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MOLECULAR CHARACTERIZATION OF THE HIGH TEMPERATURE STRESS-INDUCED SMALL HEAT SHOCK PROTEIN FROM *Capsicum annuum*

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ABSTRACT

A cDNA clone *Capsicum annuum* small Heat shock protein (*CasHsp*) was isolated from high temperature-stressed hot pepper plants. This gene contains an open reading frame (ORF) encoding a small Heat shock protein (sHsp) of 159 amino acids. CasHsp has an ACD-ScHsp26 domain. Phylogenetic analysis based on the deduced amino acid sequence of *CasHsp* cDNA revealed significant sequence similarity (94.8%) to the *Capsicum frutescens* sHsp. The putative protein hydrophilic by nature, encoded by *CasHsp* also shared 88.1% homology with a sHsp of *Nicotiana tabacum*, 84.3% homology with sHsp of *Vitis vinifera* and 79.9% homology with sHsp of *Arabidopsis thaliana*. Transcripts were preferentially induced in response to high temperature and water deficit. This demonstrates that high temperature induces a small Heat shock protein from hot pepper.

Key words: Heat shock protein, *Capsicum annuum*, environmental stress, high temperature-induced gene.

INTRODUCTION

High temperature stress is a major constraint to crop productivity in arid, semiarid, tropical and subtropical regions of the world (Valiollah *et al.*, 2007). Plant exposed to excess heat exhibit a characteristic set of cellular and metabolic responses, many of which are conserved in all organisms. Discovering the genes involved in plant responses to high temperature stress can provide new tools for genetic engineering of more tolerant field crops (Zeba *et al.*, 2006; Isbat *et al.*, 2009; Zeba *et al.*, 2009). Under high temperature stress, it is the synthesis of molecular chaperones known as Heat shock proteins (Hsps) that allow cellular proteins to avoid and/or recover from aggregation (Miernyk, 1999). This increase in expression is transcriptionally regulated. The dramatic upregulation of the heat shock proteins is a key part of the heat shock response and is induced primarily by heat shock factor (HSF) (Wu, 1995). Hsps are conserved among all living organisms. Many of these proteins function as molecular chaperones *in vitro* as part of a functional network in which the chaperones prevent the aggregation of misfolded proteins, while actively assist in their refolding denatured by heat (Veinger *et al.*, 1998; Bukau and Horwich, 1999; Mogk *et al.*, 1999; Lee and Vierling, 2000; Mayer *et al.*, 2001; Lee and Tsai, 2005). The contribution of individual chaperone to this folding network and the identity of such stress-sensitive cellular proteins are not well known (Cho and Bae, 2006). Therefore, it is important to determine that the extent of which chaperones can re-solubilize aggregates of proteins that escape the protection of holder chaperones (Tatsuta *et al.*, 1998; Mayer *et al.*, 2001; Tomoyasu *et al.*, 2001). The function of Hsps has been reported recently and much of them are in *Drosophila* and Yeast where genetic mutant can be obtained easily. These researches are not sufficient to understand plant Hsps. Based on their approximate molecular masses, Hsps are grouped into five classes: Hsp100/Clp, Hsp90, Hsp70, Hsp70/Chaperonins and small Heat shock proteins (sHsps). Small heat shock proteins (sHsp) with a molecular mass of 15-30 kDa are ubiquitous and conserved. Up to now their function has remained enigmatic. Increased expression under heat shock conditions and their protective effect on cell viability at elevated temperatures suggest that they may have a function in the formation or maintenance of the native conformation of cytosolic proteins (Jakob *et al.*, 1993). Six classes of sHsps

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have been identified in plants based on their intracellular localization and sequence relatedness (Sun *et al.*, 2002).

Here, a cDNA clones coding a small Heat shock protein from hot pepper has been reported. The gene *CasHsp* showed conservation of domains. It also compared the expression patterns of *CasHsp* to high temperature stress and water stress to envisage the conservation of sHsp in plants.

MATERIALS AND METHODS

Plant material, chemicals and bacterial strain

Hot pepper (*Capsicum annuum* cv. Bu Gang) was grown in a growth chamber providing 16 h daily light period, 24-25°C temperature, 60% relative humidity and 200 $\mu Em^{-2}s^{-1}$, white fluorescent lamp. *Escherichia coli* strain DH5α was used for recombinant DNA process. Taq DNA polymerase and DNA ligase were obtained from Promega (USA).

