MOLECULAR EVOLUTION AND DIVERSITY OF SMALL HEAT SHOCK PROTEINS GENES IN PLANTS

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Abstract

Small heat shock proteins (sHSPs) are the most abundant proteins and considered as *Cinderella* of molecular chaperon world. The present study is to understand the evolutionary process that led to the diversification of sHSPs specific to plants because of dramatic daily fluctuation in the temperature and other environmental factors which may prompt more efficient chaperon activity of sHSPs. For this purpose mRNA and protein sequences of 62 different plant sHSPs was mined from different databases and analyzed with Clustal W and MEGA 5 Beta # 7 software. Two Neighbor Joining (NJ) and two Dendrogram large congruent trees were obtained from the phylogenetic analysis of mRNA and amino acids. These analyses reveal that sHSPs encoded by one gene family are similar to each other even in different plant species. However, sHSPs belonging to different gene families show very low sequence similarity even in same plant species. These analyses also suggest that gene duplication and mutation play an important role in the evolution and diversification of sHSP.

Introduction

Every living organism responds to temperatures greater then their optimal growth temperature with increased expression of a definet class of functionally related proteins called heat shock proteins (HSPs). There are six structurally conserved distinct families of HSPs, HSP100, HSP90, HSP70, HSP60 (Chaperonins), small HSPs (sHSPs) (~17-30 kDa) and ubiquitin (8.5 kDa) (Waters et al., 1996; Vierling, 1997), sHSPs are the most abundant proteins and considered as Cinderella of HSP world (Van-den-Ussel et al., 1999, Mahmood et al., 2010) because sHSPs are also expressed under normal conditions in several organisms such as vertebrates (Liossis et al., 1998), Drosophila (McKenzie and Meselson 1977) and plants (Carranco et al., 1999). sHSPs are divided into 6 classes; three classes (CI, CII and CIII) are in the nucleus or cytosole and the other three (CIV, CV and CVI) are in the mitochondria chloroplasts and endoplasmic reticulum (Mahmood et al., 2010). This diverse family contains a α -crystalline structural domain. The significance of this α -crystalline domain is its 90 amino acid conserved region found in the C-terminal end of the sHSPs while N-terminal side contains unconserved variable length amino acid sequences (Basha et al., 2010). The α -crystalline domain contains several beta-strands organized into two beta-sheets responsible for dimer formation, the basic building block of most sHSPs. The amino-terminal extension modulates oligomerization, subunit dynamics and substrate binding, whereas the flexible carboxy-terminal extension promotes solubility, chaperoning and oligomerization, the latter by intersubunit linkage (Sun & MacRae, 2005).

sHSPs organized into large, sphere-like structures commonly consisting of 12 or 24 subunits forming two distinct types of octahedral oligomers. During the stress, some structural changes occurs resulting in increased chaperon activity (Haslbeck *et al.*, 2008). sHSP-substrate complexes with varying stability and composition can protect all substrate equally, and substrate protection is

not correlated with sHSP oligomeric stability (Basha et al., 2006).

sHSPs recognized damaged proteins and sort them into repaired form, degraded or transported across the membranes by helping them to stabilize (Nakamoto & Vigh, 2007; Toth et al., 2010). sHSPs specifically interact with the membrane lipid and control fluidity and permeability (Porta et al., 2010; Horváth & Vígh, 2010.). High temperature treatment increased the expression of HSP which inhibit tobacco mosaic viral RNA synthesis and resume when temperature decreased (Arif et al., 2005). Iqbal et al., (2010) reported that Rice sHSPs not only protect the high molecular weight proteins from heat induced but also enhance their impact in chilling tolerance. These proteins also play a fundamental role in pathology of human diseases like cancer, the neurodegenerative diseases, diabetes, prevention of apoptosis after ischemic injury, cardiac myocyte function, platelet aggregation and skeletal muscle function (Fan et al., 2005; Nakamoto & Vigh, 2007). In aged-muscle sHSPs shows a dramatic increase in expression for essential cellular response to fiber aging and might therefore be a novel therapeutic option to treat sarcopenia of old age (Doran et al., 2007).

sHSPs are also expressed during normal developmental stages like fruit maturation, pollen growth, germination and embryogenesis for the recovery of damaged and newly synthesized proteins (Mehmood *et al.*, 2010). In *Pisum sativum, Arabidopsis thaliana, Zea mays, Triticum aestivum*, sunflower, alfalfa, tobacco and tomato sHSPs are produced in a stage-specific fashion suggesting that certain sHSP genes may play specific roles in early, others during later stages of development (waters *et al.*, 1996; Waters & Rioflorido, 2007).

Gene duplication and mutation play an important role in the evolutionary process and diversification of species (Wright and Gaut, 2005). Advantageous mutation in duplicated genes may derive the gene to a new function

and the original function remains preserved in another copy (Wu et al., 1995). Genetic changes and diversification in sHSPs specific to plants is of particular interest because dramatic daily fluctuation in the temperature and other environmental factors may prompt more efficient chaperon activity of sHSPs (Kriehuber et al., 2010). The sHSPs encoded by one gene family are similar to each other even in different plant species. The sequence similarity can be up to 93% and identity up to 85% (Vierling, 1991). However, sHSPs of one plant species belonging to different families show very low sequence similarity (50-75%), and identity usually below 50%. This applies not only for the comparisons of sHSPs between divergent species, but also for comparisons between different classes of plant sHSPs (Waters et al., 1996). Even under same heat shock conditions different sHSPs of are accumulated in different variety of the same species (Iqbal et al., 2010).

