African Journal of Biotechnology Vol. 11(104), pp. 16800-16808, 27 December, 2012
Available online at http://www.academicjournals.org/AJB
DOI: 10.5897/AJB12.2855
ISSN 1684–5315 ©2012 Academic Journals

Full Length Research Paper

Molecular cloning of *HSP17* gene (*sHSP*) and their differential expression under exogenous putrescine and heat shock in wheat (*Triticum aestivum*)

Ranjeet R. Kumar[1]*, G. P. Singh[2], Sushil K. Sharma[1], Khushboo Singh[1], Suneha Goswami[1]* and Raj D. Rai[1]

[1]Division of Biochemistry, Indian Agricultural Research Institute, New Delhi 110012, India.
[2]Division of Genetics, Indian Agricultural Research Institute, New Delhi 110012, India.

Accepted 11 December, 2012

Polyamines (PAs) are low molecular weight ubiquitous nitrogenous compounds found in all the living organisms, which have been implicated in the expression of various stress-proteins against the abiotic stresses. Small heat shock proteins (sHSPs) are of particular importance in the thermotolerance and have been reported to act as molecular chaperones preventing denaturation or aggregation of the target proteins. Here, we report cloning of a small HSP of ~573 bp from C-306 cultivar of wheat (*Triticum aestivum* L), having open reading frame of 162 amino acids. *In silico* analysis showed the presence of an alpha crystalline domain (ACD), the signature domain for small HSPs. Consensus localization prediction (ConLoc) provides 98% consensus prediction of *HSP17* in the nucleus. Quantitative real time polymerase chain reaction (qRT-PCR) analysis of *HSP17* gene showed maximum (34 fold) transcript in C-306 and minimum (1.5 fold) in HD2329 cultivars of wheat in response to differential treatment of putrescine (1.5 to 2.5 mM + heat shock of 42°C for 2 h). Putrescine seems to enhance the transcript levels against the heat shock much more pronounced in thermotolerant than in the susceptible cultivars.

Key words: *Triticum aestivum*, heat stress, small heat shock protein, putrescine, HSP17, polyamine, domain, cloning.

INTRODUCTION

Anthropogenic activities have exacerbated gaseous emissions having wide and deleterious ramifications on environment of the plant. One of the consequences is increase in the global temperature and the greatest threat the climate change poses is the reduction in the crop production. Abiotic stresses including extreme temperatures, drought, flooding or chemical toxicity, pose serious threats to agricultural production (Bita et al., 2011). Plants require optimum temperature for reproduction and maximum yield (Hussain and Mudasser, 2007) and higher temperatures adversely affect the growth and yield of the crop plants especially when a temperature extreme coincides with critical stages of plant development (Craufurd and Wheeler, 2009). High temperature stress results in the membrane fluidizations and also affects plant photosynthetic functions (Lichtenthaler et al., 2005). It has been demonstrated that high temperature or low temperature adversely affects plant growth and survival, but the impact of temperature stress on the photosynthetic

*Corresponding author. E-mail: ranjeetranjaniari@gmail.com. Tel/Fax: +91-996863788.

Abbreviations: sHSP, Small heat shock protein; **ROS,** reactive oxygen species; **qRT-PCR,** quantitative real time polymerase chain reaction; **GAPDH,** glyceraldehyde 3-phosphate dehydrogenase; **ACD,** alpha crystalline domain.

apparatus is considered to be of particular significance because photosynthesis is often inhibited before other cell functions are impaired (Haldimann and Feller, 2004). Like any other organism, plants also possess defense mechanisms to respond to the adverse conditions (Parida et al., 2006). Under the biotic and abiotic stresses, plants produce various stress proteins like antioxidant enzymes, kinases, heat shock proteins etc. Under the normal conditions plants also express these proteins, but the expression is feeble, whereas, as soon as plants are exposed to the stress conditions, the expression of these stress proteins gets augmented considerably. Heat shock proteins are of particular importance in the thermotolerance reactions and act as molecular chaperones to prevent denaturation or aggregation of the target proteins, as well as to facilitate protein-refolding (Sarkar et al., 2009; Perez et al., 2009). According to the molecular weight, HSPs have been classified into small and high molecular weight HSPs that is, *HSP90*, *HSP101*, *HSP 70, HSP17, HSP23, HSP26* etc (Kotak et al., 2007). Heat stress readily alters the pattern of gene expression, as very significant part of the thermotolerance (Yang et al., 2006). It also weakens the mRNAs encoding non-heat stress induced proteins (Maestri et al., 2002). Increased expression of sHSPs is mediated at various levels such as mRNA synthesis stability and translation efficiency. It has recently been observed that the elevated expression of many stress proteins can be further accentuated with exogenous application of few elicitors like polyamines. These plants growth regulators play a vital role in enhancing the tolerance against the stress in plants (Chakarbati and Mukharjee, 2003). Putrescine is a polyamine widely present in all organisms and have been reported to plays a vital role in many physiological processes in plants. Exogenous application of putrescine is one of the approaches for enhancing the stress tolerance of plants (Alcazar et al., 2006). Because of the polycationic nature at the physiological pH, polyamines can bind strongly to the cellular constituents such as nucleic acids, proteins and membranes (Childs et al., 2003). Some recent reports have indicated the involvement of polyamines in regulating the heat stress-induced inhibition of photosynthetic efficiency (Liu et al., 2007).

The present study, a part of a program to understand the molecular mechanisms underlying the thermotolerance of wheat, is an attempt to identify, isolate and characterize small heat shock protein (*HSP17*) and to access the effect of the exogenous application of elicitor, putrescine, on the expression of the small heat shock protein.

MATERIALS AND METHODS

Plant materials and exogenous putrescine treatment

Two different cultivars of wheat (*Triticum aestivum*), namely the thermotolerant (C-306) and thermosuscepetible (HD2329) were obtained from the Division of Genetics, Indian Agricultural Research Institute (IARI) and sown in the Phytotron under the controlled conditions; T_0 (22°C, 16 h light /8 h dark cycle). Wheat plants were exposed to different treatments like: T_1 (heat shock of 42°C for 2 h), T_2 ($Put_{1.5\ mM}$ + heat shock of 42°C for 2 h) and T_3 ($Put_{2.5mM}$ + heat shock of 42°C for 2 h) at different stages of growth. The samples were collected and immediately frozen in liquid nitrogen and stored at -80°C till RNA was isolated. Plants of the same age but without pre-treatments and or heat shock served as respective controls.

