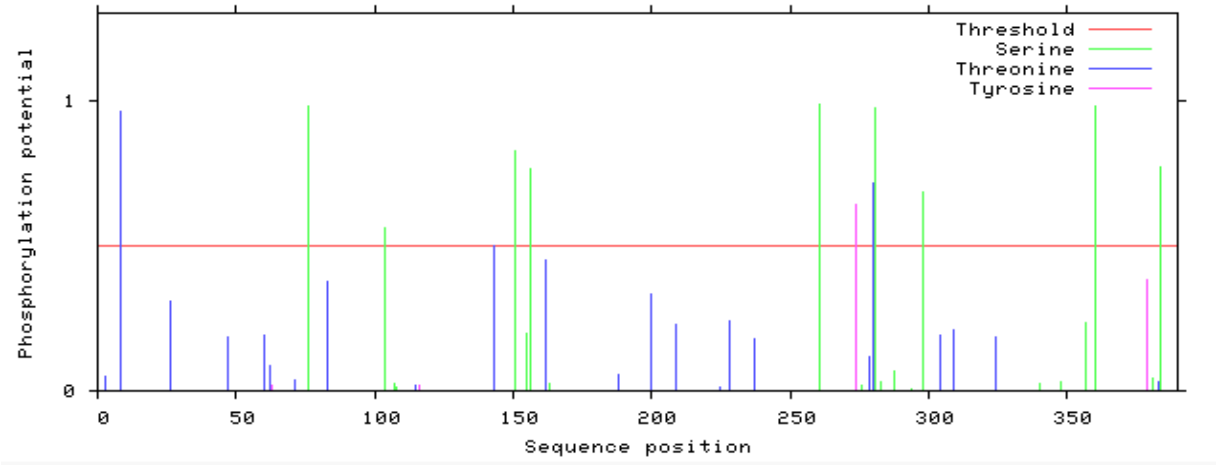

Figure 3-Phosphorylation site predicted in transcription factor using Net MPhos 2.0 server was used for identifying the kinase specific protein phosphorylation sites

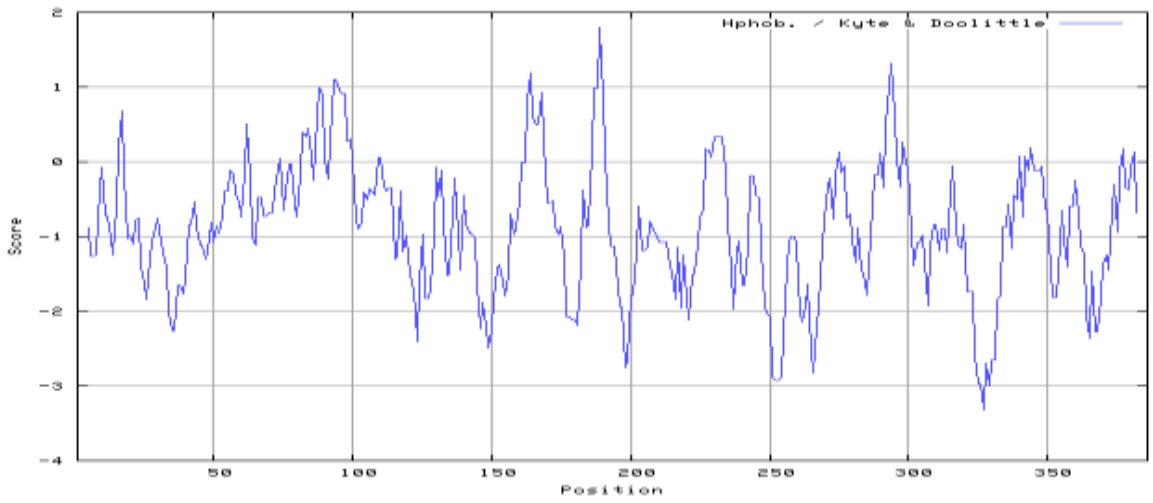

Figure 4- Hydropathy analysis of heat-responsive transcription factor using Prostate tool of expasy was used for analysis of degree of hydrophobicity or hydrophilicity of amino acids of a protein