Naz et al., (2006) reported that the most of the high and low molecular weight HSPs remain conserved in wild and hybrid rice. The conserved region of mitochondrial sHSP (MT-sHSP) genes among diploid genome of cotton species contain one single nucleotide polymorphism per 14 bp indicating higher degree of evolution among MTsHSP of different cotton species (Shaheen et al., 2009). Phylogenetic analysis of the chloroplast localized small heat shock proteins (CP sHSPs) from angiosperms, with other plant CP sHSPs and eukaryotic, archaeal, and bacterial sHSPs shows that the CP sHSPs are not closely related to the cyanobacterial sHSPs (Waters & Vierling, 1999). Yildiz & Terzi (2008) reported that heat shock treatment may be helpful to determine the genetic variability chlorophyll accumulation in and thermotolerance of cereals. So molecular and genetic basis of heat tolerance is strongly required to elucidate in cereals for identification of beneficial genes and alleles. These genes may be utilized in molecular breeding programs to produce superior cereal cultivars (Yildiz & Terzi, 2008). In the present study phylogenetic analysis of different sHSPs was carried out to analyze their evolutionary relationship among different plant species on the bases of mRNA and protein sequences.

Materials and Methods

Data collection: mRNA sequences of 62 different plants sHSPs were downloaded from the literature and NCBI Genbank database. Their species name and accession numbers are given in the Table 1.

Evolutionary analysis: Phylogenetic analysis of these mRNA sequences was conducted using different bioinformatic tools. Sequence alignment and Dendrogram tree was dragged by using online available program Clustal W (http://align.genome.jp/). Statistical selection pairing, by applying Tajima's test (Tajima, 1989) and Neighbour Joining (NJ) (Saitou & Nei 1987) methods were used for phylogenetic reconstruction and the NJ *p*-*distance* model was used for distance analysis (Nei & Kumar 2000). Base statistical robustness was performed

by using 500 bootstrap repeats for the validity of results and the whole process was developed by MEGA 5 Beta # 7 software (Kumar *et al.*, 2004). Conceptual translated amino acid sequences of plants sHSPs were also subjected to Clustal W and MEGA 5 Beta # 7 software for phylogenetic analysis.

Results and Discussions

sHSP are considered to be the most conserved among protein families. To understand the molecular evolution and diversity of sHSP genes, mRNA and protein sequences were mined from different data bases including GenBank. Sequence alignment and Dendrogram tree was dragged by using online available program Clustal W. Sequence alignment of mRNA (Fig. 1) and protein sequences (Fig. 2). HSP 16.9 that belong to different species of genus Triticum, Pennisetum and Oryza showed highly conserved sequences of mRNA and Proteins. Similarly HSP 17.6C-II, HSP 20.2 and HSP 17.4 that belong to same genus Lycopersicon also showed highly conserved sequences of mRNA and Proteins. This indicates that different sHSPs that belong to same species showed high sequence similarity and sHSP that belongs to different species remain conserved during evolution. The dragged dendrograms (Fig. 3) were the rooted trees; with tree topologies indicated the bifurcating internodes with asymmetrical branching structures. Dendrogram of the studied accessions was divided into III clusters. All clusters evolved almost at the same time with branch topologies indicated a slow and stable evolution; one possible reason might be the gradual increase in temperature of the environment. In cluster I and II sHSPs belongs to genus Triticum form a distinct evolutionary conserved group.

The overall mean distance with standard error 0.02 among all studies mRNA sequences are 0.74. This showed the mean pairwise distance and standard error for the set of sequences under study. When the values above were recalculated using the deduced protein data, the overall mean distance became higher to 0.90 (with Std. Err. 0.02) among protein sequences. Standard error estimate(s) were obtained by a bootstrap procedure (500 replicates). Mean diversity of entire studied population from mRNA sequences is 3.98 and from protein sequences is 0.83. For the entire population, the mean diversity is calculated by the formula:

$$\pi T - \frac{q}{q-1} \sum_{i=1}^{q} \sum_{j=1}^{q} XiXjdij$$

Where Xi is the estimate of average frequency of the *i*-th allele in the entire population, and q is the number of different sequences in the entire sample. Tajima's Neutrality Test was also performed (Table 2). The analysis involved 62 nucleotide and amino acid sequences. All positions containing gaps and missing data were eliminated. There were a total of 151 positions in the final dataset of mRNA and 130 positions in the final dataset of proteins. Evolutionary analyses were