Molecular cloning of *HSP17* gene

After *in silico* identification of conserved sequence regions of *HSP17* genes in cereals, a pair of degenerated primers was designed for polymerase chain reaction (PCR) amplification of the corresponding gene sequences in wheat. Total RNA was isolated from 14 days old germinating wheat seedlings which were heat shocked at 42°C for 2 h by Trizol method (Invitrogen). cDNA was synthesized using oligo dT primer (RevertAid^{TM} H minus first strand cDNA synthesis kit, Fermentas). cDNA synthesized from C-306 cultivar of wheat was used for PCR amplification of *HSP17* gene by using forward primer (HspF- 5'-CGA GAA TGG AGG GCA GGA-3';Tm 58.2°C) and reverse primer (HspR-5'-ACC AAA AGA CAG ACA GAC CA-3';Tm 55.3°C). The amplified product was cloned in $pGEMT_{Easy}$ vector (Promega) and sequenced using M13 forward and reverses primers.

In silico characterization of *HSP17* gene

The nucleotide sequence of *HSP17* was used for homology search using BLASTn tool (NCBI) (http://blast.ncbi.nlm.nih.gov/Blast.cgi). The open reading frame (ORF) of the sequence was characterized using ORF finder (http://www.ncbi.nlm.nih.gov/projects/gorf/). The conserved domain of the gene was searched using conserved domains (CD) search (NCBI) (http://www.ncbi.nlm.nih.gov/Structure/cdd/wrpsb.cgi). The *HSP17* genes reported from different plant sources were aligned using clustal W alignment (http://www.genome.jp/tools/clustalw/). The inter-cellular localization of the HSP17 was predicted using PSORT software (http://psort.hgc.jp/). The hydropathy plot was calculated using Kyte-Doolittle index (Kyte and Doolittle, 1982; http://gcat.davidson.edu/rakarnik/kyte-doolittle.htm).

RNA extraction and quantitative real time PCR (qRT-PCR)

Total RNA was extracted using the Trizol method (Invitrogen) and quantified by Quant^{IT} RNA quantification Qubit (Invitrogen). RNA integrity was verified on 1.2% agarose gels. First strand cDNA synthesis was performed using oligo dT primers and the Superscript II reverse transcriptase (Invitrogen, UK), according to the manufacturer's instructions. First-strand cDNA was diluted to a final concentration of 20 ng/µl. Primers for RT-qPCR reactions were designed from the deduced sequence corresponding to the wheat *HSP17* gene using Prime 3 primer designing software (Premier Biosoft, USA). Quantitative PCR was performed in 25 µl reactions using forward primer ($HspF_q$-5'-CAT CAA GGT GCA GGT GGA G-3'; Tm, 60.2°C) and reverse primer ($HspR_q$-5'-AGG TAC TTG GCG TCC TCC TT-3'; Tm 60.1°C), 1 µl of cDNA as template and the SYBRGreenER qPCR SuperMix Universal (Invitrogen, UK). Reactions were performed on the CFX96 Real-Time PCR system (BioRad, UK). The thermal profile for qPCR was: 3 min at 95°C, followed by 35 cycles each consisting of 95°C for 15 s, 60°C for 30 s and 72°C for 15 s followed by plate read. The primer specificity and the formation of primer-dimers were monitored by dissociation curve analysis and agarose gel electrophoresis on a 2% agarose

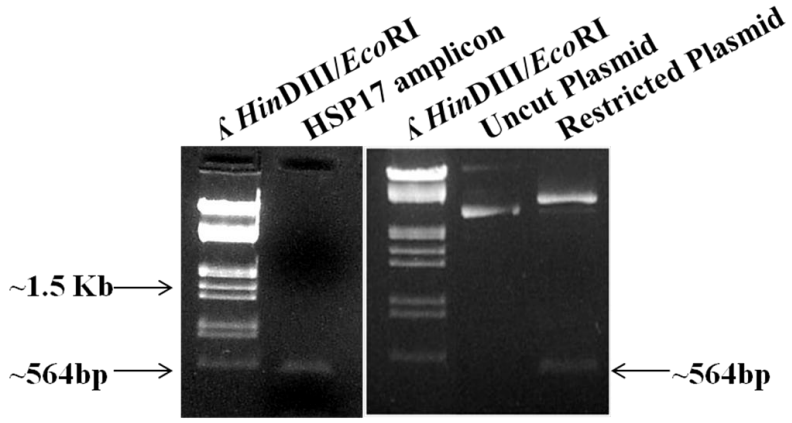

Figure 1. Reverse transcription polymerase chain reaction (RT-PCR) amplification of small heat shock protein (*HSP17*) of ~573 bp using gene specific primers from C-306 cultivar of wheat and cloned in pGEMT $_{Easy}$ vector, plasmid restricted using *Eco*RI RE showed the release of ~573 bp insert, 0.8% agarose gel was used.

gel. The expression levels of wheat *actin* gene and glyceraldehydes-3-phosphate dehydrogenase (GAPDH) were used as internal standards for normalization of cDNA template quantity using actin (accession no. AF282624) and GAPDH (accession no EF592180) specific primers. For the treatments as well as controls, expression measurements were performed using duplicate biological replications and three technical replications. Controls with no cDNA template were also included in qPCR analysis. Data analysis was performed using the software provided by BioRad, UK. The Comparative C_t (2-ΔΔCt) method was used to calculate the changes in gene expression as a relative fold difference between an experiment and calibrator sample.

Semi-quantitative real time PCR

The qRT-PCR products (25 µl) were resolved on ethidium bromide stained agarose gel (2%) for analyzing marked variations in transcript compared to the expression of *actin* and *GAPDH* genes. Two different genes (*actin* and *GAPDH*) were used as endogenous control in present investigation because it has been reported that GAPDH showed marked tissue specific variation in its expression under different treatments and has least expression stability compared to other reference genes (Maroufi et al., 2010). The mean C_t value of actin and GAPDH was used for normalizing the expression of *HSP17* gene. The gel image was taken by gel documentation system (Syngene, UK).

RESULTS AND DISCUSSION

Sequence identification of small HSP gene and *in silico* characterization

An amplicon of ~573 bp was amplified from C-306 cultivar of wheat by RT-PCR and cloned in pGEMT $_{Easy}$ vector (Figure 1). Plasmid restricted using *Eco*RI restriction enzyme releases insert of ~573 bp. BLASTn homology analysis showed maximum resemblance with HSP17.8 gene reported from *T. aestivum* L (accession no. AF350423.1) as submitted by (Basha et al., 2001). The gene was submitted to NCBI Gen Bank with accession number JN572711.