High temperature and dehydration treatments

Young hot pepper plants with 4-6 leaves were chosen for high temperature and dehydration stress. High temperature was given for 1, 2, 3 and 4 h at 42°C. Relative humidity in the incubator for heat shock treatment was maintained at 90% that was measured by a hygrometer (UEI, Korea). Control plants were held in growth room at 25°C under a 16-h photoperiod. For drought treatment, 4-6 week-old seedlings were carefully transferred from their pots onto dry paper in the growth room for different time periods. The stressed plant tissues from all completed treatments were then frozen in liquid nitrogen and stored at -70°C until nucleic acid extraction.

Preparation of cDNA library and double negative screening

A cDNA library for high temperature-stressed hot pepper was used for the study. Briefly, after the poly (A) RNA was purified at 42°C for 30 min, a unidirectional *Eco*RI/*Xho*I cDNA library was constructed in a Uni-λZAP XR vector (Stratagene, USA). The cDNA and vector recombinants were treated with Gigapck III gold packaging extracts (Stratagene) which simplifies the packaging procedure of recombinant λ phage and increases the efficiency. For double negative screening, after amplification and titrating, duplicate plaque lifts were made from 150-nm plates (~5X 10^3 pfu per plate) onto nylon membranes (Hybond-N, Amersham, UK). These lifts were denatured, neutralized, blotted, dried, and exposed to UV for cross-linking. The membranes were then hybridized with two different probes that had been made using poly (A) RNA from either heat-shocked or unstressed (control) hot pepper plants to the ^{32}P-labeled cDNA. The hybridized membranes were washed and exposed to X-ray film overnight (Sambrook *et al.*, 1989). Five hundred plaques showing high-temperature inducibility were randomly selected and subcloned into pBluescript SK(-) via the ExAssist helper phage (M13 stratagene). The cDNA were amplified in *Escherichia coli* after *in vivo* excision.

Isolation, nucleotide sequencing and analysis

Cloned plasmids were extracted and purified with a DNA-spin plasmid DNA purification kit (iNTRONE, Korea) to yield template plasmid DNA suitable for automatic nucleotide sequencing. Both strands of the high temperature stress inducible cDNA inserts in pBluescript SK(-) were sequenced using T_3 and T_7 primers on an automated DNA sequencer (ABI 3730; Applied Bio-systems, USA). Amino acid sequences were deduced from the nucleotide sequences according to the program ExPASY (**E**xpert **P**rotein **A**nalysis **S**ystem) proteomics server of the Swiss Institute of Bioinformatics, (Gasteiger *et al.*, 2003). These nucleotide and deduced amino acid sequences were searched for homologous genes and proteins in the databases, using the PC/Gene software system and BLAST network services at the National Center for Biotechnology Information (Altschul *et al.*, 1997).

Hydropathicity and Phylogenetic analysis

Hydropathicity of the deduced amino acid sequences was calculated as described by Kyte and Doolitle (1982). Multiple alignments and phylogenetic tree were done through the Gene Bee-Molecular Biology server, which is managed by the A.N. Belozersky Institute of Physico-Chemical Biology, Moscow State University, Russia.

RNA extraction and blot hybridization

Total RNA was isolated from plant tissues that previously had been frozen in liquid nitrogen (Zeba *et al.*, 2006). Briefly, these were grounded to powder and homogenized in 3 ml of extraction buffer [100 mM LiCl, 100 mM Tris-Cl (pH 8.0), 10 mM EDTA, and 1% SDS (w/v)]. Before extraction, 3 ml of phenol was added to the extraction buffer and preheated at 80°C for at least 10 min. A mixture of 3 ml of chloroform-isoamylalcohol (24:1) was added to the homogenate followed by vortexing and centrifugation at 10000 g for 25 min at 4°C. Afterward, the supernatant was transferred to a 1.5 ml chloroform-isoamyl alcohol (24:1) mixture. Precipitation was performed in an equal volume of 4M LiCl at -70°C for 2 h. After centrifugation, the pellets were washed with cold 70% ethanol and dissolved in DEPC-treated distilled water. RNA blot hybridization was conducted with α^{32P}-dCTP labeled plasmid DNAs following the standard protocol (Sambrook *et al.*, 1989). The membrane was pre-hybridized for 1 to 2 h, then hybridized at 65°C for 16-18 h in a solution containing 1M dibasic sodium phosphate (P^H 7.2), 14% (w/v) SDS and 0.5 M EDTA (P^H 7.8). The membrane was washed twice in 2X SSPE and 0.1% SDS for 15 min, once at room temperature, then at 65°C; and in 1X SSPE and 0.1% SDS at 65°C, before being exposed to X-ray film.