			A appealing	Numboug
S. No.	Species	Proteins	Accession	Ductoing
1		LICD16.5	Nucleotides	
1.	Agrostis stolonijera	HSP10.5	AF007/62	AAC01560
2.	Arabiaopsis inaliana	HSP1/.6-II	X03443 X54102	CAA45039
3. 4	Arabiaopsis inaliana	HSP21	A34102 X90504	CAA38030
4.	Arabiaopsis inaliana	HSP1/.0	A89304	CAA010/3
5. 6	Arabiaopsis inaliana	HSP25.5	NNI 124323	NP 199937
0. 7	Carica papaya	ПЗР17.3 ЦСР177	A 1 50/500 AV242075	AAK23040 A A D72704
/. 8	Dancus carota	HSP12.7	X 1 242073 X 53852	CAA37848
0. Q	Daucus carota	HSP17.8	X53851	CAA37847
9. 10	Europia hyprometrica	HSD18 3	A E 080846	A A D00185
10.	Funaria hygrometrica	HSP16 /	AF089845	A A D 09183
11.	Glycing max	HSP23 0	LI21722	AAB03096
12.	Glycine max	HSP22.0	X07188	CAA30168
14	Helianthus annuus	HSP17.9	729554	CAA82653
14.	Hordeum vulgare	HSP18.0	X64561	CAA45862
16	Hordeum vulgare	HSP17.0	Y07844	CAA69172
17	Ivcopersicon esculentum	HSP21.0	LEU66300	AAB07023
18	Lycopersicon esculentum	HSP17 6CI	AF123257	AAD30454
19	Lycopersicon esculentum	HSP17.8	AF123256	AAD30453
20	Lycopersicon esculentum	HSP177	AF123255	AAD30452
21	Lycopersicon esculentum	HSP17 6CII	LEU72396	LEU72396
22.	Lycopersicon peruvianum	HSP17.4	AY608694	AAT36481
23.	Lycopersicon peruvianum	HSP20.2	AJ225049	CAA12390
24.	Medicago sativa	HSP17.0	X98617	CAA67206
25.	Medicago sativa	HSP18.1	X58710	CAA41546
26.	Medicago sativa	HSP18.2	X58711	CAA41547
27.	Nicotiana tabacum	HSP26.0	D88584	BAA29064
28.	Orvza sativa	HSP16.9	X60820	CAA43210
29.	Oryza sativa	HSP CII	DQ180746	ABA29610
30.	Oryza sativa	HSP17.8	EU715987	ACH72824
31.	Oryza sativa	HSP26.0	AB020973	BAA78385
32.	Pennisetum glaucum	HSP16.9	X94192	CAA63902
33.	Pennisetum glaucum	HSP17.9	GQ121016	ACR78191
34.	Petroselinum crispum	HSP17.9	X95716	CAA65020
35.	Petunia hybrid	HSP21.0	X54103	CAA38037
36.	Picea glauca	HSP17.0	L47717	AAB01561
37.	Picea glauca	HSP23.5	L47741	AAB01557
38.	Pisum sativum	HSP22.0	X86222	CAA60120
39.	Pisum sativum	HSP17.7	M33901	AAA33670
40.	Pisum sativum	HSP21.0	X07187	CAA30167
41.	Rosa hybrid	HSP17.5	EF157600	ABO84842
42.	Triticum aestivum	HSP26.6B	X67328	CAA47745
43.	Triticum aestivum	HSP17.3	X58279	CAA41218
44.	Triticum aestivum	HSP26.6	X58280	CAA41219
45.	Triticum aestivum	HSP16.9B	X64618	CAA45902
46.	Triticum aestivum	HSP17.8	AF350423	AAK51797
47.	Triticum aestivum	HSP16.9A	EU649679	ACD03605
48.	Triticum aestivum	HSP16.9C	L14444	AAA34294
49.	Triticum durum	HSP26.5	AJ971373	CA196515
50.	Triticum durum	HSP17.6	AJ9/1359	CA196501
51.	Triticum monococcum	HSP16.8	AM/09/55	CAM96546
52.	Iriticum monococcum	HSP1/.0	AM/09/56	CAM96547
53. 54	Iriticum monococcum	HSP16.9A	AM700759	CAM96548
54.	I riticum monococcum	HSP16.9B	AM/09/58	CAM96549
55. 57	Iruicum monococcum	HSP26.6	AJ9/13/4	CA196516
50.	Triticum MONOCOCCUM	H5P23.5	AJ9/1303	CA19050/
57. 50	Iruicum iurgiaum Vitia vinifara	HSP20.8	AJ9/13/2 CU140701	UA190314
50. 50	v uis vinijera Vitis vinifera	ПЗГ1/.4 ЦСD17 2	GU169700	ACZ48084
59. 60	v ilis vinijera Zag mays	113F1/.3 ЦСD10 Л	V54075	AUL40003 CAA29012
61	Zeu muys Zaa mays	HSP17.5	AJ40/J NM 00115/082	NP 001148454
62	Zea mays	HSP26.0	I.28712	AAA33477
04.		1101 20.0	120/12	

 Table 1. List of plants sHSPs along with their mRNA and protein accession numbers and species name used in this study.

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Table 2. Tajima's neutrality Test for mRNA and proteins.