Isolated *HSP17* gene has an ORF from 6 to 494 which encode for 162 amino acids (Protein ID AEN95115; Figure 2a). The region of ORF from 234 to 317 bp was used for quantitative real time expression profiling using forward (HspFq) and reverse (HspRq) primers with expected transcript of 83 bp (Figure 2b). Conserved Domain Architecture Retrieval Tool (CDART) showed the presence of alpha-crystalline domain (ACD) in the sequence (Figure 3a). The ACD was predicted between 57 to 142 aa, a characteristic feature of small HSPs (Mohamed, 2010; Figure 3b). sHSPs generally active, as large oligomers consisting of multiple subunits are believed to be ATP-independent chaperones (Miernyk, 1999) that prevent aggregation and are important in refolding also in combination with other HSPs. Clustal W alignment of *HSP17* gene (accession no JN572711.1) isolated in the present investigation from wheat along with other sHSPs reported from plant sources showed the presence of three different evolutionary families of *HSP17* gene (Figure 4). The hydropathy index of *HSP17* gene amplified in the present investigation indicates that *HSP17* does not seem to be transmembrane protein. The negative region from 25 to 100 showed that this protein is globular and likely functions in the cytoplasm or within the hydrophilic region of some organelle of the plant cell (Figure S_1) which is in conformity with characteristics of orthologous proteins from *Oryza sativa* (accession no EU715987), *Hordeum vulgare* (accession no X64560), and *Lycopersicon peruvianum* (AY608694).

Transcript profiling of *HSP17* gene

In general, plant species and cultivars with high stress tolerance are endowed with a great capacity to enhance

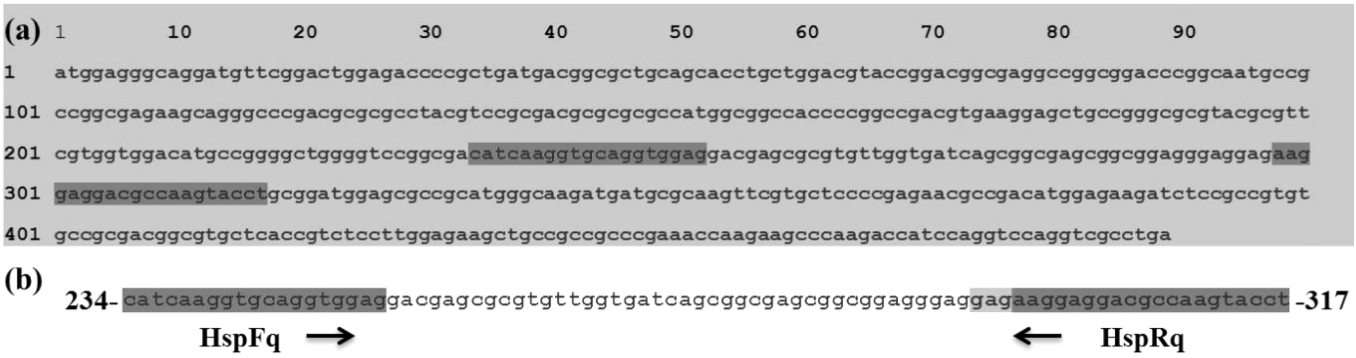

Figure 2. (a) Open reading frame (ORF) of *HSP17* gene amplified in present investigation showing the position of forward and reverse primers used in qRT-PCR expression profiling. (b) Transcript of 83bp which was amplified in quantitative real time PCR along with forward (HspFq) and reverse (HspRq) primers.

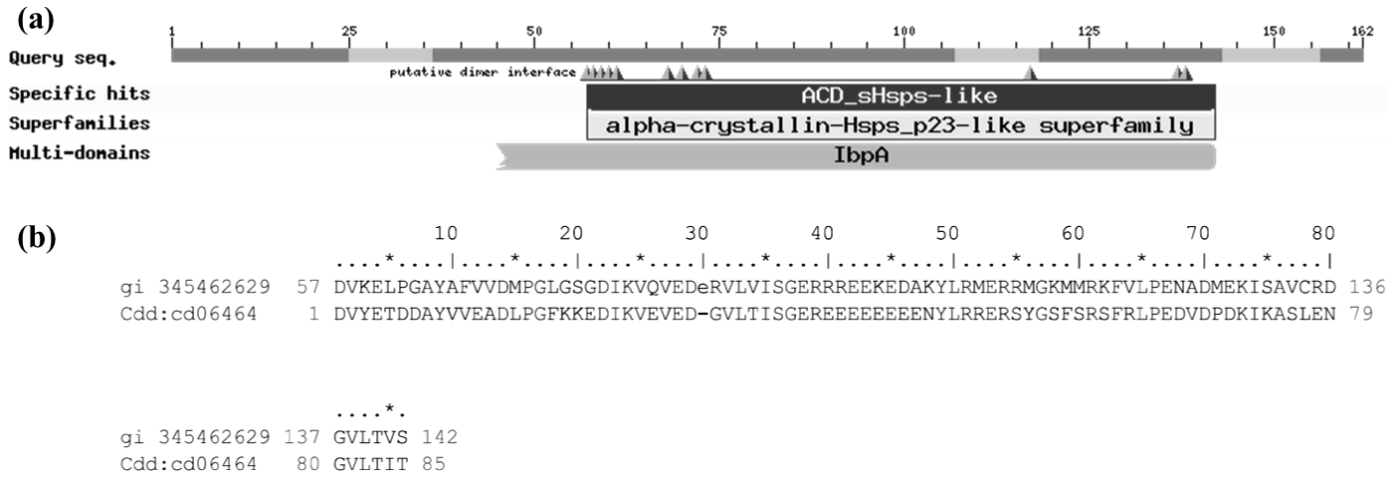

Figure 3. (a) Alpha crystalline domain (ACD) observed in the *HSP17* gene using conserved domain search tool of NCBI. (b) Amino acids characterized in the conserved region of the gene predicted to be alpha crystalline domain site along with their location

polyamines in response to environmental stresses including heat stress. Quantitative real time PCR of *HSP17* gene in C-306 (thermotolerant) showed 2.5, 3.1 and 3.3 fold increase in transcript against HS(T_1), $Put_{1.5mM}$+HS(T_2) and $Put_{2.5mM}$+HS(T_3), respectively at vegetative stage (Figure 5a). During pollination stage, 6.8, 15.2 and 16 fold increase in the expression of *HSP17* gene were observed against T_1, T_2 and T_3 treatments, respectively (Figure 5b). An increase of 6.8, 12.2 and 16 fold (milky dough; Figure 5c) and 18, 32 and 34 fold increase (seed hardening stage) were observed against T_1, T_2 and T_3 treatments, respectively (Figure 5d). Semi-quantitative real time PCR of HSP17 in case of C-306 against different putrescine treatments along with HS showed variations in the expression of HSP17 on the gel compared to that of actin and GAPDH which was used as endogenous control genes (Figure 6).

