



Short communication

Evidence for molecular evolutionary conservedness of small heat-shock protein sequence in solanaceaeous crops using *in silico* methods

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ABSTRACT

Drought and heat contribute to much of the yield decline in agricultural lands all over the world. The basic physiological responses developed against drought and heat stress overlie each other, as; both these stresses eventually lead to dehydration of the cell and to osmotic imbalance. To cope with abiotic stresses, it is necessary to understand plant responses to stresses that disturb homeostatic equilibrium at the cellular and molecular level. Although there has been remarkable progress in this with development of microarray-based expression profiling methods (together with genomic sequence data), understanding on ways to employ these data to engineer plants with improved stress-tolerance is still at a nascent stage. However, these data can be used for discovering genes, functional microsatellites and regulatory elements using *in silico* methods. In this context, single nucleotide repeat marker sequences have been identified which is associated with small heat-shock protein sequence (sHSP) for heat tolerance in *Capsicum annum*. These sHSP sequences have some structural features in common; its characteristic is that it is homologous and highly conserved. These sequences have been analyzed for molecular evolutionary conservedness in solanaceaeous crops and have been found to have a single nucleotide repeat sequence and a highly conserved sHSP sequence.

Key words: Small heat-shock protein (sHSP), evolutionary, conserved, Solanaceae, markers

Heat tolerance genes, namely, heat-shock proteins (HSP) play a vital role in stress tolerance. These are a class of functionally related proteins involved in folding and unfolding of other proteins. Their expression increases when cells are exposed to elevated temperatures. This increase in expression is transcriptionally regulated. The dramatic upregulation of heat shock proteins is a key part of the heat-shock response and is induced primarily by the heat shock factor. HSPs are found in virtually all living organisms, including plants. Many HSPs function as molecular chaperones where these direct a protein into a particular pathway by excluding alternate pathways. HSPs plays a critical role in protein-protein interactions (such as folding) and assist in establishing of proper protein-shape conformation (Ellis, 1993; Georgopoulos and Welch, 1993; Welch, 1993).

High levels of heat-shock proteins were produced by exposure to different kinds of environmental stresses, including ultraviolet light, nitrogen deficiency and water deprivation. Thus, heat-shock proteins are also referred to

as stress proteins. HSPs range in size from about 16 to over 100kDa (Vierling, 1991; Waters *et al*, 1996) and are classified into five groups based on molecular weight and function.

Heat-shock proteins are named according to their molecular weight. For example, Hsp60, Hsp70 and Hsp90 (the most widely-studied HSPs) refer to families of heat-shock proteins of the order of 60, 70, and 90kDa size. The small Hsp (sHsp), or Hsp20, family of heat-stress proteins is a nearly ubiquitous family of stress proteins that range in size from approximately 16–42kDa (Scharf *et al*, 2001). Increased expression of these under heat-shock conditions and their protective effect on cell viability at elevated temperatures suggests that these may have a function in formation or maintenance of native conformation of the proteins (Jakob *et al*, 1993). During high-temperature stress, molecular chaperones are believed to act by preventing irreversible protein denaturation harmful to the cell (Parsell and Lindquist, 1993).

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Genome sequence of the Solanaceae crops, i.e., *Solanum lycopersicum*, and *Capsicum annuum* has been explored for abiotic-stress tolerance genes using several *in silico* methods. The EST collection of *Capsicum annuum* and *Solanum lycopersicum* database (approximately 33,875 sequences in *Capsicum annuum* and 2,65,760 sequences in *Solanum lycopersicum*) has been explored for repeat sequences. Good quality repeat sequences of around 2500 in *Capsicum annuum* and 12900 in *Solanum lycopersicum* have been identified. These sequences are subjected to

several *in silico* methods for identifying conserved sequences. A sequence of 314bp in *Capsicum annuum* has shown a high degree of similarity with sequences in several solanaceous crops. The sequence has been identified as a small heat-shock protein sequence in *Capsicum annuum*, having Single Nucleotide Repeat (SNP) that could be a potential unique marker for Heat-shock protein sequence. Further, the sequence has been blasted against EMBL nucleotide sequence database and the result is presented in Fig 1.

