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Original Research Paper

Comparative Analysis of Heat Shock Transcription Factor in Solanum *Tuberosum* and Solanum *Lycopersicum*

Engineering

KEYWORDS : Potato, tomato, synteny, Transcription factor

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ABSTRACT

Heat shock transcription factors are one of the important transcription factors involved in heat stress and several other abiotic stresses like drought and cold. HSF regulates expression of HSPs and controls high temperature stress, damage and biological process. In this study we identified the HSF genes in potato and tomato and compared their gene structure, evolutionary relationship, motif and domains with the help of several bioinformatics software. 17 members of potato and 24 members of tomato were identified from Plant Transcription Factor database. Potato and tomato genes were distributed unevenly in 9 and 10 chromosomes of 12 chromosomes respectively. In terms of Gene structure, potato HSF genes retain 1 intron except 2 genes while in tomato 9.3% of genes consist 2 intron which suggests gain of intron during evolutionary process. Comparative phylogenetic analysis of HSF genes revealed 4 groups with several orthologous and paralogous genes. Conserved domains and cis-regulatory elements were also searched for both species of solaneaceae family. Segmental duplications were found to be involved in family expansion and evolution. Our comparative study may be helpful in understanding of HSF genes in solaneaceae family and evolutionary pathways of HSFs in potato and tomato.

INTRODUCTION

Potato, one of the most important non-grain food crops of the world ranks fourth in the world after wheat, rice and maize and plays an important role in the food chain (Anonymous 1999). During 1998, the global area under potato cultivation was approximately 18 million ha with a total production of about 295 million tonnes. By 2020, it has been estimated that demand for potato will increase by 40%. Potato is composed of 80% water, 18% carbohydrates and 2-3% protein. It plays an important role in human diet as it consists of carbohydrates, vitamins, minerals and proteins and also some trace elements which are necessary for human diet. Potato needs specific cooler temperature for its growth. Increase in temperature decreases the yield of potato (Bita and Gerats 2013). The expected yield potential of potato is 120 tons/ha while the average yield of potato is just 15 tons/ha due to various abiotic stress like high temperature, drought etc. Yield of potato can be enhanced by tolerance to different abiotic stresses. To cope up with high temperature there must be a heat shock system available in all species in which different proteins are involved (Li et al. 2014). Tolerance against different abiotic stresses is governed by plant's capability to express a set of genes whose expression is often regulated by specific transcription factors (TFs). Among different transcription factor families, Heat shock transcription factor (HSF) is one of an important transcription factor family in plants responsible for high-temperature tolerance in plants (Nover et al. 2001, Akerfelt et al. 2010, Hayashida et al. 2011). In plants the size of HSF transcription factor family is large as compared to other organisms (Czarnecka et al. 2004). HSF regulates the expression of Heat shock proteins (Sorger 1991). In signal transduction pathway, HSF's are present at the terminal end and acts as transcriptional regulators which help in activation of genes of different abiotic stresses such as high temperature, drought etc (Baniwal et al. 2004). Heat shock transcription factors binds with heat shock elements (HSE) to regulate the transcription of heat shock proteins (HSP). HSPs are involved in the protection of cells against stress impairment and also involved in folding of proteins (Morimoto et al. 1994, Schoffl et al. 1998, Hartl and Hayer-Hartl 2002). Various studies have showed the involvement of the HSF family in high temperature stress as well as in other abiotic stresses in different crops (Guo et al. 2008, Chauhan et al. 2011, Yoshida et al. 2011, Hahn et al. 2011, Mishra et al. 2002). As heat stress affects the yield of potato, so in this study we performed an insilico analysis of the HSF gene family in potato and compared it with the tomato HSF genes. Comparative analysis was performed to have an idea about their expansion and evolutionary history, explore their heat stress responses as elicited by naturally increased temperature.

