Cloning and *In silico* characterization of HSP90 gene from wheat (*Triticum aestivum* L)

Ranjeet Ranjan Kumar[1]*, Suneha Goswami[1], Khushboo Singh[1], Sushil Kumar Sharma[1] and Raj Deo Rai[1]

[1]Division of Biochemistry, Indian Agricultural Research Institute (IARI), New Delhi

HSP90 is an essential heat shock protein found in all eukaryotes studied so far. This protein is known for its role in facilitating maturation of signaling molecules, manage protein folding, cell cycle control, protein degradation and protein trafficking. In plants HSP90 is localized in cytosol, chloroplast, mitochondria and ER. In present investigation an amplicon of ~2.5 kb was amplified from C-306 wheat cultivar. BLASTn analysis showed 96% homology with HSP90 of *Triticum aestivum* (Accession number GQ240780.1). *In silico* characterization of HSP90 revealed that it has an ORF from 62 to 2164 encoded for 700 amino acids. The sequence was submitted in Gen Bank with accession no JN052206. Clustal W analysis of HSP90 with HSP90 sequence (reported from wheat) showed large variability in HSP90 nucleotide sequence. Based on phylogeny analysis, all HSP90 reported till date from wheat has been classified into four different subgroups and HSP90 belongs to subgroup IV showing same origin as that of *Triticum aestivum* (Accession number GQ240780.1 and GQ40779.1). There is a need to exploit the stages and tissues specific expression profiling of HSP90 in order to enhance the thermo tolerance capacity of wheat.

Keywords: HSP90, *In silico*, Wheat, Phylogeny.

INTRODUCTION

In plants expression pattern of a number of genes modulated in response to various acute environmental changes. This results in altered biochemical and physiological activity of the cell and developmental pursuit of the organism. When plants were exposed to high temperatures a new expression pattern was observed by the biosynthesis of heat shock proteins (Hsps) (Lindquist and Craig, 1988; Vierling, 1991; Miernyk, 1999). The heat-shock response in plants is similar to that of other organisms. Trimerization and activation of heat shock factors (HSFs) regulate the induction of heat shock genes. HSFs act in the promoter region of the HSP genes through a well-defined and highly conserved heat shock element (HSE). Hsp are essential components of cells and developmental processes under normal physiological conditions (Rutherford and Lindquist, 1998) besides its role in heat stress. Earlier results reveal that most Hsps serve as molecular chaperones (Georgopoulos and Welch, 1993; Ma et al., 2022; Bukau and Horwich, 1998; Pratt et al., 2001).

The HSP90s are highly conserved and abundant cytosolic proteins in eukaryotes (Prasinos C. et.al. 2004). Unlike other heat shock proteins, the chaperone activity of the HSP90 is exerted on a number of target substrates or client proteins including steroid hormone receptors, cell cycle kinases, signal transduction pathway components, proteolytic machinery, and microtubule dynamics (Czar et al., 1997; Nathan et al., 1997; Garcia-Gardena et al., 1998; Holt et al., 1999; Pratt et al., 2001). However, the high abundance of HSP90 compared with that of its client proteins, the high levels of expression in response to stress, and the existence of endoplasmic

Abbreviations: HSP: heat shock protein; PCR: polymerase chain reaction; RT: reverse transcription

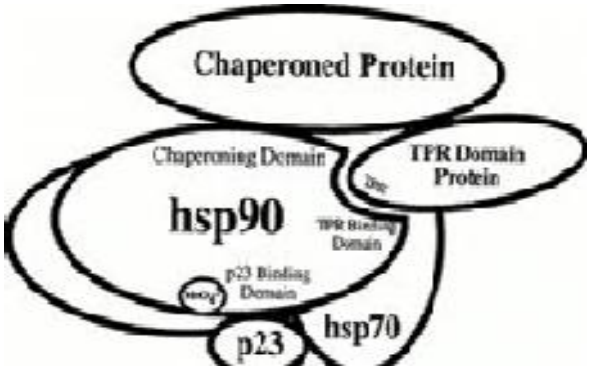


Figure 1. HSP90 acting as multichaperone machine together with Hsp70 and other cohort of co-chaperones