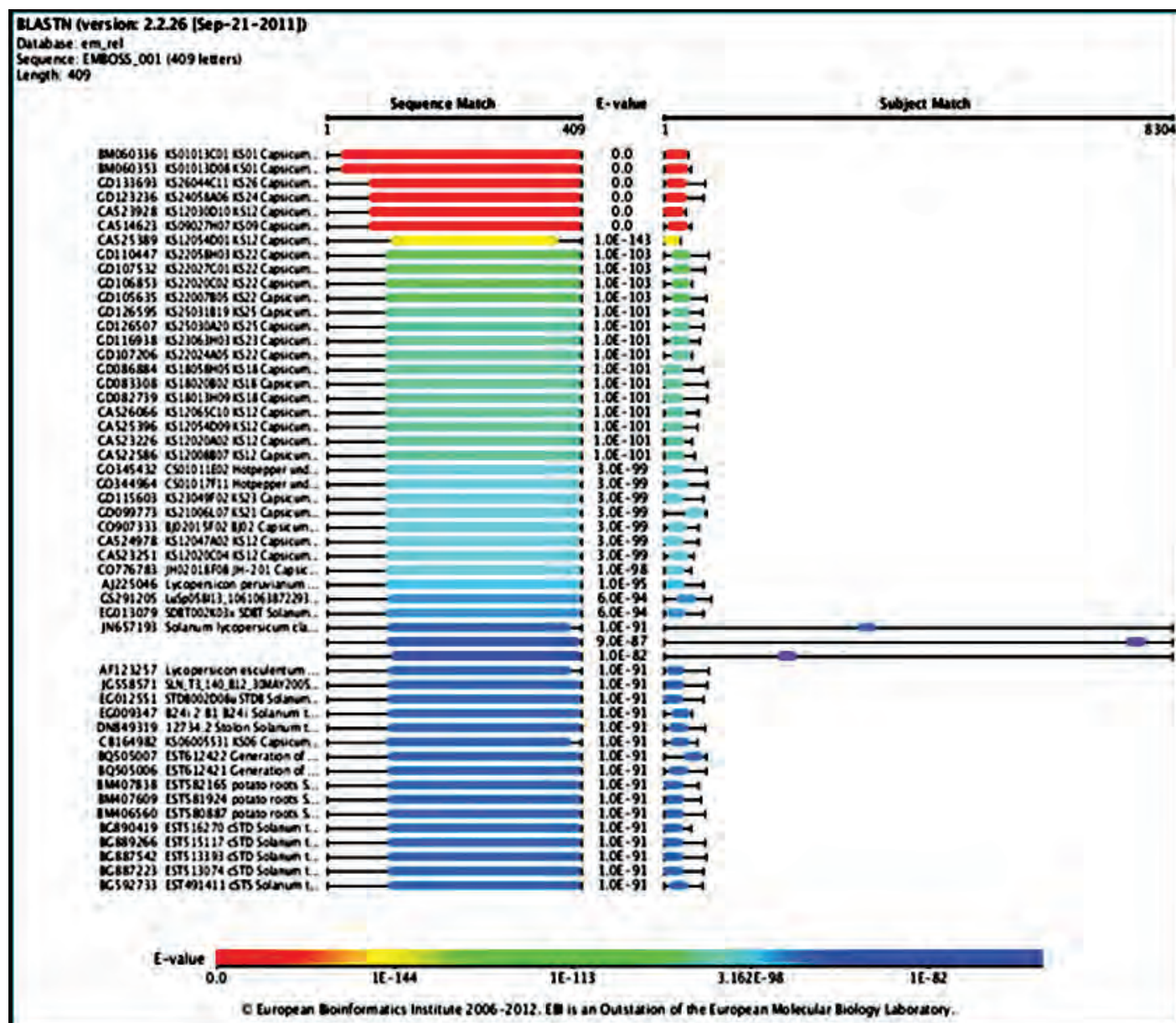


Fig 1. The above figure shows the most homologous sequences on top (shown in red) and the less similar ones (in blue). Sequence-match and subject-match are shown on the left side and right side, respectively. The sequence in red colour has zero error values (E values) while the sequence in blue colour has a small error value of 1×10^{-29} . Most homologous sequences are from *Capsicum annuum*, hot pepper, *Solanum tuberosum* and *Solanum lycopersicum*