RESULTS AND DISCUSSION

Nucleotide sequences and deduced amino acid sequences of the of *CasHsp*

From the expressed sequence tag (EST) analysis of a cDNA library, which was prepared from heat-shocked hot pepper, a cDNA clone has been identified, HTI9 (High Temperature Induced 9) encoding a small Heat shock protein. This gene named as *CasHsp* (*Capsicum annuum* small Heat shock protein); its nucleotide sequence is presented in Fig 1.

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1   ggcacgaggacgctacaatcaaagaaaaattcaagctctgtaattgttcatctttgtcttc
63    ttcaaacgacaaagtccccaaaaaatgtctctgattccaagcttcttcggtggtcgcagg
                                  M  S  L  I  P  S  F  F  G  G  R  R    12
123   agcaacatcttcgacccattctccctcgacttatgggatccgttcgaaggcttcccaatt
       S  N  I  F  D  P  F  S  L  D  L  W  D  P  F  E  G  F  P  I    32
183   tcaagcacaatcgccaacacccccctcctctgctcgtgaaacctctgctttcgcaaatgca
       S  S  T  I  A  N  T  P  S  S  A  R  E  T  S  A  F  A  N  A    52
243   agaatcgattggaaagagaccccacaagcgcacatcttcaaagtagacgttccggggatc
       R  I  D  W  K  E  T  P  Q  A  H  I  F  K  V  D  V  P  G  I    72
303   aagagagaggaagtgaaagtcgaagttgaagaaggaaggatactacagataagcggtgag
       K  R  E  E  V  K  V  E  V  E  E  G  R  I  L  Q  I  S  G  E    92
363   aggagcagagagcaagaggagaagaatgatcagtggcaccgtatggagaggagcagtggc
       R  S  R  E  Q  E  E  K  N  D  Q  W  H  R  H  E  R  S  S  G   112
423   aagtttttgaggagatttaggctgccggagaatacgaaaacaggggaaattaaggcagct
       K  F  L  R  R  F  R  L  P  E  N  T  K  T  G  E  I  K  A  A   132
483   atggaggatggagtgctcactgtaactgttccgaaagaagaggagaagaaacctgaggtc
       M  E  D  G  V  L  T  V  T  V  P  K  E  E  E  K  K  P  E  V   152
543   aaggcaattgacatatctggttaaacaagtgcatgcagatcagctgtttttgttgaaagt
       K  A  I  D  I  S  G  *
603   cctttgtctattgtgtttggtgtaatctgaattgtgtgtgtgtaattatctaagttgtgg
663   gtcttggctttgtagttgtaaacaagaaattgttgtatgagcttgattaatgaataattt
723   cctttacatttatttaaaaaaaaaaaaaaaaaa
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Fig. 1 Nucleotide sequence and deduced amino acid sequence for a hot pepper sHsp cDNA clone, *CasHsp*. Residues corresponding to highly conserved sHsp domain are in bold and shadow phases. The protein -coding region are in uppercase letters and the 5′ and 3′ flanking regions are in lower letters. Putative open reading frame (ORF) of *CasHsp* is shown below the nucleotide sequence in amino acids. The ORF started with ATG and ended with TAA. In the 3′ untranslated region, putative polyadenylation sequence is underlined. Nucleotide numbers are on the left side and the amino acid numbers are on the right side.