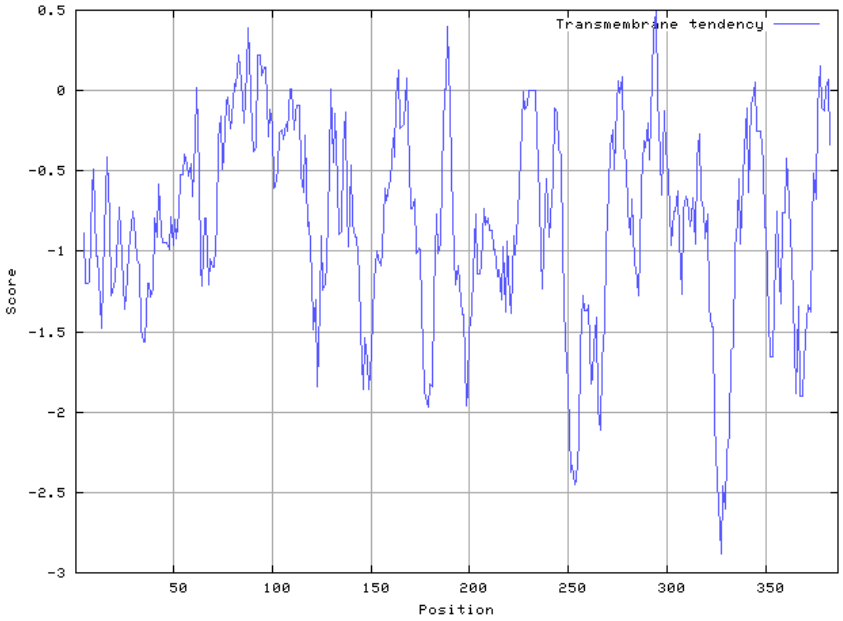

Figure 5- Tran membrane analysis of heat-responsive transcripton factor using Prot Scale tool of expasy was used for analysis trans membrane helices in protein

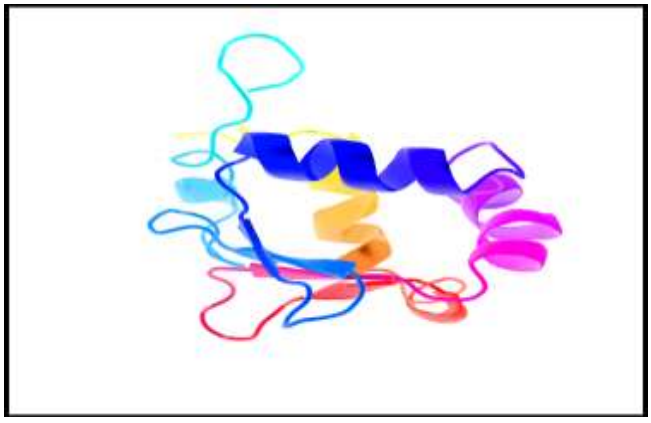

Figure 6- 3D structure of the cloned heat-responsive transcription factor using Phyre2 software; gene has structure similar to heat shock factor DNA binding domain

CONCLUSION:

Heat-responsive factor plays very important role in providing tolerance heat stress in plant. These transcription factor help to regulate the expression of heat shock protein under stress conditions. In- silico characterization showed the presence of HSF_DNA-bnding domain in the sequence predicted to be functional domain of the gene. BlastN search showed maximum homology with clone NIASHv2013N08 (acc. no. AK363263.1) reported from Hordeumvulgare. Heat-responsive transcription factor can b used for developing or enhancing the tolerance capacity of the plant against different abiotic stresses using the tool of genetic engineering.

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