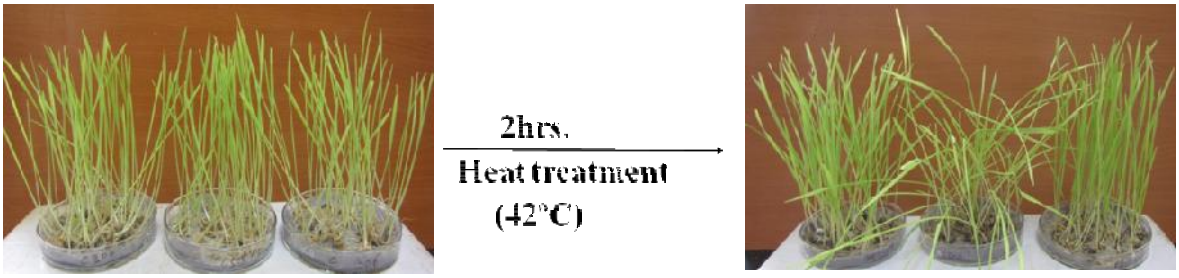


Figure 2. Change in phenotypic character of wheat cultivars exposed to heat shock

reticulum (ER), chloroplasts, and mitochondria homologues suggests that this is a very narrow view of the Hsp90 cellular activity and further indicates that it may contribute to additional functions in the cytosol and organelles under physiological or stress conditions.HSP90 acts as part of a multichaperone machine together with Hsp70 and co-operates with a cohort of co-chaperones, including Hip (Hsp70 interacting protein), Hop (Hsp70/Hsp90 organizing protein), p23 and Hsp40 (a DnaJ homolog) (Figure 1).

Evidence showed participation of Hsp90s in different developmentally, hormonally, and morphogenetically regulated processes (Dhaubhadel et al., 1999; Ludwig-Muller et al., 2000; Berardini et al., 2001; Muessig et al., 2002). Plants acquire thermotolerance to lethal high temperatures if first exposed to moderately high temperature or if temperature is increased gradually to an otherwise lethal temperature. Heat stress due to high ambient temperatures is a serious threat to crop production worldwide (Hall, 2001).Immediately after exposure to high temperatures and perception of signals, changes occur at the molecular level altering the expression of genes and accumulation of transcripts, thereby leading to the synthesis of stress-related proteins as a stress tolerance strategy (Iba, 2002). Expression of heat shock proteins (HSPs) is known to be an important adaptive strategy in this regard (Feder and Hoffman, 1999).

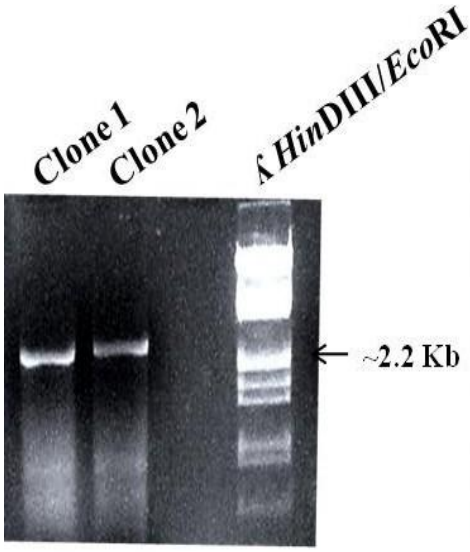
MATERIAL AND METHODS

Plant Material and heat shock treatment

The thermotolernat cultivar (C-306) of wheat (*Triticum aestivum*) was selected for the experiment because of its diverse nature of tolerance against different abiotic stresses and is also a popular cultivar in north India. Seeds were collected from Division of Genetics, Indian Agricultural Research Institute (IARI) and sown in phytotron under controlled conditions (22˚C, 16hlight/8h dark cycle). Fourteen days-old (14 old) seedl i ngs were subjected to heat shock treatment of 42°C for 2h (Figure 2). Plants of the same age that were not exposed to thermal stress were included as controls.Samples were immediately frozen in liquid nitrogen and maintained at -80°C.