Sequences	m	S	p _s	Θ	π	D
mRNA	62	151	1.000000	0.212935	0.737320	8.624317
Proteins	62	130	1.000000	0.212935	0.896111	11.196385
m = number of	of sites	S, S = 1	Number of seg	regating sites, $p_s = S/m$, $\Theta = p_s/a$	π = nucleotide diversity, and D	is the Tajima test
statistic		- ,				
suusue						
T. aestiv	7um I	HSP1	7.8	GACATGGAGAAGATC	TCCGCCGTGTGCCGCGACGG	CGTGCTCACCGTCTC
Tdurum_	HSP:	17.6		GACATGGAGGAGATC	TCCGCCGTGTGCCGCGACGG	CGTGCTCACCGTGAC
Vvinife	era_l	HSP1	7.4	AATACTGATAAGATC	TCCGCGGTTTGTCAAGATGG	AGTACTGACCGTCAC
Vvinife	era_l	HSP1	7.3	AATACTGACAAGATC	TCCGCGATTTGTCAGGATGG	GGTGTTGACCGTCAC
L. escule	encur	m HS	P17.8		AAGGCGTCTATGGAGAATGG	AGIGCITACIGITAC
T. aestiv	7um I	HSP1	6.9A	AAGGTGGAGGAGGTG	AAGGCCGGGCTGGAGAACGG	CGTGCTCACCGTCAC
Tmonoco	occur	m_HS	P17.0	AAGGTGGAGGAGGTG	AAGGCCGGGCTGGAGAACGG	GGTGCTCACCGTCAC
Taestiv	7um_I	HSP1	6.9B	AAGGTGGAGGAGGTG	AAGGCCGGGCTGGAGAACGG	GGTGCTCACCGTCAC
Tmonoco	occur	m_HS	P16.9B	AAGGTGGAGGAGGTG	AAGGCCGGGGCTGGAGAACGG	GGTGCTCACCGTCAC
T. monoco		m HS	P16.9A	AAGGTGGAGGAGGTG	AAGGCCGGGGCTGGAGAACGG	GTGCCCACCGTCAC
T. aestiv	zum I	HSP2	6.6B	GACAAGAGCCAGGTG	CGCGCCGAGCTCAAGAACGG	CGTGCTGCTCGTGTC
Tdurum_	HSP2	26.5		GACAAGAGCCAGGTG	CGCGCCGAGCTCAAGAACGG	CGTGCTGCTCGTGTC
Tmonoco	occur	m_HS	P26.6	GACAAGAGCCAGGTG	CGGGCCGAGCTCAAGAACGG	CGTGCTGCTCGTGTC
Taestiv	Jum I	HSP2	6.6A	GACAAGAGCCAGGTG	CGGGCCGAGCTCAAGAACGG	CGTGCTGCTCGTGTC
L. escule	entur	m HS	P17.6CT	AAGATGGATCAAGTT	AAGGCTTCAATGGAGAACGG	AGTGCTTACTGTTAC
Taestiv	7um_I	HSP1	6.9C	AAGGTGGAGGAGGTG	AAGGCCGGGCTGGAGAACGG	CGTGCTCACCGTCAC
Taestiv	7um_I	HSP_	17.3	GACATGGAGAAGATC	T-CGCCGTGC-#CGCGACGG	CGTGCTCACCGTGAC
Rhybric	1_HSI	P17.	5	AATGCTGATACCATT	TCTGCTGTTTGCCAAGATGG	GGTTCTGACTGTGAC
Hvulgar	Ce_H	SPI/	.0	AAGGTTGATGAAGTG	AAGGCCGGGCTGGAGAACGG	TGTGCTCACCGTCAC
C. papava	a HSI	P17.	7	AACACTGATGCGATA	TCCGCGGTTTGTCAGGACGG	TGTTTTTGACCGTCAC
Osativa		I		GACGTCGACAAGATC	TCCGCCGTGTGCCAGGACGG	CGTGCTCACCGTCAC
Pglauca	a_HSI	P17.	0	AACCTAGAGGCCATA	TCTGCCGCGTGTCAGGATGG	AGTGCTCACCGTCAC
Psativu	um_HS	SP17	.7	AATATTGAAGCTATC	TCTGCTATTTCTCAAGATGG	TGTTCTTACGGTTAC
Pglaucu	IM_H:	SP17	.9	AAGACGGACCAGATC	AGGGCGTCCATGGAGAACGG	CGTGCTCACCGTCAC
0. sativa	a HSI	P16.	9	AAGGTGGACCAGGTG	AAGGCCGGACTGGAGAACGG	CGTGCTCACCGTCAC
Lescule	ntur	m_hs	p17.6CII	AATACTGATGCAATT	TCTGCAGTTTGTCAAGATGG	AGTTCTGACTGTTAC
Lperuvi	Lanur	m_HS	P20.2	AATACTGATGCAATT	TCTGCAGTTTGTCAAGATGG	AGTTCTGACTGTTAC
Lperuvi		m_HS	P1/.4	CACAAGAGCAAGTC	TCTGCAGTTTGTCAAGATGG	AGTTCTGACTGTTAC CCTCCTCCTCCTCAC
A. stolor	i_nsi nifei	ra H	SP16.5	AAGGTGGAGGAGGTG	AAGGCCGGGGCTGGAGAACGG	CGTGCTCACCGTCAC
Z. mays H	ISP1	8.0		GACGTCGACAAGGTC	GCCGCCGTGTGCAGGGACGG	CGTGCTCACGGTGAC
Hannuus	_HSI	P17.	9	AATACGGATAAGATA	TCGGCGATTTGTCAAGACGG	AGTGCTTACGGTGAC
_Athali	lana	_HSP	17.6-11	GATTTGGACAAGATC	TCTGCTGTTTGTCATGACGG	TGTGTTGAAGGTTAC
Atnalla M gativa	ana_i		1.6	AACACTGATGCTGTT	TCTCCGGCTTGTAATGGCGG	TGTGTTGGAAGTGAC
M. sativa	A HSI	P18.	1	AAAATGGATCAAGTG	AAAGCTGCAATGGAGAATGG	TGTTCTCACTGTCAC
Msativa	a_HSI	P18.	2	AAAATGGATCAAGTG	AAAGCTGCAATGGAGAATGG	TGTTCTCACTGTCAC
Zmays_H	ISP2	6		GACAAGAGCAAGGTG	CGGGCCGAGCTCAAGAACGG	CGTGCTCCTCGTCAC
Fhygrom	netr:	1Ca_ 017	HSP16.4	AATTTGGATAAAATT	GCAGCCTCCTGTGTCAATGG	CATCCTAACAGTTAC
D. carota	A HSI	P18.	0	AAGATGGACCAGGTG	AAGGCTICAATGGAGAACGG	AGTGTTAACTGTTAC
P. crispu	im HS	SP17	.9	GATTTGGAGAATATC	AATGCGGTCTGTCAGGATGG	TGTGTTGAGTGTTAC
Dcarota	a_HSI	P17.	8	AAGGTGGATGAGGTG	AAAGCTGCTATGGCGAATGG	GGTGGTTACTGTTAC
Fhygrom	netr	ica_	HSP18.3	AATGCTGATGCCATT	ACTGCCAACTGTGTGGATGG	CGTGTTGACGGTCAC
Lescule	entur	m_HS	P21	GIGAAGGATAAAATC	AAAGCGGAAC'I'GAAGAA'I'GG	AGTTCTTTTCATCTC
P. hvbrid	la H	SP21		GACAAAGATAAAGTT	AAAGCAGAATTGAAGAATGG	AGTTCTTTTAATCTC
A. thalia	ina I	HSP2	1	GAGAAAGACAAGATC	AAAGCTGAGCTCAAGAACGG	AGTCCTCTTTATCAC
Gmax_HS	5P22	_		GAGAAGGACAAGGTT	AAGGCAGAGTTGAAAAATGG	TGTGCTTTATATCAC
Psativu	IM_HS	SP21	E.	GAGAAAGAGAAGGTT	AAGGCTGAGTTGAAAGATGG	TGTGCTTTATATTAC
Fgrauca	4_HSI	ezs.	5	AAGUTUGAUUATATU	AAAGUUUAGATGAAGAATGG	TGTGCTCAAAGTTAC
O. sativa	A HSI	P17.	8	GCGGCGGACGAGGCG	AGCGCGCGGGATGGCGGACGG	GGTGCTCACGGTGAC
Zmays H	ISP1	7.5		AGGGACGAGTTCGTC	A-AGAAGACGGCAGCAGCGT	AATAATGCACATCAT
Tmonoco	occur	m_HS	P23.5	AAGATGGACAAGATC	AAGGCCGAGATGAAGAATGG	CGTGCTCTGGGTCAC
Psativu	ım_H	SP22	.0	AAGATTGATGTGATT	AAAGCTGAGATGAAAAACGG	CGTGCTCAAGGTTAC