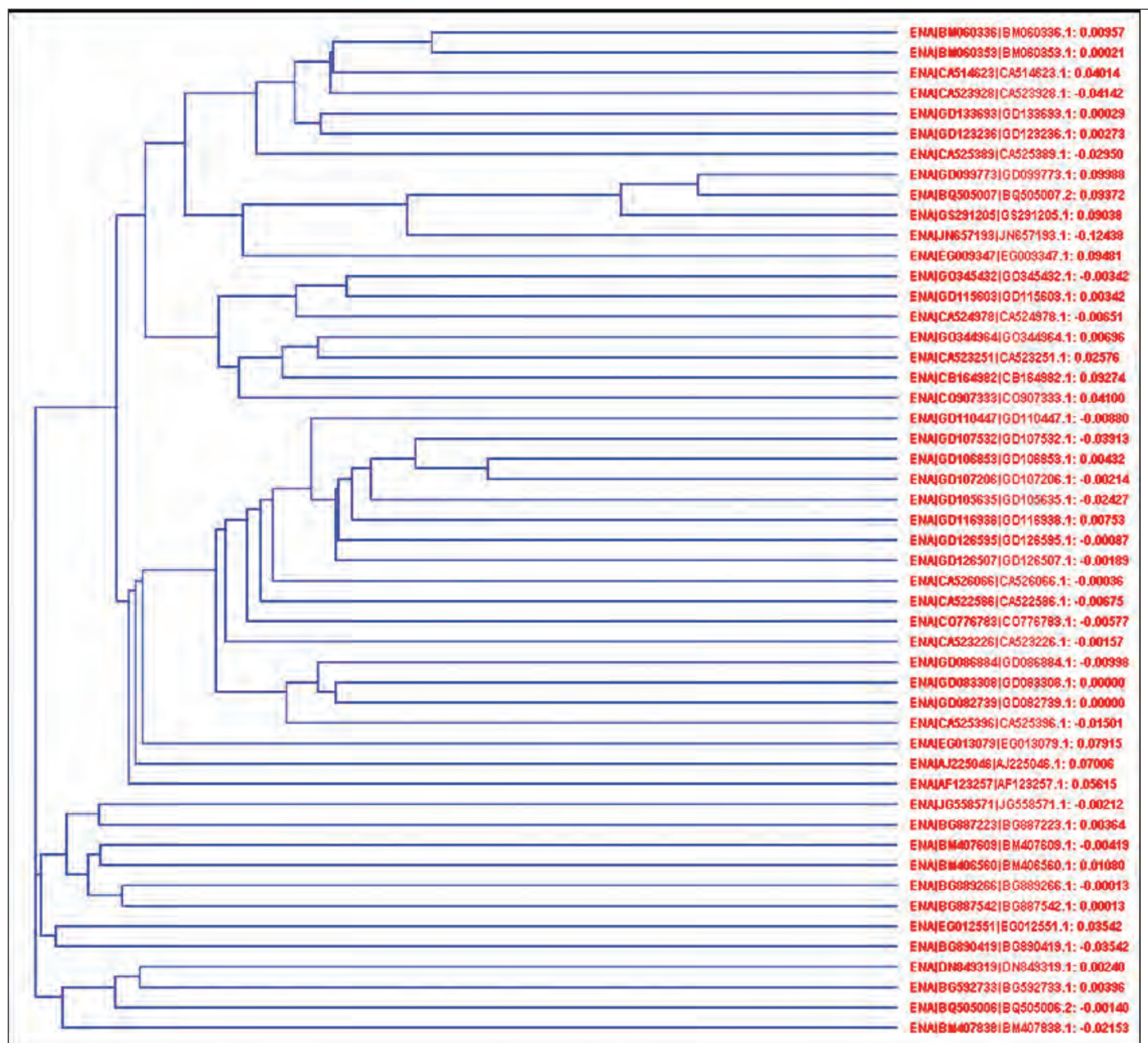


Fig 2. The above cladogram from EMBL shows that sHSP sequence is conserved in most of the Solanaceae species. The sequence is strongly conserved in *Capsicum annuum*, *Solanum tuberosum*, *Lycopersicon peruvianum*, *Lycopersicon esculentum* and hot pepper

The identified sequence has been classified as small heat-shock protein Class I mRNA sequence of *Capsicum annuum*, similar to GenBank id EU311413.1. Further, a unique signature (marker) has been identified having a 21bp single nucleotide repeat sequence. This may qualify as a marker sequence associated with heat -shock proteins in *Capsicum annuum*. The 314 bp HSP sequence in Table 1 was subjected to BLAST analysis for homologous sequences across genomes.