Isolation and cloning of HSP90 in wheat

After an *In silico* identification of conserved sequence regions of HSP90 genes in cereals,a pair of degenerated primers was designed for PCR amplification of the corresponding gene sequence in wheat. Total RNA were isolated from 14 days old germinating wheat seedlings by Trizol method (Invitrogen). cDNA were synthesized using oligo dT primer (RevertAid^{TM} H minus First Strand cDNA synthesis kit, Fermentas). cDNA synthesized from wheat



(a)

MATETETFAFQAEINQLLSLIINTFYSNKEIFLRELISNSSDALDKIRFESL
TDKSKLDAQPELFIHIIPDKATNTLTIVDSGIGMTKSDLVNNLGTIARSGT
KEFMEALAAGADVSMIGQFGVGFYSAYLVAERVVVTTKHNDDEQYVW
ESQAGGSFTVTRDTSGEQLGRGTKMVLYLKDDQMEYLEERRIKDLVK
KHSEFISYPISLWTEKTTEKEISDDEDEEEKKDTEEGKVEDVDEEKEEKE
KKKKKIKEVSHEWNLVNKQKPIWMRKPEEINKEEYAAFYKSLTNDWE
EHLAVKHFSVEGQLEFKAVLFVPKRAPFDLFDNKKKANNIKLYVRRVFI
MDNCEELIPEYLSFVKGIVDSEDLPLNISRETLQQNKILKVIRKNLVKKC
IELFFEINKEDYNKFYEAFSKNLKLGIHEDSQNRTKIAELLRYHSTKSGD
ELTSLKDYVTRMKEGQNEIYYITGESKKAVENSPFLEKLKKKGYEVIYM
VDAIDEYAIGQLKEFEGKKLVSATKEGLKLDESEDEKKKQEELKEKFEG
LCKVIKEVLGDKVEKVIVSDRVVDSPCCLVTGEYGWTANMERIMKAQA
LRDSSMAGYMSSKKTMEINPENAIMDELRKRADADKNDKSVKDLVML
LFETSLLTSGFSLEDPNTFGTRIHRMLKLGLSIDEDDEAPENDTDMPPLE
DDAGESKMEEVD*

(b)

Figure 3. (a) Reverse Transcription PCR amplification of heat shock protein using HSP90 gene specific primers, (b) Open reading frame of amplified and cloned HSP90 gene which was submitted in NCBI GenBank with accession no JN052206.1

cultivars were used for PCR amplification of HSP90 gene by using the degenerate primers (hsp90-F: 5'-TCC CGC ACG CTT CTC CT-3' and hsp90-R: 5'-AAC TGT TCC ACG AGT ACC ACA-3'). The amplified product was cloned in pGEMT$_{easy}$ vector (Promega) and sequenced using M13 forward and reverses primers.

In silico analysis of HSP90 gene

The isolated HSP90 gene was submitted in NCBI GenBank(http://www.ncbi.nlm.nih.gov) using Bankit(http://www.ncbi.nlm.nih.gov/WebSub/?tool=genbank). The translational characterization of isolated gene was carried out using Expasy tool (http://expasy.org/tools/). Open Reading Frame of the isolated gene was characterized using ORF Finder (http://www.ncbi.nlm.nih.gov/projects/gorf/). Different HSP90 gene reported from plant and non-plant sources were retrieved from GenBank and were aligned along with HSP90 isolated in present investigation using ClustalW 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. Delay divergent sequences were set at 30% robustness of the phylogenetic trees was assessed from the bootstrap value for each internal node of the neighbour joining tree by calculating 1000 random samplings (Felsenstein 1985). Conserved domain search was carried out using Simple Modular Architecture Retrieval Tool (SMART) and phosphorylating sites were scanned using NetPhos 2.0 server.

RESULTS AND DISCUSSION

Sequence identification of HSP90 gene from wheat

An amplicon of approximately 2.2 kb was amplified from C-306 cv of wheat by RT-PCR and cloned in maintenance vector(Figure 3a). The sequencing of amplicon showed the presence of 2323 nucleotides in the sequence. BLASTn homology analysis showed that the amplified sequence has 96% resemblance with HSP90 gene of wheat. The gene was submitted to NCBI GenBank with accession number JN052206.

In silico Characterization

HSP90 gene isolated in present investigation has an ORF of 700 aa with start codon at 62bp and stop codon at 2164bp(Figure 3b). ClustalW showed variability in HSP90 sequence and phylogeny of HSP90 gene of wheat sequences collected from GenBank classified it into four different subgroups. HSP90 belongs to subgroup having the same origin as that of *Triticum aestivum* (Accession no. GQ240780.1 and GQ40779.1) (Figure 4). HSP 90 can create heritable traits in brewer's yeast (*Saccharomyces cerevisiae*) by affecting a large portion of the yeast genome. It appears that HSP90 of chloroplasts derives from the endoplasmic reticulum specific isoform while mitochondrial HSP90 homologs affiliate with a eubacterial lineage other than α subdivision of proteobacteria. The finding has led to the conclusion that HSP90 has played a key role in genome evolution.