MATERIALS AND METHODS

Identification and characterization of Tomato and Potato HSF genes

To identify the HSF family genes in Solanum tuberosum and Solanum lycopersicum, we searched and obtained genes annotated as HSF in Plant Transcription Factor Database (Rian˜o-Pacho´n et al. 2007). The protein sequences of all the proteins were retrieved from Phytozome (http://www.phytozome.net). We found 25 genes for potato and 26 genes for tomato from Plant Transcription factor Database. To further refine our search, domains were identified for each protein using pfam database (http://pfam.xfam.org/) with expectation cut-off value of 1.0. The genes in which specific HSF family domain was available only those genes were selected for further study, rest of the genes were discarded. So, finally we selected 17 HSF genes for potato and 24 HSF genes of tomato for comparative study.

Physiochemical Properties

To have information about the nature or the physiochemical properties of HSF proteins, we predict grand average of hydropathy (GRAVY), isoelectronic point (pI), Molecular weight and instability index by using ProtParam tool available on Expert Protein Analysis System (ExPASy, http://web.expasy.org/compute_pi) (Gasteiger et al. 2003). The instability index depicts the stability of the protein. If the value of instability index is less than 40 than the protein is stable in nature while if the value is greater than 40 then it indicates unstable nature of protein. The GRAVY score indicates whether the protein is polar or non- polar in nature. If the GRAVY score is positive then it indicates that the protein is hydrophobic.

Chromosomal distribution and duplication events analysis

The 17 HSF genes of potato and 24 genes of tomato were mapped on 12 chromosomes of potato and tomato respectively using MapChart. The duplication events I.e., tandem and segmental duplication were obtained by performing BLASTN of HSF nucleotide sequences. Sequence identity cut off was set above 80%. Those genes which were located closely on the same chromosome were defined as tandem duplicates while those located on other chromosomes or not adjacent to each other on the same chromosome were defined as segmental duplicates.

Gene structure and motif prediction

To perform structural analysis, coding and genomic sequences of all the genes belonging to potato and tomato were retrieved from Phytozome (http://www.phytozome.net). To illustrate exon-intron structure of HSF genes, genomic and coding sequences of HSF were aligned by Gene Structure Display Server (GSDS server, http://gsds.cbi.pku.edu.cn) (Guo et al. 2007). The conserved motif of HSF proteins were analyzed by Multiple maximization for Motif Elicitation analysis tool (MEME, http://meme.sdsc.edu, v4.9.0) (Bailey et al. 2007). The MEME suite was performed with the following parameters, maximum number of motifs=5, motif width=12 to 60 and E-value < 0.01.

Cis-regulatory elements

For the identification of cis-regulatory elements, 1kb upstream region sequences were retrieved from Phytozome (http://www.phytozome.net). These sequences were manually searched into PlantCare (http://bioinformatics.psb.ugent.be/webtools/plantcare/html/) database to find cis-acting elements in potato and tomato.

Phylogenetic analysis

To generate the phylogentic trees HSF transcription factor family genes, multiple sequence alignment of the genes was done using ClustalW. Phylogenetic tree was built by using Neighbour-Joining method with 1000 rapid bootstrap replicates with the help of MEGA v6.06 (Tamura et al. 2013). In neighbour joining analysis, pairwise deletion and Poisson correction options were used. The developed phylogenetic tree was visualized using iTOL (http://itol.embl.de/) software.

Synteny analysis

The synteny among 2 species of solanaceae family i.e., Solanum tuberosum and Solanum lycopersicum was carried out by performing BLASTN search against genomic sequences of tomato chromosomes. A cut-off bit score of 75 and E-value less than 1e-05 were considered as optimum for BLASTN analysis. The synteny among potato and tomato was visualised using Circos v0.63software. (http://circos.ca) (Krzywinski et al. 2009).