Fig. 1. mRNA sequence alignment of 62 sHSP from different plant species. Box mark conserved region. *Highly conserved region; **Completely conserved region.

conducted in MEGA 5 (Saitou & Nei, 1987; Nei & Kumar, 2000; Tamura *et al.*, 2007). Maximum Likelihood fits of 24 different nucleotide substitution models was also sketched.

The evolutionary history was inferred using the Neighbor-Joining method (Figs. 4 and 5). The bootstrap consensus tree inferred from 500 replicates is taken to represent the evolutionary history of the taxa analyzed. Branches corresponding to partitions reproduced in less than 50% bootstrap replicates are collapsed. The evolutionary distances were computed using the p-distance method and are in the units of the number of base and amino acid differences per site. The analysis involved 62 nucleotide and amino acid sequences. All positions containing gaps and missing data were eliminated. There

were a total of 151 positions in the final dataset of mRNA and 130 positions in the final dataset of proteins. Evolutionary analyses were conducted in MEGA 5 (Saitou and Nei, 1987; Nei and Kumar, 2000; Tamura et al., 2007). The evolutionary history was also inferred using the Maximum Parsimony method. For mRNA and Proteins, the consistency index is 0.107743 & 0.465045, the retention index is 0.330649 & 0.541383 and the composite index is 0.035625 & 0.251767 for all sites respectively. All Evolutionary analysis revealed that different sHSPs evolve prior to the divergence of the plant species. The branch topology of cytosolic, mitochondrial, chloroplast and Endoplasmic reticulum families are highly supported by Bootstrap analysis of mRNA and Protein sequences (Figs. 4 and 5). The molecular evolution

Ν.	tabacum_HSP26
Р.	_hybrida_HSP21
ь.	esculentum HSP21
G.	max HSP22
Р.	sativum HSP21
А.	thaliana HSP21
т.	durum HSP26.5
т.	monococcum HSP26.6
т.	aestivum HSP26.6B
т.	aestivum HSP26.6A
т.	turgidum HSP26.8
z.	mays HSP26
ο.	sativa HSP26
G.	max HSP23.9
Р.	sativum HSP22
А.	thaliana HSP23.5
Р.	glauca HSP23.5
т.	aestivum HSP 17.3
т.	durum HSP17.6
т.	aestivum HSP17.8
ο.	sativa CII
z.	mays HSP18.0
ь.	esculentum hsp17.6CII
ь.	peruvianum HSP20.2
ь.	peruvianum HSP17.4
с.	papaya HSP17.7
Р	sativum HSP17.7
v.	vinifera HSP17.4
v	vinifera HSP17.3
R.	hybrid HSP17.5
м.	sativa HSP17.0
Р.	crispum HSP17.9
н.	annuus HSP17.9
А.	thaliana HSP17.6-II
А.	thaliana HSP17.6
F.	hygrometrica HSP16.4
Р.	glauca HSP17.0
г.	hygrometrica HSP18.3
т.	monococcum HSP16.9B
т.	aestivum HSP16.9B
т.	monococcum HSP16.8
т.	monococcum HSP17.0
т.	monococcum HSP16.9A
т.	aestivumHSP16.9A
т.	aestivum_HSP16.9C
н.	vulgare_HSP17.0
Α.	_stolonifera_HSP16.5
Р.	_glaucum_HSP16.9
ο.	sativa_HSP16.9
н.	vulgare_HSP18.0
ь.	_esculentum_HSP17.7
ь.	esculentum_HSP17.8
ь.	esculentum_HSP17.6CI
м.	_sativa_HSP18.1
м.	sativa_HSP18.2
с.	papaya_HSP17.5
D.	_carota_HSP18.0
D.	carota_HSP17.8
Р.	_glaucum_HSP17.9
ο.	_sativa_HSP17.8
т.	_monococcum_HSP23.5
z -	mays HSP17 5