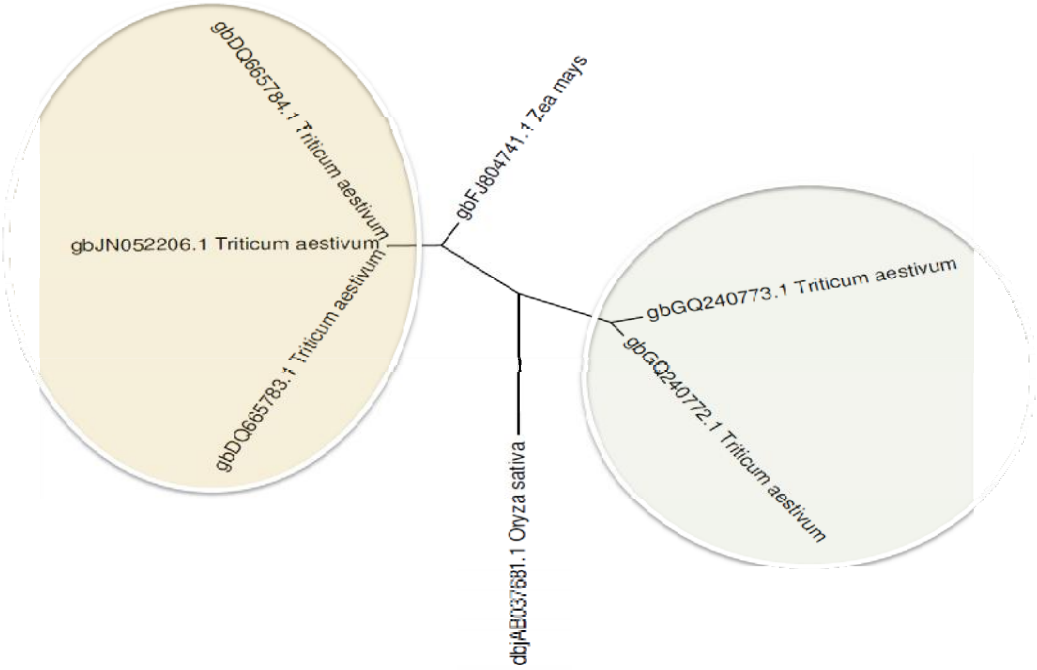


Figure 4. Phylogeny analysis of heat shock protein90 gene isolated in present investigation with that of other HSP90 genes reported from wheat