Although, as compared to the heat tolerant C-306, the abundance of *HSP17* transcript in case of HD2329 (thermosusceptible) was very low at all the stages of growth against different treatments. During the vegetative stage (HD2329), a decrease in the expression was observed against HS (T_1) (Figure 7a), whereas no change in the transcript level could be detected against $Put_{1.5\ mM}$ + HS (T_2) treatment. However, 1.2 fold increases in the expression was observed in response to $Put_{2.5mM}$+HS (T_3). Quantitative real time PCR showed 1.2,1.3 and 1.2 fold pollination (Figure 7b); 1.5, 1.1 and 1.4 fold milky dough (Figure 7c) and 1.1, 1.5 and 1.3 fold seed hardening (Figure 7d) increase in the transcript of HSP17 in response to T_1, T_2 and T_3 treatments, respectively. The abundance of *HSP17* transcript was very low in the susceptible cultivar (HD2329) as compared to the tolerant one (C-306). Semi-quantitative real time PCR of *HSP17* in case of HD2329 showed altered expression of gene visualize on the gel against different putrescine treatments followed by HS as compared to that of endogenous control genes (*actin* and

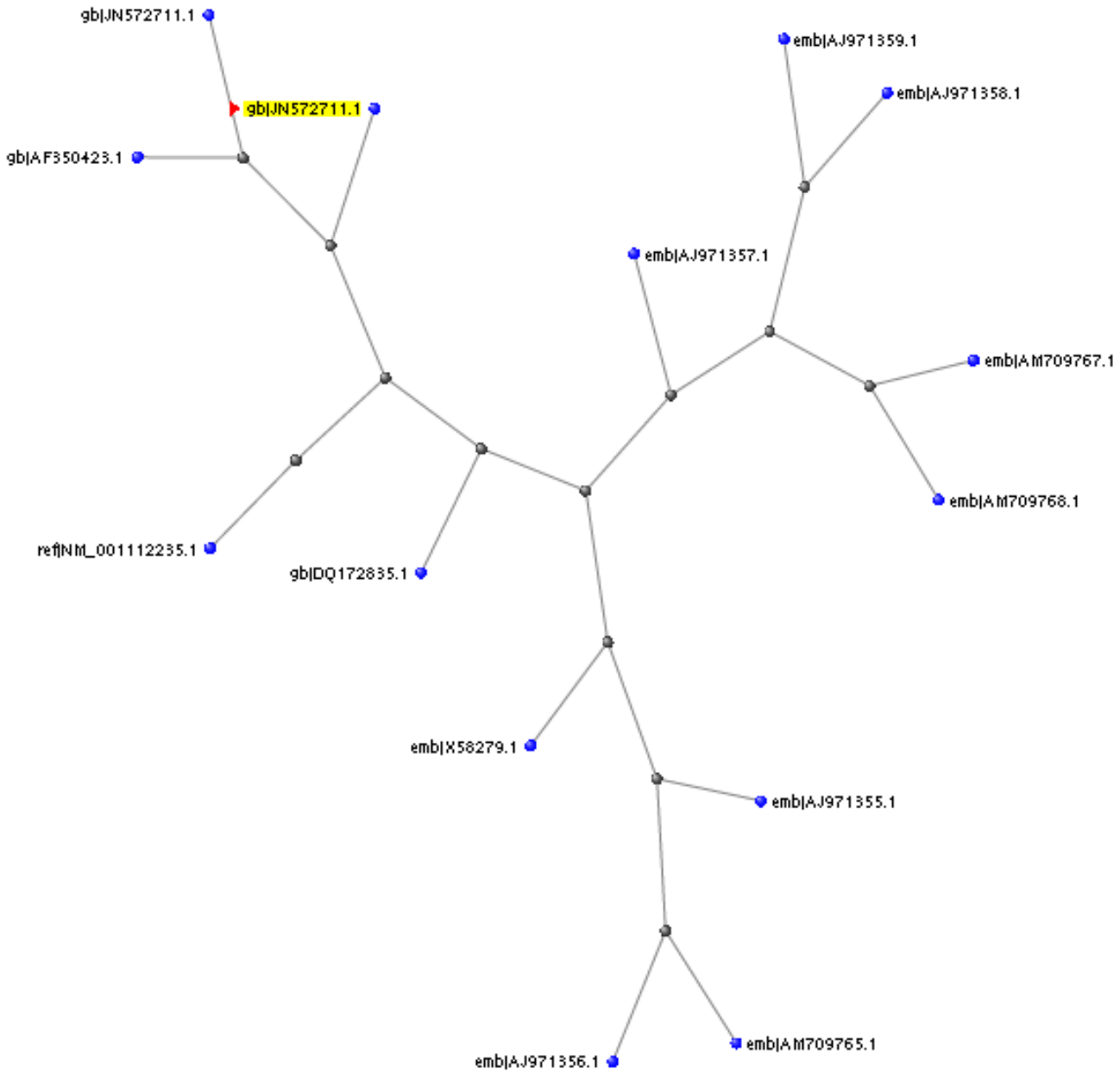

Figure 4. Clustal W alignment of *HSP17* gene amplified from C-306 cultivar of wheat along with other small heat shock protein (*HSP17*) genes reported from plant and non-plant sources.

GAPDH; Figure 8). The extent of sHSPs accumulation depends on temperature and the duration of stress periods. Expression analysis shows that the wheat chloroplastic sHSP (HSP26) is highly inducible by heat stress in almost all the vegetative and generative tissues and is also expressed constitutively in certain developmental growth stages (Chauhan et al., 2012).