Sequence identity of over 85% with HSP sequence

mostly places it in Solanaceae family. Most similar sequences belong to small Heat Shock Protein (sHSP) having similar transcription factors. Also, it was found that this is highly conserved in solanaceae species. BLAST result sequences having identity of over 85% were subjected to phylogenetic analysis. Results revealed that the query sequence was highly similar to that in *Capsicum annuum* and *Solanum tuberosum*. These sHSP sequences are highly conserved during the evolutionary process and these sequences together with similar groups indicate stress-related functional conservedness.

Further, these sequences were analyzed for functional conservedness across genomes using the Web-based program ClustalW from EMBL to assess phylogenetic distance between species. Result of the analysis is given below:

It is clearly evident from distance values (Fig 2) that sHSP sequence associated with heat tolerance is present across the genomes by being conserved during evolution. Different sHSPs that belong to the same species (*Capsicum annuum*) showed high sequence-similarity (Table 2) and sHSP belonging to different species remained conserved during evolution. HSP sequences were further analyzed using JALVIEW lite, a Web-based software from EMBL,

for Multiple Sequence Alignment (MSA) of several similar biological sequences for evolutionary relationship (by which they share a lineage, and are descended from a common ancestor). Most multiple sequence alignment programs are made using heuristic methods. From the resulting MSA sequence, homology can be inferred to identify sequences shared by evolutionary origins and their conservation. Multiple sequence alignments of sHSP sequence and its conservedness is given below:

Visual depiction of alignment in Fig. 3 illustrate mutation events such as single nucleotide changes, (that appear as differing characters in a single alignment column) and insertion or deletion mutations, i.e., indels or gaps (that