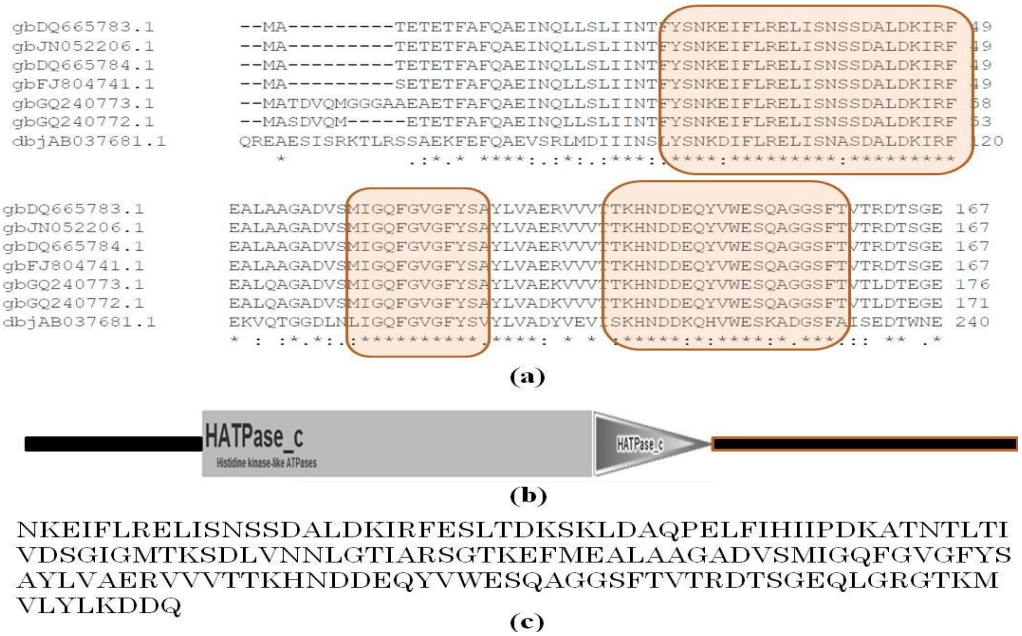


Figure 5.(a) Clustal W alignment of HSP90 gene isolated in present investigation with that of other HSP90 genes reported from plant and non-plant sources, (b) HATPase_C domain observed in the HSP90 gene predicted to be the functional domain using SMART software, (c) Amino acid sequence of the stretch of HSP90 gene predicted to code for functional domain (HATPase)

The sequence isolated in present investigation showed maximum homology with DQ665784.1 and DQ665783.1 reported from *Triticum aestivum* L and has been kept in one group.The sequence of HSP90 reported from maize and rice showed totally different phylogenetic origin. Three prominent conserved domain were characterized in the HSP90 genes reported from plant sources(Figure 5a).

The domain YSNKDIFLRELISN(S/A) SDALDKIRF were observed in all most all the sequences of HSP90

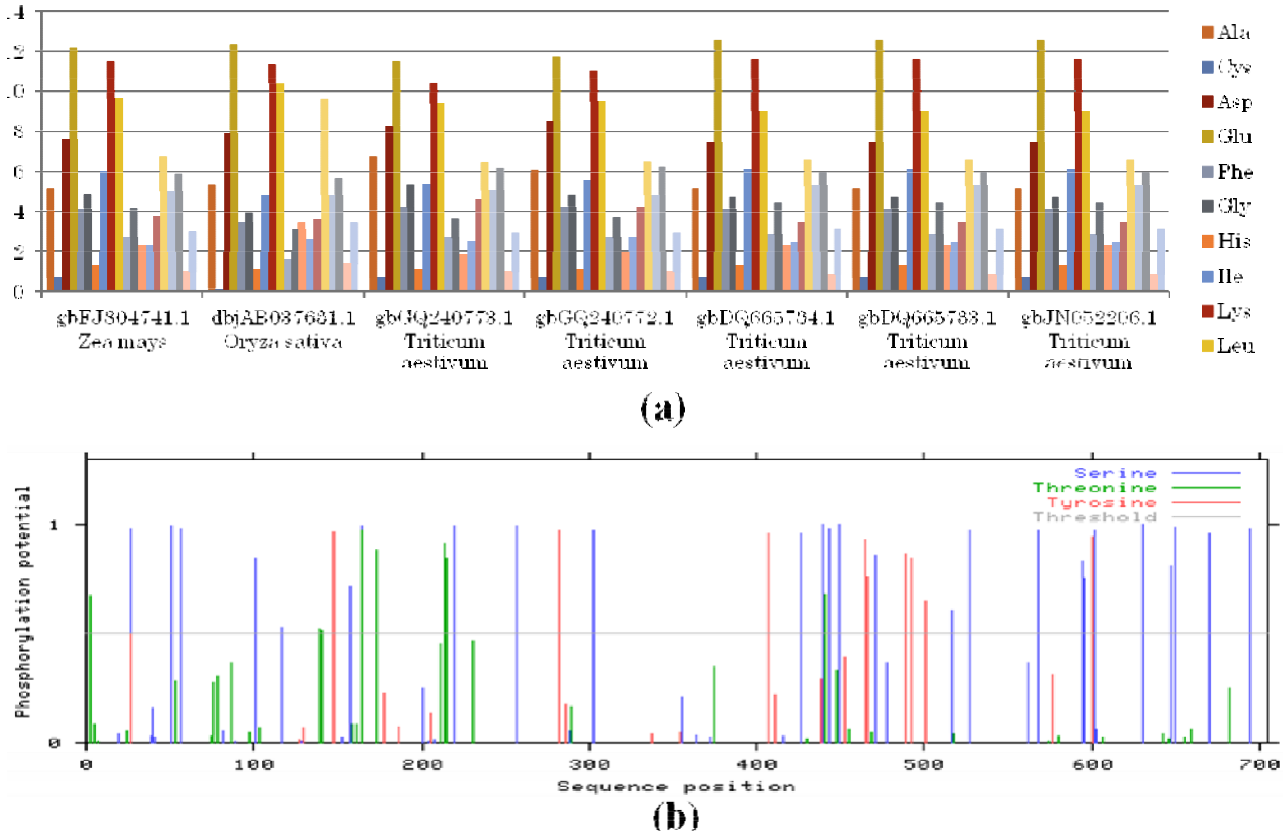


Figure6.(a) Diversity in amino acid composition of HSP90 cloned in present investigation with that of other HSP90 genes reported from plant sources, (b) Phosphorylating sites predicted in HSP90 amino acid sequence using NetPhos 2.0