KDDENEIKMRFDMPGLSKDEVKVSVEDDLLVIKGEY	к
KDDENEIKMRFDMPGLSKEEVKVSVEDDVLVIKGEH	К
HDDENEIKMRFDMPGLSKEDVKVSVENDMLVIKGEH	К
KDEEHEIRMRFDMPGLAKEDVKVSVEDDMLVIKGGH	K
KDEEHEIRMRFDMPGVSKEDVKVSVEDDVLVIKSDH	R
KEEEHEIKMRFDMPGLSKEDVKISVEDNVLVIKGEQ	К
▲ MEDEKEVKMRFDMPGLSREEVRVMVEDDALVIRGEH	K
MEDDKEVKMRFDMPGLSREEVKVMVEDDALVIRGEH	К
MEDEKEVKMRFDMPGLSREEVRVMVEDDALVIRGEH	К
MEDEKEVKMRFDMPGLSREEVRVMVEDDALVIRGEH	K
MEDDKEVKMRFDMPGLSREEVKVMVEGDALVIRGEH	K
VEDEKEVKMRIDMPGLARDEVKVMVEDDTLVIRGEH	К
MEDDKEVRMRFDMPGLSREEVKVMVEDDALVIRGEH	К
RETEDALHLRVDMPGLAKEDVKISVEQNTLIIKGE	G
RETEDALFLRLDMPGLGKEDVKISVEQNTLTIKGEE	G
KEKDDALHLRIDMPGLSREDVKLALEQNTLVIRGEG	Е
VEDKEALHLRVDMPGLGKEDIKVYAEENALVIKGE	
KELPGAYAFVVDMPGLGSGDIKVQVEDERVLVISG	
KELPGAYAFVVDMPGLGSGDIKVQVEDERVLVISG	
KELPGAYAFVVDMPGLGSGDIKVQVEDERVLVISG	
KDUPGAYAFVVDMPGLKSSDIKVQVEEERLLVISG	
KELPGAYAFVVDMPGLGTGDIRVQVEDERVLVVSG	
KEYPNSYVFVVDMPGLKSGDIKVQVEEDNVLLISG	
KEYPNSYVFVVDMPGLKSGDIKVQVEEDNVLLISG	
KEYPDSYVFVVDMPGLKSGDIKVQVEEDNVLLISG	
KEYPNSYVFVIDMPGLKSGDIKVQVEDDNVLQISG	
KEHPNSYVFMVDMPGVKSGDIKVQVEDENVLLISG	
KEYPNSYTFIVDMPGLKSGDIKVQVEDDNVLVISG	
KEYPNSYAFIIDMPGLKSGDIKVQVEDDNVLVISG	
KEIPNSYVFVIDMPGLKSGDIKVQVEDDNVLLISG	
KENPNSYVFVIDMPGLKSGDIKVQVEDDNVLVISG	
KEYPNSYVFVVDMPGLKSGDIKVQVEEDNVLVVSG	
KEQPNSYVFIVDMPGLKSGDIKVQVERDNVLVISG	
IEHPNAYAFVVDMPGIKGDEIKVQVENDNVLVVSG	
IEHPDAYVFAVDMPGIKGDEIQVQIENENVLVVSG	
KELPDAYIFVADMPGLKSADVKVQLENDNVLVIGG	
KEYPNSYVFIIDMPGLKSNDIKVQVEDENVLNISG	
KEIKDAYLFVADVPGLQKTDIEVQVENENVLTMRG	
♣KETPEAHVFKPDLPGVKKEEVKVEVEDGNVLVVSG	
KETPEAHVFKADLPGVKKEEVKVEVEDGNVLVVSG	
KEAPEAHVFKADLPGVKKEEVKVEVEDGNVLVVSG	
KETPEAHVFKADLPGVKKEEVKVEVEDGNVLVVSG	
KETPEAHVFKADLPGVKKEEVKVEVEDGNVLVVSG	
KETPERHVFKADLPGVKKEEVKVEVEDGNVLVVSG	
KETPEAHVFKADLPGVKKEEVKVEVEDGNVLVVSG	
GRRLEAHVFKADLPGVKKEEVKVEVEDGNVLIVSG	
KETPEAHVFKADLPGVKKEEVKVEVEGGNVLVVSG	
KETPEVHVFKADLPGVKKEEVKVEVEDGNVLVISG	
KETPESHVFKADLPGVKKEEVKVEVEEGNVLVISG	
GRRAVAHVFKADLPGVKKEEVKVEVEDGNVLVVSG	
KETPEAHVFKVDLPGLKKEEVKVEVEEDRVLQISG	
KETPEAHVFKVDLPGLKKEEVKEEVEEDRVLQISG	
KETPEAHVFKADLPGLKKEEVKVEVEEDRVLQISG	
KETPEAHVFKADLPGLKKEEVKVEIEDDRVLQISG	
KETPEAHVFKADLPGMKKEEVKVEIEDDRVLQISG	
EETPEAHVFRADLPGLKKEEVKVELEDDRVLQISG	
KETPQAHVFKADLPGLKKEEVKVEVEEGKVLOISG	
KETPQAHVFKADLPGLKKEEVKVELEEGKVLQISG	
KETPEAHVFKADVPGLKKEEVKVEVEDGNVLOISG	
	NNAAKAGK
RETPVAHVFEMDLPGLAKDQVAVENVDGHILRVRAGGEHEDA	
RETPVAHVFEMDLPGLAKDQVAVEVVDGHILRVRAGGEHEDA KEDDDAVYLKVPMPGLTKEHVEVRADKNILVTKGEG	