Polyamines like putrescine are reported to be involved in the plant defense to the environmental stresses (Bouchereau et al., 1999) and pre-treatments of putrescine increase the expression of HSPs to overcome the heat stress and help the plants to survive under the stress. In case of C-306, a significant increase in the transcript of *HSP17* was observed against heat stress of 42°C for 2 h at vegetative, pollination, milky dough and seed hardening stages. On pre-treatment of putrescine (1.5 and 2.5 mM) with heat stress, a many fold increase in the transcript was observed at almost all the stages of growth with maximum increase at seed hardening stage. Similarly, in HD2329 (thermosusceptible), a decrease in the transcript was observed at vegetative stage followed by slight increase at pollination, milky dough and seed

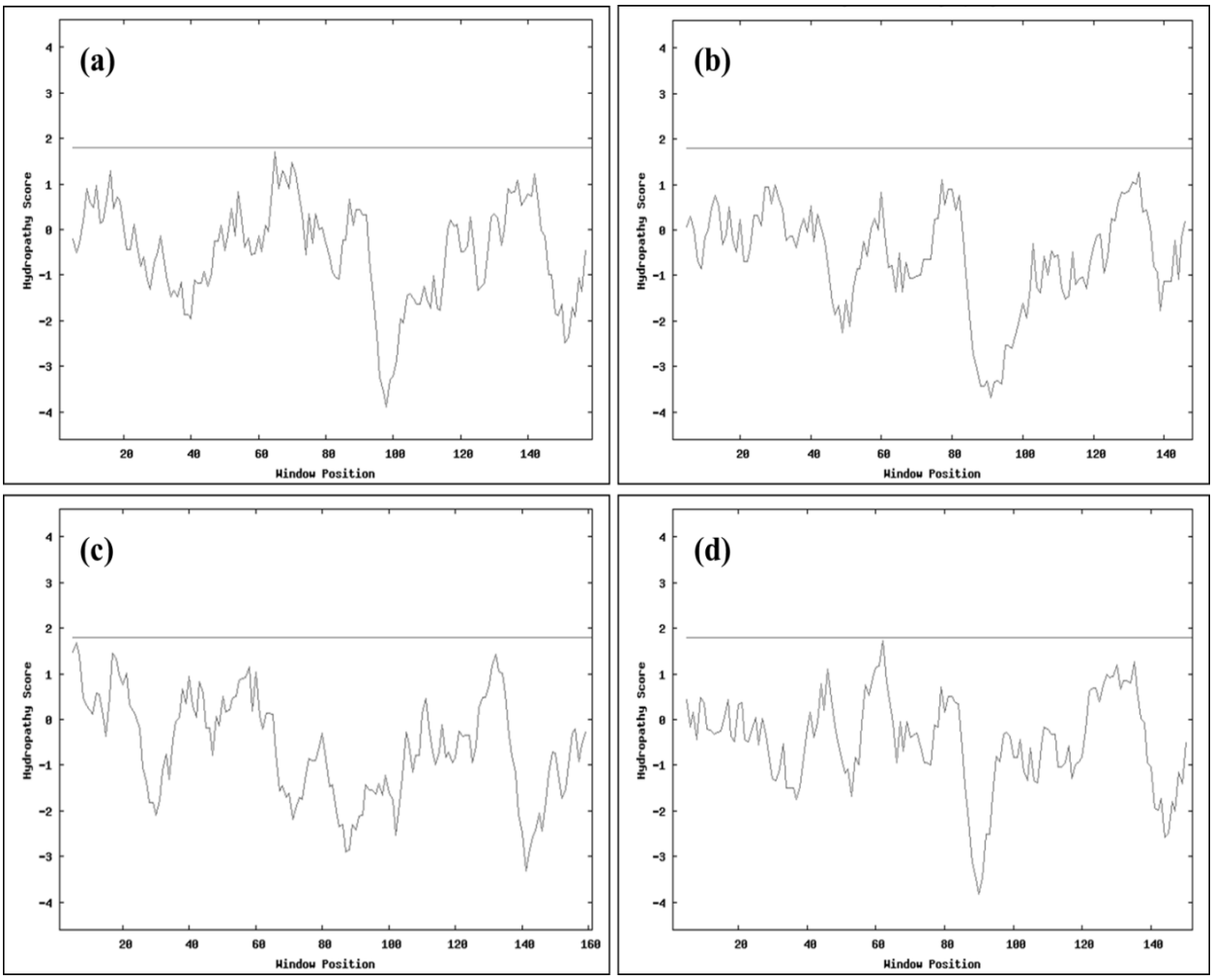

Figure S_1. Kyte-Doolittle hydropathy plot for small heat shock protein genes. **(a)** *HSP17* isolated from *Triticum aestivum* (accession no JN572711). **(b)** *Oryza sativa* (accession no EU715987). **(c)** *Hordeum vulgare* (accession no X64560). **(d)** *Lycopersicon peruvianum* (AY608694). This graph is the hydropathy plot for the amino acid sequence of the open reading frame (ORF). Points above zero indicate hydrophobic residues while points below zero represent hydrophilic residues. The window size is 7. The lack of strong peaks crossing the red line at 1.8 suggests that this protein is likely not an integral membrane protein.

hardening stages against heat stress (HS, 42°C for 2 h). Putrescine treatment along with heat shock leads to altered expression of *HSP17*, but the change was very feeble and non-significant at different stages of growth.

The capacity to survive heat shock varies with plant species, genotype and developmental stage (Hong and Vierling, 2001). Plants cannot survive a heat burst, but they can survive to some extent, the gradual increase in temperature within the context of their natural environment. Exogenous application of polyamines have been found to reduce the levels of hydrogen peroxide, malondialdehyde content and raised the level of antioxidants in Chickpea (Nayyar and Chander, 2004). He et al. (2008) found that putrescine induced the changes of endogenous polyamines in the photosynthetic apparatus to some extent, might be involved in the reduction of H_2O_2 contents and membrane lipid peroxidation under the salt stress. Several studies have reported that exogenous polyamines application is involved in improving drought tolerance also (Alcazar et al., 2010). The heat tolerant wheat cv. PBW 343 exhibited higher content of antioxidants and activities of antioxidative enzymes, while lower content of lipid peroxides as compared to the heat-sensitive cv. HD 2329 under differential putrescine treatment (Asthir et al., 2012). Putrescine has been observed to enhance the expression of small heat shock protein gene as observed in present investigation. NnHSP17.5 from *Nelumbo nucifera* and over-expression of HSP26 enhanced seedling thermotolerance in transgenic Arabidopsis