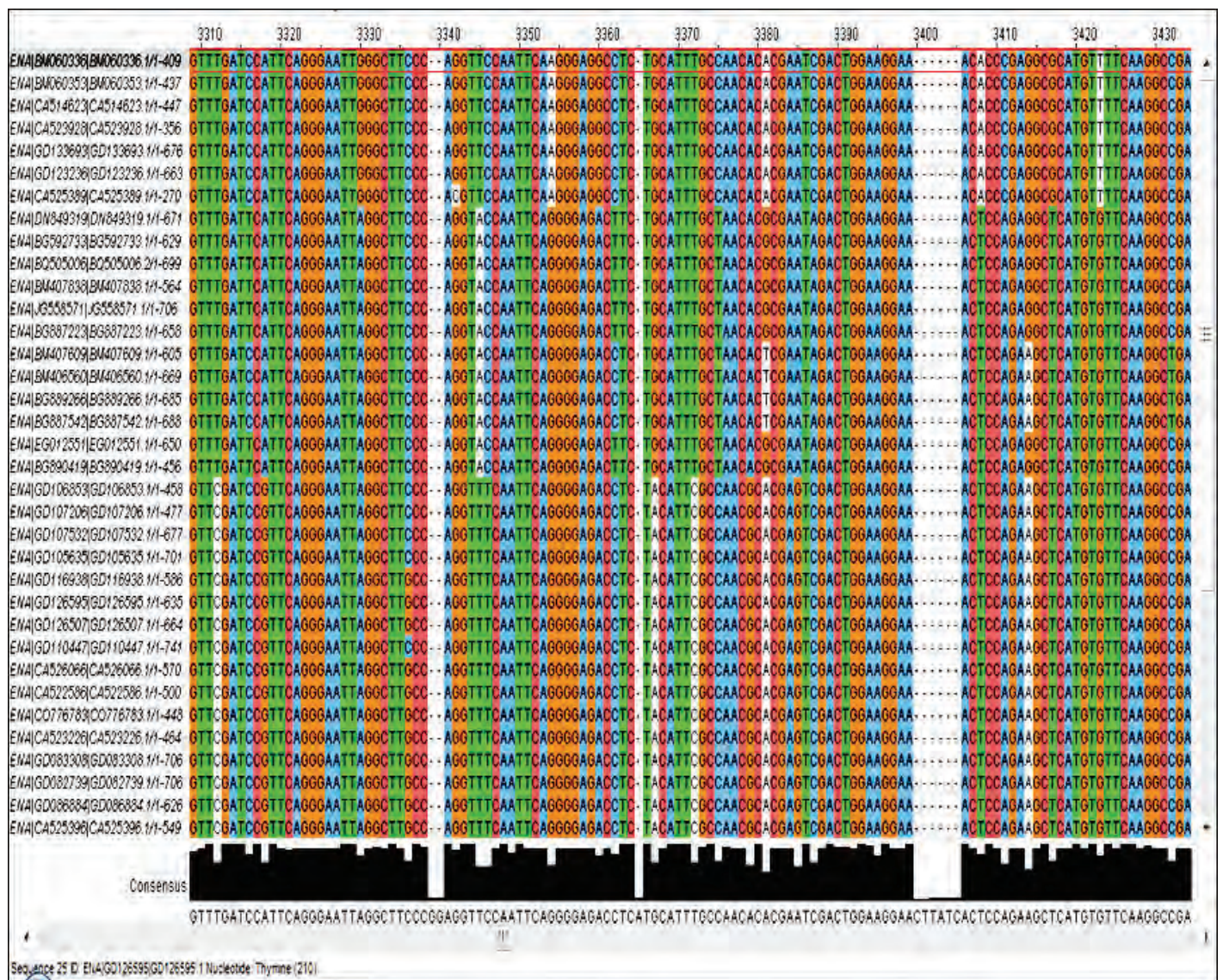


Fig 3. The above diagram shows alignment of sHSP sequence. JALVIEW lite software displays sequence consensus of *Capsicum annuum* cDNA, which shows evolutionary conservation with other solanaceaeous crops

appear as hyphens in one or more of the sequences in the alignment). The consensus sequence is shown at the bottom as a black bar, where height of the black bar depicts sequence consensus. Greater height of the black bar denotes high consensus while lesser height denotes low consensus. From the above MSA, it is evident that the identified sHSP sequence is highly conserved in *Capsicum annuum* and *Solanum tuberosum*.

From the above results, it is clear that the 314bp sHSP sequence (Table 1) shows evolutionary conservedness in Solanaceae crops. These sHSP sequences have similar structural features in all the crops and BLAST result revealed that it is highly conserved in the same species (Table 2). The single-nucleotide marker sequence "TTTTTTTTTTTTTTTTTTTTTTTT" - 72bp from 314bp sHSP, sequence is a potential unique signature associated with small

Table 1. Single Nucleotide Repeat sequence, a unique marker, and sHSP conserved sequence from solanaceous crops

Sequence	Feature	Length/locus
TTTTTTTTTTTTTTTTTTTTTTTT	Single nucleotide repeat sequence	21 bp sequence. (Starting from -72bp of 314 bp sHSP sequence)
AAGCTCACTGAAAATGTCGCTAATCCCAAGAA TCTTCGGCGATCGACGAAGCAGCAGCATGTT GATCCATTCTCAATCGACATGTTTGATCCATTC AGGGAATTGGGCTTCCCAGGTTCCAATTCAAG GGAGGCCTCTGCATTTGCCAACACACGAATCG ACTGGAAGGAAACACCCGAGGCGCATGTTTT AAGGCCGATCTCCAGGGCTTAAGAAGGAGGA AGTCAAAGTAGAGATCGAAGAGCATAGGGTAC TTCAGATTATCGGAGAGAGGAATGAGGAGAAA GAAGATAAGAGTGATACTTGGCATC	Conserved sHSP Sequence	314 bp

Table 2. The sHSP sequence is conserved in same species, i.e., *Capsicum annuum*, with 100% identity and >89% identical with other Solanaceae species such as *Solanum tuberosum*, hot pepper and *Lycopersicon peruvianum*