Fig. 2. Protein sequence alignment of 62 sHSP from different plant species. Box mark conserved region. *Highly conserved region; **Completely conserved region.

of the sHSPs in plants can be considered as the best example of gene evolution. Darwinian selection reflects different selective constraints for sequence evolution rate among members of a gene family (Waters *et al.*, 1996). Variation in branch length indicates difference in rate of evolution. The phylogenetic relationships reveal that sHSPs are evolved due to gene duplication, sequence divergence and gene conversion at different levels (Waters & Rioflorido, 2007). Phylogenetic analysis of a representative 62 sHSPs from plants reveals a close relationship between plant species, which appears to exhibit polydispersity (Fu *et al.*, 2006). Phylogenetic analysis of the chloroplast localized small heat shock proteins (CP sHSPs) from angiosperms, with other plant CP sHSPs and eukaryotic, archaeal, and bacterial sHSPs shows that the CP sHSPs are not closely related to the cyanobacterial sHSPs (Waters & Vierling, 1999). Thus, they most likely evolved via gene duplication from a nuclear-encoded cytosolic sHSP and not via gene transfer from the CP endosymbiont (Vierling, 1991). The evolutionary relationships among all of the plant sHSPs identified and homologues from bacteria and other eukaryotes are consistent with the hypothesis that the land plant chloroplast and mitochondrion sHSPs did not originate from the endosymbionts of the chloroplast and mitochondria (Waters & Rioflorido, 2007). This tremendous diversification of small heat shock proteins in plants may reflect adaptations to stresses unique to plants



Fig. 3. Dendrogram tree of 62 mRNA and protein sequences with branch length.



Fig. 4. Neighbor-Joining tree of mRNA sequences with bootstrap values from different plant species using p-distance method.



Fig. 5. Neighbor-Joining tree of protein sequences with bootstrap values from different plant species using p-distance method.

(Vierling, 1991). The gene duplication that gave rise to the sHSP genes families certainly occurred before the divergence of the monocots and dicots, a minimum of 150 million years ago (Doyle & Donoghue, 1993).

References

- Arif, M., M. Ibrahim, A. Ahmad and S. Hassan. 2005. Elimination of *Citrus tristeza* Closterovirus from Citrus bud-wood through thermotherapy. *Pak. J. Bot.*, 37(2): 423-430.
- Basha, E., C. Jones, V. Wysocki, E. Vierling. 2010. Mechanistic differences between two conserved classes of small heat shock proteins found in the plant cytosol. *J. Biol. Chem.* 285: 11489-11497.
- Basha, E., K.L. Friedrich and E. Vierling. 2006. The N-terminal arm of small heat shock proteins is important for both chaperone activity and substrate specificity. *J. Biol. Chem.*, 281(52): 39943-39952.
- Carranco, R., C. Almoguera and J. Jordano. 1999. An imperfect heat shock element and different upstream sequences are required for the seed-specific expression of a small heat shock protein gene. *Plant Physiol.*, 121: 723-730.
- Doran, P., J. Gannon, O. K. Connell and K. Ohlendieck. 2007. Aging skeletal muscle shows a drastic increase in the small heat shock proteins aB-crystallin/HspB5 and cvHsp/HspB7. *Eur. J. Cell Biol.*, 86(10): 629-640.
- Doyle, J.A. and M.J. Donoghue. 1993. Phylogenies and angiosperm diversification. *Paleobiol.*, 19: 141-67.
- Fan, G.C., X. Ren, J. Qian, Q. Yuan, P. Nicolaou, Y. Wang, W.K. Jones, G. Chu and E.G. Kranias. 2005. Novel cardioprotective role of a small heat-shock protein, Hsp20, against ischemia/reperfusion *injury*. *Circulation*, 111(14): 1792-9.
- Fu, X., W. Jiao and Z. Chang. 2006. Phylogenetic and biochemical studies reveal a potential evolutionary origin of small heat shock proteins of animals from bacterial class A. J. Mol. Evol., 62: 257-266.
- Haslbeck, M., A. Kastenmuller, J. Buchner, S. Weinkauf and N. Braun. 2008. Structural Dynamics of Archaeal Small Heat Shock Proteins. J. Mol. Biol., 378(2): 362-374.
- Horváth, I. and L. Vígh. 2010. Stability in times of stress. *Nature*, 463(7280): 436-438
- Iqbal, N., S. Farooq, R. Arshad and A Hameed. 2010. Differential accumulation of high and low molecular weight heat shock proteins in Basmati rice (*Oriza sativa* L.) cultivars. *Genet. Resour. Crop. Evol.*, 57: 65-70.
- Kriehuber, T., T. Rattei, T. Weinmaier, A. Bepperling, M. Haslbeck and J. Buchner. 2010. Independent evolution of the core domain and its flanking sequences in small heat shock proteins. *FASEB J.*, 24(10): 3633-3642.
- Kumar, S., K. Tamura and M. Nei. 2004. Mega-3, integrated software for molecular evolutionary genetics analysis and sequence alignment. *Bri. Bioinform.*, 5: 150-163.
- Liossis, S.N., C.S. Via and G.C. Tsokos. 1998. The alter ego of heat shock proteins. *Clin. Immunol. Immunopathol.*, 86: 235-236.
- Mahmood, T., W. Safdar, B.H. Abbasi and S.M.S. Naqvi. 2010. An overview on the small heat shock proteins. *Afr. J. Biotechnol.*, 9(7): 727-949.
- McKenzie, S.L. and M. Meselson. 1977. Translation in vitro of Drosophila heat-shock messages. J. Mol. Biol., 117: 279-283.