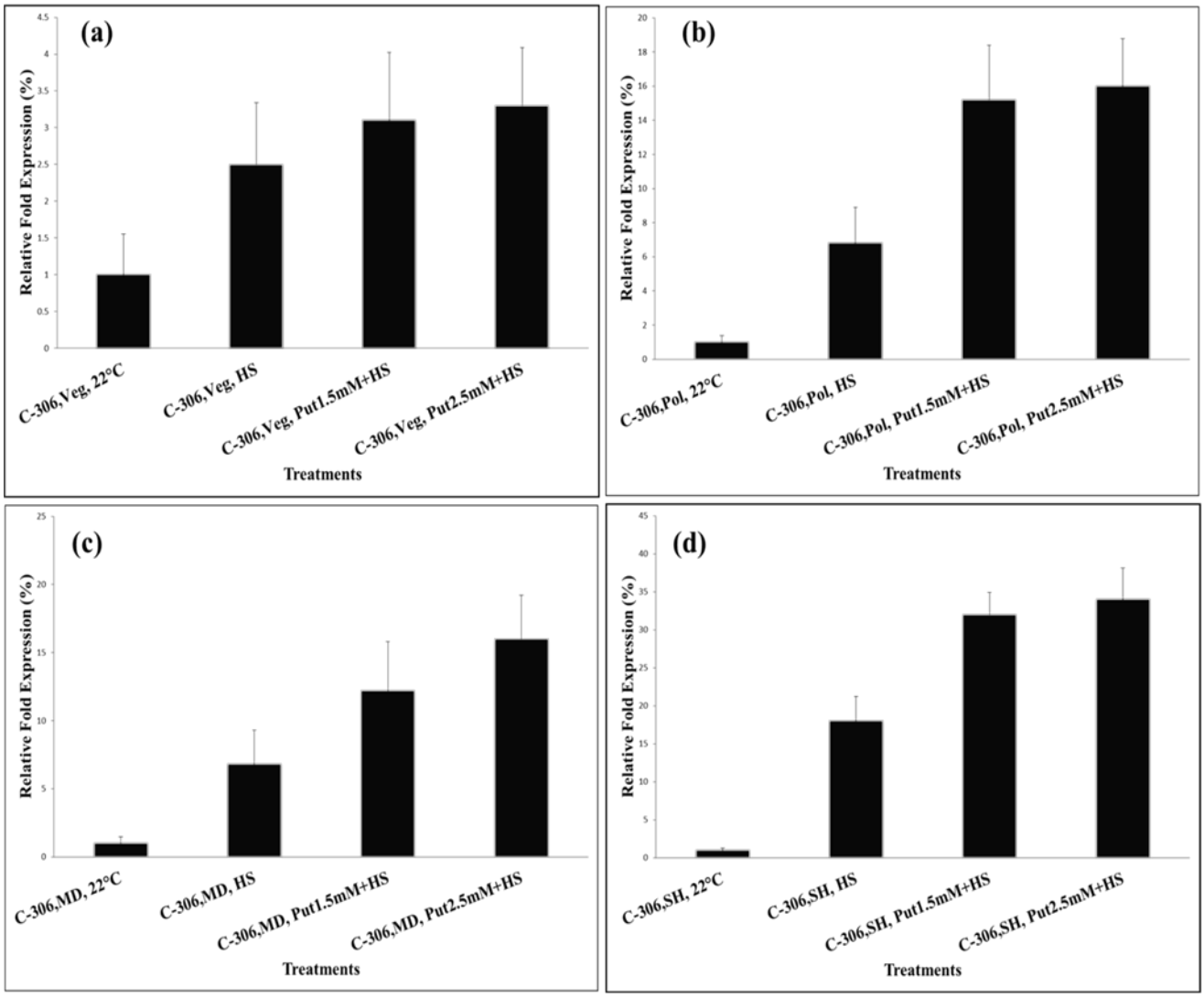

Figure 5. Quantitative real time expression analysis of *HSP17* gene in C-306 (thermotolerant) cultivar of wheat at different stages of growth against putrescine (1.5 and 2.5 mM) and differential heat shock treatment (HS- 42°C for 2h); (a) Vegetative (Veg), (b) Pollination (Pol), (c) Milky dough (MD), (d) Seed hardening (SH). *Actin* and *GAPDH* genes were used as endogenous control for normalizing the expression of sHSP gene.

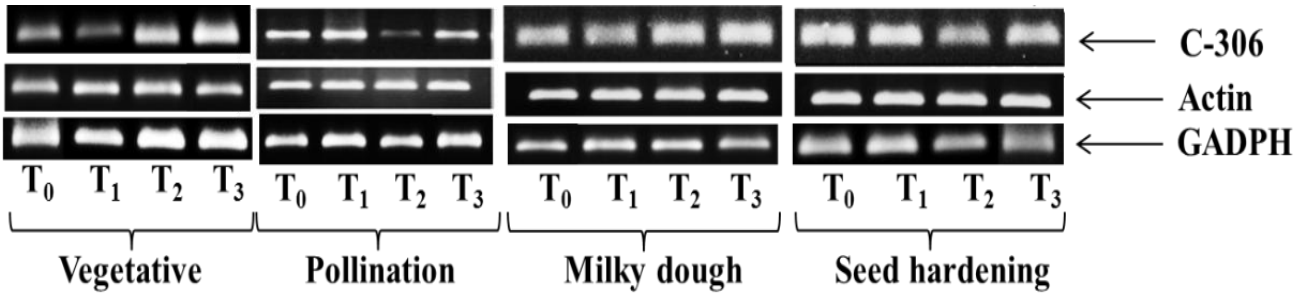

Figure 6. Semi-quantitative real time expression study of *HSP17* gene in C-306 cultivar of wheat against putrescine (1.5 and 2.5 mM) and heat shock (HS, 42°C for 2 h) at different stages of growth (vegetative, pollination, milky dough and seed hardening), T_0, control; T_1, heat stress; T_2, $Put_{1.5}$ + HS; T_3, $Put_{2.5}$ + HS. Expression of *actin* and *GAPDH* genes were used as endogenous control gene for normalizing the expression of *HSP17* gene.