RESULTS AND DISCUSSION

Identification and physiochemical properties of HSF gene family

Genome wide analysis led to the identification of 41 HSF proteins in potato and 26 HSF proteins in tomato using Plant transcription Factor Database. To further refine our search we find domains of these genes using pfam and selected only those genes which were significant. So finally we selected 17 genes for potato and 24 genes for tomato as shown in Table 1. In previous studies, 21, 25, 28, 16, 25 HSF's were found in Arabidopsis, rice, populous, Medicago and maize (Miller and Mittler 2006, Guo et al. 2008, Lin et al. 2011, Wang et al. 2011). In order to understand nature of these HSF proteins, we analysed their physiochemical properties. Molecular weight of HSF proteins in potato varies from 8.5 kDa and 54.4 kDa with an average value of 39.1 ka while in tomato it ranges from 16.6kDa to 57.7 kDa with an average value of 39.5. It has been observed that if the hydropathy score of a protein is negative then it is soluble in nature while if the hydropathy score is positive then the protein is insoluble in nature (Kyte and Doolittle 1982). In our study, GRAVY score of all proteins in potato as well as in tomato have negative value which indicates that all of these genes are soluble in nature, a character that is necessary for transcription factors. Isoelectronic point (pI) is the point at which net charge of the protein is zero and the protein is least soluble at this point. The average pI of HSF transcription factor genes in potato and tomato was 6.0 and 6.5 respectively. The minimum and maximum pI was 4.71 (PGSC0003DMG400017484) and 9.58 (PGSC0003DMG400029718) in potato while 4.7 (Solyc09g059520.2) and 9.4 (Solyc06g053960.2) in case of tomato. In previous studies also, wide range of isoelectronic point has been noticed in cucumber, soybean etc (Zhou et al. 2013, Li et al. 2014). We have also analyzed the stability of the proteins by calculating instability index of each protein. Instability index is the measure of protein to determine its stability. It has been observed that if instability index of a protein is greater than 40 then it is unstable while if it is less than 40 then the protein is stable in nature (Rogers et al. 1986). It has been observed that most of the HSF transcription factor genes are unstable I nature both in potato as well as in tomato. In potato, 94.1 % of the HSF genes are unstable while 91.7 % in tomato.

Table 1: Summary of physio-chemical properties of HSF transcription factors in Potato and Tomato.

Gene Name	Abbreviation	Chr	Mol wt. (kDa)	pI	GRAVY	Instability Index
PGSC0003D-MG400028414	ST_HSF1	2	46.22	5.23	-0.76	46.07
PGSC0003D-MG400029718	ST_HSF2	2	23.85	9.58	-0.85	49.15
PGSC0003D-MG400041361	ST_HSF3	2	46.95	4.92	-0.81	47.6
PGSC0003D-MG400003160	ST_HSF4	3	54.32	4.85	-0.44	59.56
PGSC0003D-MG401027812	ST_HSF5	3	46.21	5.16	-0.77	44.3
PGSC0003D-MG400027283	ST_HSF6	4	28.7	8.68	-0.75	60.24
PGSC0003D-MG400027036	ST_HSF7	6	54.42	5.17	-0.58	54.79
PGSC0003D-MG400032793	ST_HSF8	7	41.76	5.28	-0.83	56.45
PGSC0003D-MG400003053	ST_HSF9	8	35.02	4.93	-0.47	54.35
PGSC0003D-MG401002683	ST_HSF10	9	52.91	4.81	-0.45	55.73
PGSC0003D-MG400017484	ST_HSF11	9	45.54	4.71	-0.59	56.07
PGSC0003D-MG402019343	ST_HSF12	9	42.03	5.53	-0.78	54.59
PGSC0003D-MG401019343	ST_HSF13	9	39.71	5.23	-0.75	48.7
PGSC0003D-MG400019344	ST_HSF14	9	8.55	5.99	-0.33	32.73
PGSC0003D-MG400034428	ST_HSF15	11	29.39	7.31	-0.69	48.32