which gives us a prediction that this domain might be playing the important role in the functional activity of the HSP90 in response to heat stress. Other than this, some other potential domains were also observed like KHNDD(X)Q(X)VWES(X)A(X)GSF and IGQFGVGFYS which may be the potential phosphorylating sites for the HSP90 during heat stress.An functional domain characterization of HSP90 showed the presence of HATPase domain in the amino acid sequence (Figure 5b). The amino acid sequence of HATPase domain was also retrieved using SMART tool (Figure 5c).However, recent studies have uncovered structures of full length HSP90 from bacteria (Shiau et al., 2006), yeast (Ali et al., 2006) and mammals (Dollins et al., 2007). Their domain architecture is similar, but they exhibit large subdomain rotations relative to each other. PgHSP90 isolated from *Pennesitum typhoides* possesses the five-conserved amino acid signature sequence motifs characteristic of the Hsp90 family and a C-terminus MEEVD penta-peptide characteristic of the cytosolic Hsp90 isoform (Reddy et al., 2010). HSP 90 genes reported from different plant sources were retrieved from Gen bank and were characterized along with the HSP isolated in present investigation for their diversity in amino acid compositions (Figure 6a) The percentage of glutamic acid in almost all the HSPs were highest (>12%) followed by aspartic acid compare to alanine whose percentage was found to be lowest. The percentage of acidic amino acid in all the HSP90 sequences were more compare to neutral and basic amino acids. Fares et al. (2006) research on coevolution analysis and functional data for heat-shock proteins, HSP90 and GroEL, highlight that almost all detected coevolving sites are functionally or structurally important and he proposed that new amino acid sites are important for interdomain functional communication.

The characterization of isolated HSP90 gene sequence for the phosphorylation sites using Netphos 2.0 software revealed the presence of 39 potential phosphorylating sites in the sequence which gives us idea about the functional nature of the isolated HSP gene (Figure 6b).

In all eukaryotes tested, Hsp90 is essential, abundant at normal temperatures, and induced by stress. Under physiological conditions, Hsp90 dynamically interacts with a diverse but highly select set of inherently unstable ‘client’ proteins (for example, kinases and transcription factors). It keeps these metastable proteins poised for activation until they are stabilized by conformational changes, such as those associated with signal transduction. The requirement of many principal regulatory proteins for Hsp90 renders entire pathways sensitive to decreases in its function (Salathia et al., 2007).

CONCLUSION

HSP90 plays very important role in providing tolerance against different abiotic stresses in plant. It acts as a sensor in transferring the signal of heat stress as well as protects the native protein from denaturation. Here, we have cloned a HSP90 gene from wheat having ORF of 700aa. *In silico* characterization showed the presence of HATPase domain in the sequence predicted to be functional domain of the gene. The amino acid sequence composition showed the abundance of glutamic acid in almost all the HSP90s reported from plant sources. This HSP belongs to one of the most important family of heat stable protein and there is a need to further exploit HSP90 gene by over-expressing it in cereals crops like wheat in order to see the actual effect of this protein on tolerance capacity of the plant against different abiotic stresses. This gene can be used for developing or enhancing the tolerance capacity of the plant against different abiotic stresses using the tool of genetic engineering.

ACKNOWLEDGMENT

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

REFERENCES

Ali MM, Roe SM, Vaughan CK, Meyer P, Panaretou B, Piper PW, Prodromou C, Pearl LH (2006). Crystal structure of an Hsp90-nucleotide-p23/Sba1 closed chaperone. Nature 440:1013-1017.

Berardini TZ, Bollman K, Sun H, Poethig RS (2001). Regulation of vegetative phase change in Arabidopsis thaliana by cyclophilin 40. Sci. 291:2405–2407.

Bukau B, Horwich AL (1998). The Hsp70 and Hsp60 chaperone machines. Cell 92: 351–366.complex. Nature 440:1013–1017.