Source	Length	Score	Identity	E value
KS01013C01 KS01 <i>Capsicum annuum</i> cDNA, mRNA sequence	409	387	100.0	0.0
KS01013D08 KS01 <i>Capsicum annuum</i> cDNA, mRNA sequence	437	383	99.0	0.0
KS26044C11 KS26 <i>Capsicum annuum</i> cDNA, mRNA sequence	676	337	99.0	0.0
KS24058A06 KS24 <i>Capsicum annuum</i> cDNA, mRNA sequence	663	337	99.0	0.0
KS12030D10 KS12 <i>Capsicum annuum</i> cDNA, mRNA sequence	356	337	99.0	0.0
KS09027H07 KS09 <i>Capsicum annuum</i> cDNA, mRNA sequence	447	337	99.0	0.0
CS01011E02 Hot pepper under oxidative stress <i>Capsicum annuum</i> cDNA 5', mRNA sequence	699	188	90.0	3.0E-99
CS01017F11 Hot pepper under oxidative stress <i>Capsicum annuum</i> cDNA 5', mRNA sequence	708	188	90.0	3.0E-99
<i>Lycopersicon peruvianum</i> mRNA for Hsp20.1 protein	656	182	89.0	1.0E-95
SDBT002K03x SDBT <i>Solanum tuberosum</i> cDNA clone SDBT002K03, mRNA sequence	654	179	89.0	6.0E-94
<i>Solanum lycopersicum</i> Class I small heat-shock protein 20.1 (SI20.1shsp) and Class I small heat-shock protein 17.6 (SI17.6 shsp) genes	8304	175	89.0	1.0E-91
<i>Lycopersicon esculentum</i> 17.6 kD Class I small heat-shock protein (HSP17.6) mRNA	732	175	89.0	1.0E-91
STDB002D08u STDB <i>Solanum tuberosum</i> cDNA clone STDB002D08, mRNA sequence	650	175	89.0	1.0E-91
B24i 2 B1 B24i <i>Solanum tuberosum</i> cDNA, mRNA sequence	470	175	89.0	1.0E-91
12734.2 Stolon <i>Solanum tuberosum</i> cDNA clone 12734 5', mRNA sequence	671	175	89.0	1.0E-91
KS06005531 KS06 <i>Capsicum annuum</i> cDNA, mRNA sequence	551	175	89.0	1.0E-91
EST612422 Generation of a set of potato cDNA clones for microarray analyses mixed potato tissues <i>Solanum tuberosum</i> cDNA clone STMGB34 3' end, mRNA sequence	697	175	89.0	1.0E-91
EST582165 potato roots <i>Solanum tuberosum</i> cDNA clone cPRO32M12 5' end, mRNA sequence	564	175	89.0	1.0E-91
EST581924 potato roots <i>Solanum tuberosum</i> cDNA clone cPRO31N3 5' end, mRNA sequence	605	175	89.0	1.0E-91
EST580887 potato roots <i>Solanum tuberosum</i> cDNA clone cPRO28I11 5' end, mRNA sequence	669	175	89.0	1.0E-91
EST516270 cSTD <i>Solanum tuberosum</i> cDNA clone cSTD18G8 5' sequence, mRNA sequence	456	175	89.0	1.0E-91
EST515117 cSTD <i>Solanum tuberosum</i> cDNA clone cSTD13D9 5' sequence, mRNA sequence	685	175	89.0	1.0E-91
EST513393 cSTD <i>Solanum tuberosum</i> cDNA clone cSTD6E7 5' sequence, mRNA sequence	688	175	89.0	1.0E-91
EST513074 cSTD <i>Solanum tuberosum</i> cDNA clone cSTD3N22 5' sequence, mRNA sequence	658	175	89.0	1.0E-91
EST491411 cSTS <i>Solanum tuberosum</i> cDNA clone cSTS2A16 5' sequence, mRNA sequence	629	175	89.0	1.0E-91

heat-shock protein sequence in *Capsicum annuum*. Further, distance value from phylogenetic analysis reveals that the sequence has been highly conserved across genomes during evolution. MSA also proves alignment conservedness and that mutations occurred during the process of evolution. The sHSP sequence shows a high degree of similarity between *Capsicum annuum* and *Solanum tuberosum* followed by *Solanum lycopersicum*, *Solanum peruvianum* and *Lycopersicon esculentum*. Presence of this sHSP sequence in a crop reveals tolerance to heat and other stress-inducible conditions.

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