The 755-bp *CasHsp* contains a 86-b 5′ untranslated sequence and a 189-b 3′ untranslated region flanking the putative open reading frame. This ORF encodes a small Heat shock protein of 159 amino acids, beginning at the initiation codon ATG (Position 87) and ending at the stop codon TAA (Position 564) of the cDNA. *CasHsp* shows one ACD_ScHsp26_like domain, between 53 and 136. sHsps are small stress induced proteins with monomeric masses between 12 -43 kDa, whose common feature is the Alpha-crystalline domain (ACD). Alpha crystalline domain (ACD) found in *Saccharomyces cerevisiae* (Sc) small heat shock protein (Hsp)26 and similar proteins. sHsps are molecular chaperones that suppress protein aggregation and protect against cell stress, and are generally active as large oligomers consisting of multiple subunits (Benesch *et al.*, 2008). ScHsp26 is temperature-regulated, it switches from an inactive to a chaperone-active form upon elevation in temperature. It associates into large 24-mers storage forms which upon heat shock disassociate into dimers. These dimers initiate the interaction with non-native substrate proteins and re-assemble into large globular assemblies having one monomer of substrate bound per dimer. This group also contains *Arabidopsis thaliana* (Ath) Hsp15.7, a peroxisomal matrix protein which can complement the morphological phenotype of *S. cerevisiae* mutants deficient in Hsps26. AthHsp15.7 is minimally expressed under normal conditions and is strongly induced by heat and oxidative stress. Also belonging to this group is wheat HSP16.9 which differs in quaternary structure from the shell-type particles of ScHsp26, it assembles as a dodecameric double disc, with each disc organized as a trimer of dimers.

Characteristics of the protein coded by *CasHsp*

The putative ORF of *CasHsp* showed high homology with the previously reported sHsps of other species. The putative protein encoded by *CasHsp* shares homology with *C. frutescens* (accession no. AAQ19680; Guo *et al.*, 2005) (94.8% homology), *N. tabacum* (accession no. AAD49336; Park and Hong, 1998) (88.1% homology), *V. vinifera* (accession no. CAN60868; Velasco *et al.*, 2007) (84.3% homology) and *A. thaliana* (accession no. NP_200780) (79.9% homology) (Fig 2).

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                    *++****+*+ **********+*+*+********+.++*+++.*+++**++**.*+********
HTI9           ( 1) MSLIPSFFG-GRRSNIFDPFSLDLWDPFEGFPISSTIANTPSSARETSAFANARIDWKET
C.frutescens   ( 1) MSMIPSFFG-GRRSNIFDPVSLDLWDPFEGFPISSTIANTPSSARETSAFPNARIDWKET
N.tabacum      ( 1) MSLIPSFFD-GRRSNIFDPFSLNIWDPFEGFPFSGTVANIPTSTRETAAFSSARIDWKET
V.vinifera     ( 1) MALISSVLGxGRRSNIFDPISLDIWDPFEGFPFSTTLANVPNTARETSAFVNTRIDWKET
A.thaliana     ( 1) MSLIPSIFG-GRRSNVFDPFSQDLWDPFEGFFTPSSALANASTARDVAAFTNARVDWKET

                     *++******+*+***+*+**********++*****+***++*+*****+**++**+*****+****
HTI9           ( 60) PQAHIFKVDVPGIKREEVKVEVEEGRILQISGERSREQEEKNDQWHRMERSSGKFLRRFR
C.frutescens   ( 60) PQAHIFKVDVPGIKREEVKVQVEEGRILQITGERSREQEEKNDQWHRMERSSGKFLRRFR
N.tabacum      ( 60) PESHVFKVDLPGIKKEEVKVEVEEGRVLQISGERSREQEEKNDKWHSMERSSGKFLRRFR
V.Vinifera     ( 61) PEAHVFKADLPGLKKEEVKVEVEEGRVLKISGERTKEQEEKNDKWHRVERSSGKFLRRFR
A.Thaliana     ( 60) PEAHVFKADLPGLKKEEVKVEVEDKNVLQISGERSKENEEKNDKWHRVERASGKFMRRFR