- Nakamoto, H. and L. Vigh. 2007. The small heat shock proteins and their clients. *Cell. Mol. Life Sci.*, 64: 294-306.
- Naz, F., S. Farooq, R. Arshad, M. Afzaal and M. Akram. 2006. Discriminating upland and lowland rice genotypes through proteomic approach. *Pak. J. Bot.*, 38(5): 1731-1738.
- Nei M. and S. Kumar. 2000. Molecular Evolution and Phylogenetics. Oxford University Press, New York, pp. 241-287.
- Porta, A., Z. Török, I. Horvath, S. Franceschelli, L. Vígh and B. Maresca. 2010. Genetic modification of the Salmonella membrane physical state alters the pattern of heat shock response. J. Bacteriol., 192(7): 1988-1998.
- Saitou, N. and M. Nei. 1987. The neighbor-joining method: A new method for reconstructing phylogenetic trees. *Molecular Biology and Evolution*, 4: 406-425.
- Shaheen, T., M. Asif, Y. Zafar and Mehboob-ur-rahman. 2009. Single nucleotide polymorphism analysis of MT-sHSP Gene of Gossypium arboreum and its relationship with other diploid cotton genomes, G. hirsutum and Arabidopsis thaliana. Pak. J. Bot., 41(1): 177-183.
- Sun, Y. and T.H. MacRae. 2005. Small heat shock proteins: Molecular structure and chaperone function. *Cell Mol. Life Sci.*, 62(21): 2460-2476.
- Tajima, F. 1989. Statistical methods to test for nucleotide mutation hypothesis by DNA polymorphism. *Genetics*, 123: 585-595.
- Tamura, K., J. Dudley, M. Nei and S. Kumar. 2007. MEGA4: Molecular Evolutionary Genetics Analysis (MEGA) software version 4.0. *Mol. Biol. Evol.*, 24: 1596-1599.
- Toth, M.E., S. Gonda, L. Vígh and M. Santha. 2010. Neuroprotective effect of small heat shock protein, Hsp27, after acute and chronic alcohol administration. *Cell Str. Chaperon.*, 15(6): 807-817.
- Van-den-Ussel, P., D.G. Norman and R.A. Quinlan. 1999. Molecular chaperones: Small heat shock proteins in the limelight. *Current Biol.*, 9(3): 103-105.
- Vierling, E. 1991. The heat shock response in plants. Ann. Rev. Plant Physiol Plant Mol. Biol., 4: 579-620.
- Vierling, E. 1997. The small heat shock proteins in plants are members of an ancient family of heat induced proteins. *Acta Physiol. Plan.*, 19(4): 539-547.
- Waters, E.R., G.J. Lee and E. Vierling. 1996. Evolution, structure and function of the small heat shock proteins in plants J. Exp. Bot., 47(296): 325-338.
- Waters, E. R. and E. Vierling. 1999. Chloroplast small heat shock proteins: Evidence for atypical evolution of an organelle-localized protein. Proceedings of the National Academy of Sciences of the United States of America, 96(25): 14394-14399.
- Waters, E.R. and I. Rioflorido. 2007. Evolutionary analysis of the small heat shock proteins in five complete algal genomes. J. Mol. Evol., 65: 162-174.
- Waters, E.R., G.J. Lee and E. Vierling. 1996. Evolution, structure and function of the small heat shock proteins in plants. J. Exp. Bot., 47(296): 325-338.
- Wright, S.I. and B.S. Gaut. 2005. Molecular population genetics and the search for adaptive evolution in plants. *Mol. Biol. Evol.*, 22: 506-519.
- Wu, C. 1995. Heat shock transcription factors: structure and regulation. Ann. Rev. Cell Develop. Biol., 11: 441-69.
- Yildiz, M. and H. Terzi. 2008. Evaluation of acquired thermotolerance in wheat (*triticum aestivum* and *T. Durum*) cultivars grown in turkey *Pak. J. Bot.*, 40(1): 317-327.

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