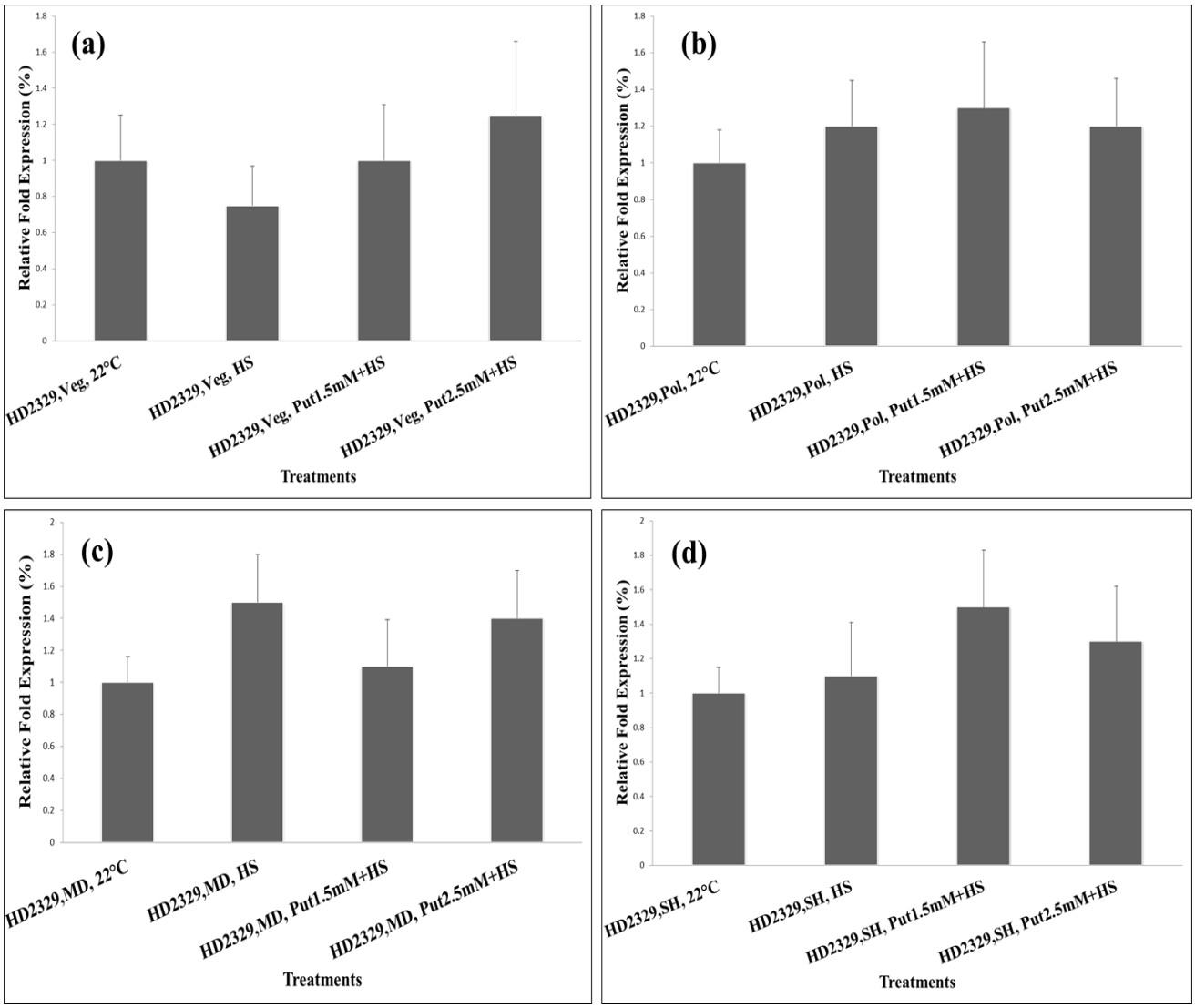

Figure 7. Quantitative real time expression analysis of *HSP17* gene in HD2329 (thermosusceptible) cultivar of wheat at different stages of growth against putrescine (1.5 and 2.5 mM) and differential heat shock treatment (HS, 42°C for 2 h): (a) Vegetative (Veg), (b) Pollination (Pol), (c) Milky dough (MD), (d) Seed hardening (SH); *actin* and *GAPDH* genes were used as endogenous control for normalizing the expression of *HSP17* gene.

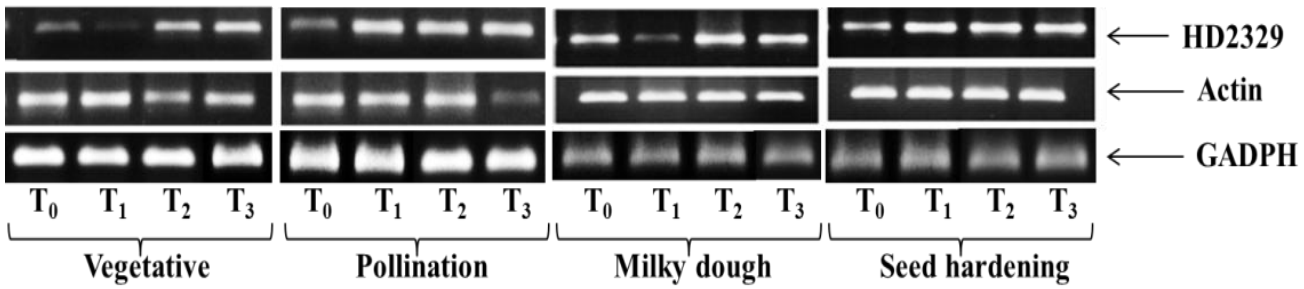

Figure 8. Semi-quantitative real time expression study of *HSP17* gene in HD2329 cultivar of wheat against putrescine (1.5 and 2.5 mM) and differential heat shock (42°C for 2 h) at different stages of growth (vegetative, pollination, milky dough and seed hardening), T_0, control; T_1, heat stress; T_2, $Put_{1.5}$ + HS; T_3, $Put_{2.5}$ + HS. Expression of *actin* and *GAPDH* genes were used as endogenous control gene for normalizing the expression of *HSP17* gene.

(Zhou et al., 2012; Xue et al., 2010).

Conclusions

Small heat shock proteins play very important role in protecting the plant from various abiotic stresses. Here, we have cloned a full length gene of small HSP (*HSP17*) from C-306 cultivar of wheat having alpha crystalline domain (ACD) in the sequence. Expression analysis showed the abundance of HSP17 transcript in thermotolerant cultivar (C-306) compared to susceptible (HD2329) in response to exogenous application of putrescine and heat shock at different stages of growth and development. The transcript accumulation was observed high in response to $Put_{1.5\ mM}$ (C-306) and $Put_{2.5\ mM}$ (HD2329) under heat stress. Putrescine modulates the expression of various stress associated proteins and in turn enhances the thermotolerance capacity of the plant under heat stress. However, application of advanced molecular biology and proteomic approaches will further help elucidate the mechanisms of polyamines in various plant processes involved in the stress tolerance.