                      ****.**+.****+********.***.++******+*****
HTI9           ( 120) LPENTKTGEIKAAMEDGVLTVTVPKEEEKKPEVKAIDISG-
C.frutescens   ( 120) LPENTKMGEIKAAMENGVLTVTVPKEEEKRSEVKAIDISG-
N.tabacum      ( 120) LPENIKMEEIKATMENGVLTVTVPKMEEKKPEVKAIDISG-
V.vinifera     ( 121) LPENAKMDEVKATMENGVLTVRVPKEEVKKPEVKAIEISG-
A.thaliana     ( 120) LPENAKMEEVKATMENGVLTVVVPKAPEKKPQVKSIDISGan
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Fig. 2 Comparison of the deduced amino acid sequence of the *CasHsp* with the similar protein of *C. frutescens* (Accession no. AAQ19680), *N. tabacum* (Accession no. AAD49336) and *V. vinifera* (Accession no. CAN60868) and *A. thaliana* (Accession no. NP_200780). Signs at the top of the alignment are as follows: '.' is less than mean value plus one SD. '+' is less than mean value plus two SDs. '*' is more than mean value plus two SDs. These definitions are based on Dayoff matrix.

Phylogenetic analysis revealed that *CasHsp* and the sHsp of *C. frutescens* are most similar to each other, and are not allied with the *N. tabacam, V. vinifera* and *A. thaliana* sHsps (Fig 3). Hydropathy value by the method of Kyte and Doolittle (1982) over a window of nine amino acid positions, it was found that CasHsp is very hydrophilic (Fig 4). Hydropathy plots for *CasHsp* and some other sHsp

members from other species commonly revealed distinguished differences particularly at 40-130 residues. However, *CasHsp* and sHsp of *C. frutescens* were more similar whereas sHsp of *N. tabacum*, *Vitis vinifera* and *A. thaliana* showed dissimilarity with them. These sHsp members involved strong hydrophilic regions in the carboxy-terminus (Fig 4).

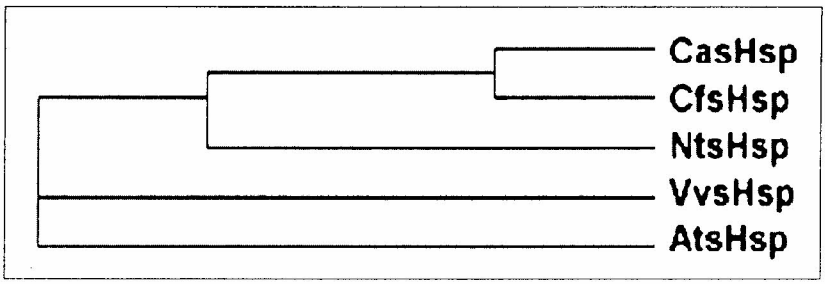

Fig. 3 Phylogenetic tree of *CasHsp* (159 amino acids) and sHsps from other species as, *C. frutescens* sHsp (159 amino acids), *N. tabacum* sHsp (159 amino acids), *Vitis vinifera* sHsp (160 aminoacids) and *A. thaliana* sHsp (161 amino acids).