Constantinos P, Konstantinos K, Despina S, Polydefkis H (2004). Tight regulation of expression of two Arabidopsis cytosolic Hsp90 genes during embryo development. J Exp. Bot. 56:633–644.

Czar MJ, Galigniana MD, Silverstein AM, Pratt WB (1997). Geldanamycin, a heat shock protein 90-binding benzoquinone ansamysin, inhibits steroid-dependent translocation of the glucocorticoid receptor from the cytoplasm to the nucleus. Biochem. 36:7776–7785.

Dhaubhadel S, Chaudhary S, Dobinson KF, Krishna P (1999).Treatment with 24-epibrassinolide, a brassinosteroid, increases the basic thermotolerance of Brassica napus and tomato seedlings. Plant Mol. Biol. 40:333–342.

Dollins DE, Warren JJ, Immormino RM, Gewirth DT (2007). Structures of GRP94-nucleotide complexes reveal mechanistic differences between the Hsp90 chaperones. Mol. Cell 28:41–56.

Fares MA, Travers SAA (2006). A Novel Method for Detecting Intramolecular Coevolution: Adding a Further Dimension to Selective Constraints Analyses. Genet. 173:9-23.

Feder ME, Hoffman GE (1999). Heat-shock proteins, molecular chaperones, and the stress response: evolutionary and ecological physiology. Annu.Rev.Physiol. 61:243–282.

Garcia-Gardena G, Fan R, Shah V, Sorrentino R, Cirino G, Papapetropoulos A, Sessa WC (1998). Dynamic activation of endothelial nitric oxide synthase by Hsp90. Nat. 39:821–824.

Georgopoulos C, Welch WJ (1993). Role of the major heat shock proteins as molecular chaperones. Ann. Rev. Cell Biol. 9:601–634.

Hall AE (2001). Crop Responses to Environment. CRC Press

Holt SE, Aisner DL, Baur J , Tesmer VM, Dy M, Ouellette M, Tranger JB, Morin GB, Toft DO, Shay JW , Wright WE, White MA (1999). Functional requirement of p23 and Hsp90 in telomerase complexes. Genes Dev. 13:817–826.

Iba, K (2002). Acclimative response to temperature stress in higher plants: approaches of gene engineering for temperature tolerance. Annu. Rev. Plant Biol. 53:225–245.

Lindquist S, Craig EA (1988). The heat-shock proteins. Ann. Rev. Genet. 22:631–677.

Ludwig-Muller J, Krishna P, Forreiter C (2000). A glucosinolate mutant of Arabidopsis is thermosensitive and defective in cytosolic hsp90 expression after heat stress. Plant Physiol. 123:949–958.

Ma L, Gao Y, Chen Z, Li J, Zhao H, Deng XW (2002). Genomic evidence for COP1 as a repressor of light-regulated gene expression and development in Arabidopsis. Plant Cell 14:2383–2398.

Miernyk JA (1999). Protein folding in the plant cell. Plant Physiol. 121:695–703.

Muessig C, Fischer S, Altmann T (2002). Brassinosteroid-regulated gene expression. Plant Physiol. 129:1241–1251.

Nathan DF, Vos MH, Lindquist S (1997). In vivo functions of the Saccaromycescerevisiae Hsp90 chaperone. Proceedings of the National Academy of Sciences, USA 94: 12949–12956.

Pratt WB, Krishna P, Olsen LJ (2001). Hsp90-binding immunophilins in plants: the protein movers. Trends Plant Sci. 6:54–58.

Reddy MK, Reddy PS, Mallikarjuna G, Kaul T, Chakradhar T, Mishra RN, Sopory SK (2010). Molecular cloning and characterization of gene encoding for cytoplasmic Hsc70 from Pennisetumglaucum may play a protective role against abiotic stresses. Mol. Genet. Gen. 283:243–254.

Salathia N, Queitsch C (2007). Molecular mechanisms of canalization: Hsp90 and beyond. J. Biosci. 32:457–463.

Shiau AK, Harris SF, Southworth DR, Agard DA (2006). Structural analysis of E. coli hsp90 reveals dramatic nucleotide-dependent conformational rearrangements. Cell 127:329–340.

Vierling E (1991). The roles of heat-shock proteins in plants. Ann. Rev. Plant Physiol. Plant Mol. Biol. 42:579–620.