ACKNOWLEDGEMENTS

The author sincerely thanks Indian Agricultural Research Institute (IARI) and Indian Council of Agriculture Research (ICAR) for providing financial support under institutional project.

REFERENCES

Alcazar R, Marco F, Cuevas JC, Patron M, Ferrando A, Carrasco P (2006). Involvement of polyamines in plant response to abiotic stress. Biotechnol. Lett. 28:1867–1876.

Alcazar R, Planas J, Saxena T, Zarza X, Bortolotti C, Cuevas J, Bitrian M, Tiburcio AF, Altabella T (2010). Putrescine accumulation confers drought tolerance in transgenic Arabidopsis plants over-expressing the homologous *Arginine decarboxylase 2* gene. Plant Physiol. Biochem. 48:547–552.

Asthir B, Koundal A, Bains NS (2012). Putrescine modulates antioxidant defense response in wheat under high temperature stress. Biol. Plant 56(4):757-761.

Basha E, Lee GJ, Demeler B, Vierling E (2004). Chaperone activity of cytosolic small heat shock proteins from wheat. Eur. J. Biochem. 271(8):1426-1436.

Bita CE, Zenoni S, Vriezen WH, Mariani C, Pezzotti M, Gerats T (2011). Temperature stress differentially modulates transcription in meiotic anthers of heat-tolerant and heat-sensitive tomato plants. BMC Genomics 12:384. doi:10.1186/1471-2164-12-384

Bouchereau A, Aziz A, Larher F, Martin-Tanguy J (1999). Polyamines and environmental challenges: recent developments. Plant Sci. 140:103-125.

Chauhan H, Khurana N, Nijhavan A, Khurana JP, Khurana P (2012). The wheat chloroplastic small heat shock protein (sHSP26) is involved in seed maturation and germination and imparts tolerance to heat stress. Plant Cell Environ. 35(11):1912-1931.

Childs AC, Mehta DJ, Gerner EW (2003). Polyamine-dependent gene expression. Cell Mol. Life Sci. 60:1394–1406.

Craufurd PQ, Wheeler TR (2009). Climate change and the flowering time of annual crops. J. Exp. Bot. 60(9):2529-2539.

Haldimann P, Feller U (2004). Inhibition of photosynthesis by high temperature in oak (*Quercus pubescens*) leaves grown under natural conditions closely correlates with a reversible heat-dependent reduction of the activation state of ribulose-1, 5-bisphosphate carboxylase/oxygenase. Plant Cell Environ. 27:1169-1183.

He LX, Nada K, Kasukabe Y, Tachibana S (2008). Enhanced susceptibility of photosynthesis to low-temperature photoinhibition due to interruption of chilling induced increase of S-adenosylmethionine decarboxylase activity in leaves of spinach (*Spinacia oleracea* L.). Plant Cell Physiol. 43:196-206.

Hong SW, Vierling E (2001). Hsp101 is necessary for heat tolerance but dispensable for development and germination in the absence of stress. Plant J. 27:25-35.

Hussain SS, Mudasser M (2007). Prospects for wheat production under changing climate in mountain areas of Pakistan – An econometric analysis. Agric. Sys. 94(2):494–501.

Kyte J, Doolittle R (1982). A simple method for displaying the hydropathic character of a protein. J. Mol. Biol. 157:105-132.

Lichtenthaler HK, Langsdorf G, Lank S, Buschamann (2005). Chlorophyll fluorescence imaging of photosynthetic activity with the flesh lamp fluorescence imaging system. Photosynthetica 43:355-369.

Liu JH, Kitashiba H, Wang J, Ban Y, Moriguchi T (2007). Polyamines and their ability to provide environmental stress tolerance to plants. Plant Biotechnol. 24:117-126.

Maestri E, Klueva N, Perrotta C, Gulli M, Nguyen HT, Marmiroli N (2002). Molecular genetics of heat tolerance and heat shock proteins in cereals. Plant Mol. Biol. 48:667-681.

Maroufi A, Bockstaele EV, Loose MD (2010). Validation of reference genes for gene expression analysis in chicory (*Cichorium intybus*) using quantitative real-time PCR. BMC Mol. Biol. 11:15.

Miernyk JA (1999). Complexity of the heat stress response in plants. Plant Biol. 10:310-316.

Mohamed HAW (2010). Plant heat-shock proteins: A mini review. J. King Saud University (Science), doi:10.1016/j.jksus.2010.06.022.

Nayyar H, Chander S (2004). Protective Effects of polyamines against oxidative stress induced by water and cold stress in Chickpea. J. Agron. Crop Sci. 190:355-365.

Parida AK, Jithesh MN, Prashanth SR, Sivaprakash KR (2006). Antioxidative response mechanisms in halophytes: their role in stress defence. J. Genet. 85(3):237-245.

Perez DE, Hoyer JS, Johnson AI, Moody ZR, Lopez Z, Kaplinsky NJ (2009). BOBBER1 is a non-canonical Arabidopsis small heat shock protein required for both development and thermotolerance. Plant Physiol. 151:241-252.

Sarkar NK, Kim YK, Grover A (2009). Rice sHsp genes: genomic organization and expression profiling under stress and development. BMC Genomics 10:393.

Xue Y, Peng R, Xiong A, Li X, Zha D, Yao Q (2010). Over-expression of heat shock protein gene hsp26 in *Arabidopsis thaliana* enhances heat tolerance. Biol. Plant 54:105-111.

Yang KA, Lim CJ, Hong JK, Park CY, Cheong YH, Chung WS, Lee KO, Lee SY, Cho MJ, Lim CO (2006). Identification of cell wall genes modified by a permissive high temperature in Chinese cabbage. Plant Sci. 171:175-182.

Zhou Y, Chen H, Chu P, Li Y, Tan B, Ding Y, Tsang EWT, Jiang L, Wu K, Huang H (2012). NnHSP17.5, a cytosolic class II small heat shock protein gene from *Nelumbo nucifera*, contributes to seed germination vigor and seedling thermotolerance in transgenic Arabidopsis. Plant Cell Rep. 31:379-389.