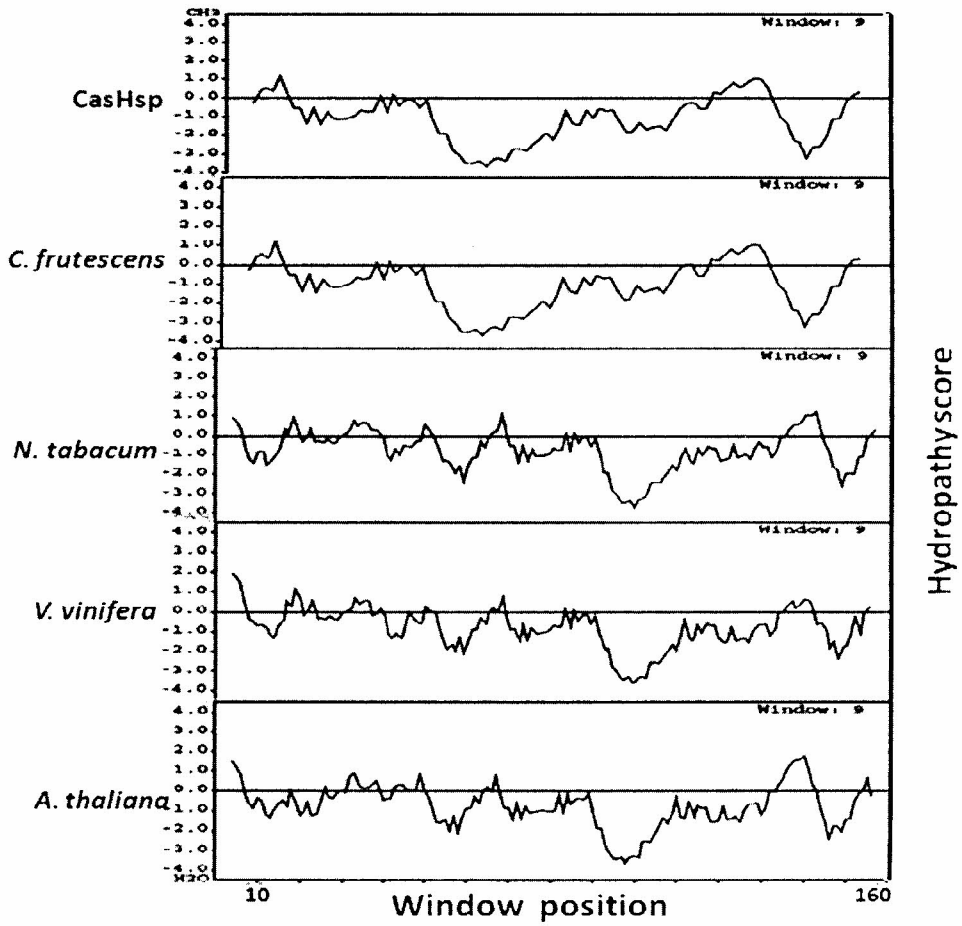

Fig. 4 Hydropathy plot for *CasHsp* and some sHsps from other species. Negative value on the y-axis represents hydrophilicity, and the positive value represents hydrophobicity. Numbers on the x-axis are position of the amino acid starting from the amino-terminus.

Expression patterns of *CasHsp* upon heat shock and dehydration stress in *C. annuum*

To analyze the induction kinetics of *CasHsp* transcripts young hot pepper plants were treated with high temperature and drought stresses. After one hour of heat shock at 42°C, weak transcript appeared at the size of 0.48 Kb (Fig 5a). Within two hour of heat shock transcript level increased and remained at a steady state until 4 hour. No expression was induced under non-heat shock condition. The northern band for the transcript corresponded to the expected transcript size for *CasHsp*. Next, the expression profile of *CasHsp* under drought conditions has been examined. Transcript was first detected after 30 minutes of dehydration stress (Fig 5b). Expression continued to be gradual strong until 4 hour and then declined and was very mild at 12 hour of stress.

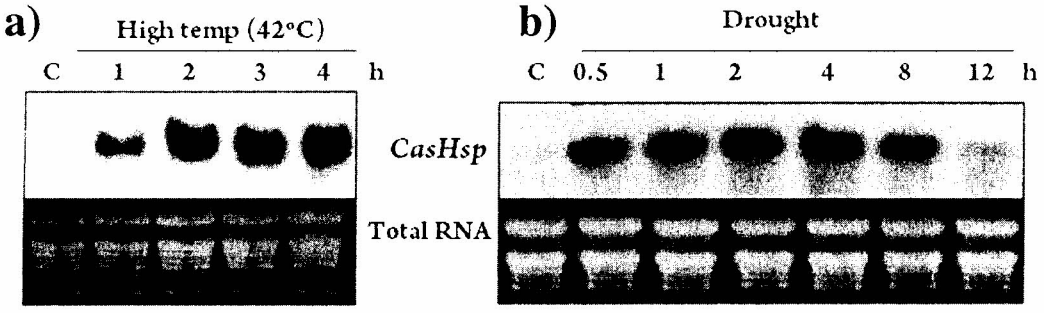

Fig. 5 Induction kinetics of *CasHsp* in response to adverse environmental conditions in hot pepper. Four- week-old plants were subjected to **a)** high temperature and **b)** drought. Treated plants were harvested at indicated time periods and total RNAs were isolated. Total RNAs (20μg) were separated by electrophoresis on 1.2% formaldehyde-agarose gel and blotted to Hybond-N^+ nylon membrane. To ensure equal loading of RNA, gel was stained with ethidium bromide after electrophoresis. To confirm transfer of RNA to membrane filter, both gel and membrane were viewed under UV light at end of transfer. Filter was hybridized to ^{32}P-labeled *CasHsp* cDNA clone visualized by autoradiography.

environmental stresses point to the hypothesis that these proteins play an important role in stress tolerance. The function of sHsps as molecular chaperones is supported by *in vitro* and *in vivo* assays. This review summarizes recent knowledge about plant *sHsp* expression and its functions. Only a few previous researches centered on heat shock protein and heat shock transcription factor (HSFs) on plant (Cho and Hong 2004; Yoon *et al.*, 2005). HSFs are constitutively present and under non-stressed conditions, they exist as monomer forms that usually bind to heat shock protein 70 (Hsp70). HSFs can be activated by heat shock, a process that usually accompanies the dissociation of Hsp70 and homotrimerization of HSFs (Ahn *et al.*, 2001). However, it can be concluded, as high temperature is a major abiotic stress that severely damages crop productivity, extensive research is necessary on plant at molecular level. The possible function of these high temperature stress-inducible genes has been suggested based on the reports for their homolog. Ectopic expression of *CasHsp* will provide more information of its function, not only for tolerance but also of novel cis-acting promoter elements involved in various stresses. Functional analysis of *CasHsp* is still necessary.

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REFERENCES

Ahn, S. G, Liu, P. C. C, Klyachko, K., Morimoto, R. I. and Thiele, D. J. 2001. The loop domain of heat shock transcription factor 1 dictates DNA-binding specificity and responses to heat stress. *Genes Dev.* 15: 2134-2145.

Altschul, S. F., Madden, T. L., Schaffer, A. A., Zhang, Z., Miller, W. and Lipman, D. J. 1997. Gapped BLAST and PSI BLAST: A new generation of protein database search programs. *Nucleic Acids Res.* 25: 3389-3402.

Bukau, B. and Horwich, A. L. 1998. The Hsp70 and Hsp60 chaperone machines. *Cell.* 6; 92(3): 351-366.

Benesch, J. L., Ayoub, M., Robinson, C. V. and Aquiliana, J. A. 2008. Small heat shockprotein activity is regulated by variable oligomeric substructure. *J. Biol. Chem.* 283: 28513-28517.

Cho, E. K. and Bae, S. 2006. ATP-independent Thermoprotective Activity of *Nicotiana tabacum* Heat Shock Protein 70 in *Escherichia coli. J. Biochem. Mol. Biol.* 40:107-112.

Cho, E. K. and Hong, C. B. 2004. Molecular cloning and characterization and characterization of three heat shock protein 70 genes from Nicotiana tabacum. *J. Plant Biol.* 47:149-159.

Gasteiger, E., Gattiker, A., Hoogland, C., Ivanyi, I., Appel, R. D. and Bairoch, A. 2003. ExPASY: The proteomics server for in-depth protein knowledge and analysis. *Nucleic Acids Res.* 31:3784-3788.

Guo, S. J., Chen, N., Guo, P. and Meng, Q. W. 2005. cDNA cloning and expression of a cytosolic small heat shock protein gene (CaHSP18) from *Capsicum annuum. Zhi Wu Sheng Li Yu Fen Zi Sheng Wu Xue Xue Bao.* 31(4):409-416.

Isbat, M., Zeba, N., Kim, S. R., and Hong, C. B. 2009. A BAX inhibitor-1 gene in *Capsicum annuum* is induced under various abiotic stresses and endows multi-tolerance in transgenic tobacco. *J. Plant. Physiol.* 166(15):1685-1693.

Jakob, U., Gaestel, M., Engel, K. and Buhner, J. 1993. Small heat shock proteins are molecular chaperones. *J. Biol. Chem.* 168(3):1517-1520.

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

Lee, G. J. and Vierling, E. 2000. A small heat shock protein cooperates with heat shock protein 70 systems to reactivate a heat-denatured protein. *Plant Physiol.* 122: 189-198.

Lee, S. Y. and Tsai, F. T. F. 2005. Molecular chaperones in protein quality control. *J. Biochem. Mol. Biol.* 38: 259-265.

Mayer, M. P., Brehmer, D., Gassler, D. S. and Bukau, B. 2001. Advances in protein chemistry: Protien folding in the cell. *Academic press.* 59:1-44.

Miernyk, J. A.1999. Protein folding in the plant cell. *Plant Physiol.* 121: 695-703.

Mogk, A., Tomoyasu, T., Goloubinoff, P., Rudiger, S., Roder, D., Langen, H. and Bakau, B. 1999. Identification of thermolabile *E.coli* proteins: prevention and reversion of aggregation by Dnak and ClpB. *J. EMBO,* 18: 6934-6949.

Park, S., M. and Hong, C., B. 1998. Comparison of the structure and expression pattern for a low molecular weight heat-shock protein cDNA clone from *Nicotiana tabacum. Mol. Cells.* 8(5): 594-599.

Sambrook, J., Fritsch, E. F. and Maniatis, T. 1989. Molecular Cloning: A Laboratory Manual, 2nded. Cold Spring Harbor Laboratory Press, New York.

Sun, W., Van Montagu, M. and Verbruggen, N. 2002. Small heat shock proteins and stress tolerance in plants. *Biochim. Biophys. Acta.* 1577 (1): 1-9.

Tatsuta, T., Tomoyasu, T., Bukau, B., Kitagawa, M., Mori, H., Karata, K. and Ogura, T. 1998. Heat shock regulation in the ftsH null mutant of Escherichia coli: Dissection of stability and activity control mechanisms of σ 32 *in vivo. Mol. Microbiol.* 30: 583-593.

Tomoyasu, T., Mogk, A., Langen, H., Goloubinoff, P. and Bukau, B. 2001. Genetic dissection of the roles of chaperones and proteases in protein folding and degradation in the *Escherichia coli* cytosol. *Mol. Microbiol.* 40: 397-413.

Valiollah, M., Bihamta, M. and Zali, A. 2007. Evaluation of Screening techniques for heat tolerance in wheat. *Pakistan J. Biol. Sci.* 10(6): 887-892.

Velasco, R., Zharkikh, A., Troggio, M., Cartwright, D. A., Cestaro, A., Pruss, D., Pindo, M., Fitzgerald, L. M., Vezzulli, S., Reid, J., Malacarne, G., Iliev, D., Coppola, G., Wardell, B., Micheletti, D., Macalma, T., Facci, M., Mitchell, J.T., Perazzolli, M., Eldredge, G., Gatto, P., Oyzerski, R., Moretto, M., Gutin, N., Stefanini, M., Chen, Y., Segala, C., Davenport, C., Dematte, L., Mraz, A., Battilana, J., Stormo, K., Costa, F., Tao, Q., Si-Ammour, A., Harkins, T., Lackey, A., Perbost, C., Taillon, B., Stella, A., Solovyev, V., Fawcett, J. A., Sterck, L., Vandepoele, K., Grando, S. M., Toppo, S., Moser, C., Lanchbury, J., Bogden, R., Skolnick, M., Sgaramella, V., Bhatnagar, S. K., Fontana, P., Gutin, A., Van de Peer, Y., Salamini, F. and Viola, R. 2007. A high quality draft consensus sequence of the genome of a heterozygous grape vine variety. *PLoS One.* 2(12):e1326.

Veinger, L., Diamant, S., Buchner, J. and Goloubinoff, P. 1998. The small heat-shock protein IbpB from Escherichia coli stabilizes stress-denatured proteins for subsequent refolding by a multichaperone network. *J. Biol. Chem.* 273: 11032-11037.

White, H. E., Orlova, E. V. and Chen, S. 2006. Multiple distinct assemblies reveal conformational flexibility in the small heat shock protein Hsp26. *Sturcture.* 14(7): 1197-1204.

Wu, C. (1995). Heat shock transcription factors: structure and regulation. *Ann. Rev. of cell and dev. Boil.* 11: 441–469.

Yoon, H., Kim, K. P., Park, S.M. and Hong, C. B. 2005. Functional mode of NtHSP17.6, a cytosolic small heat shock protein, from *Nicotiana tabacum. J. Plant Biol.* 48:120-127.

Zeba, N., Ashrafuzzaman, M. and Hong , C. B. 2006. Molecular characterization of *Capsicum annum* RING Zinc Finger Protein 1 (*CaRZFP1*) gene induced by abiotic stresses. *J. Plant. Biol.* 49(6):484-490.

Zeba, N., Isbat, M., Kwon, N., Lee, M. O., Kim, S. R. and Hong, C. B. 2009. Heat-inducible C3HC4 type RING zinc finger protein gene from *Capsicum annuum* enhances growth of transgenic tobacco. *Planta.* 229(4):861-871.