PGSC0003D-MG400043234	ST_HSF16	11	28.71	9.3	-0.65	47.63
PGSC0003D-MG400000380	ST_HSF17	12	40.97	5.86	-0.61	66.82
Soly-c09g059520.2	SL_HSF1	9	44.92	4.68	-0.62	52.43
Soly-c09g009100.2	SL_HSF2	9	56.1	4.82	-0.55	54.9
Soly-c02g079180.1	SL_HSF3	2	47.14	4.95	-0.77	45.09
Soly-c08g080540.2	SL_HSF4	8	35.29	4.99	-0.43	52.33
Soly-c03g097120.2	SL_HSF5	3	54.03	5.07	-0.41	55.16
Soly-c08g076590.2	SL_HSF6	8	55.02	5.16	-0.67	61.98
Soly-c08g005170.2	SL_HSF7	8	57.7	5.16	-0.57	57.67
Soly-c02g072000.2	SL_HSF8	2	46.17	5.23	-0.73	50.95
Soly-c09g065660.2	SL_HSF9	9	42.44	5.28	-0.82	58.96
Soly-c06g072750.2	SL_HSF10	6	54.34	5.32	-0.62	53.81
Soly-c03g006000.2	SL_HSF11	3	46.08	5.33	-0.8	44.55
Soly-c07g040680.2	SL_HSF12	7	40.87	5.33	-0.76	57.33
Soly-c03g026020.2	SL_HSF13	3	37.39	5.34	-0.74	68.07
Soly-c09g082670.2	SL_HSF14	9	41.76	5.38	-0.96	43.54
Soly-c12g098520.1	SL_HSF15	12	53.38	5.4	-0.69	58.51
Soly-c02g090820.2	SL_HSF16	2	33.26	5.75	-0.75	37.03
Soly-c12g007070.1	SL_HSF17	12	41.17	6.05	-0.63	65.11
Soly-c11g064990.1	SL_HSF18	11	29.83	7.29	-0.74	51.99
Soly-c10g079380.1	SL_HSF19	10	29.92	7.59	-0.87	50.78
Soly-c02g072060.1	SL_HSF20	2	38.41	7.63	-0.89	44.57
Soly-c04g078770.2	SL_HSF21	4	40.93	7.72	-0.65	54.99
Soly-c04g016000.2	SL_HSF22	4	27.57	8.87	-0.7	56.27
Soly-c02g078340.2	SL_HSF23	2	24.24	9.39	-0.87	46.08
Soly-c06g053960.2	SL_HSF24	6	16.64	9.44	-0.8	31.89

Chromosomal location of HSF genes

Seventeen HSF genes of potato and 24 HSF genes of tomato were mapped on their chromosomes to examine the association between genetic divergence within HSF gene family and duplication in potato and tomato. The distribution of HSF genes was not uniform in potato as well as in tomato. Some chromosome and chromosomal regions have high density of genes while some regions lack the genes. The HSF family genes were unevenly mapped on 9 chromosomes of 12 chromosomes in potato and on 10 chromosomes in tomato. Most of the HSF genes lie only on the short arm of the chromosome. As depicted in Figure1, In potato chromosome 9 has highest number of genes i.e., 5 while in tomato maximum number of genes were found on chromosome 2 i.e., 5. No genes were found on chromosome 1, 5 and 10 in potato while in tomato, chromosome 1 and 5 lacks genes for HSF transcription factor. In various previous studies it has been observed that the HSF genes were distributed unevenly on their chromosomes. In soybean also, the HSF genes were unevenly distributed on 15 chromosomes out of 20 chromosomes (Li et al. 2014). Gene duplication is a process in which new genes are formed which disperses in the genome (Lynch et al. 2000). In Solanum tuberosum, we found only 3 tandem duplication and 1 segmental duplication while in case of solanum lycopersicum only 2 segmental duplication were obtained but no tandem duplication were identified.

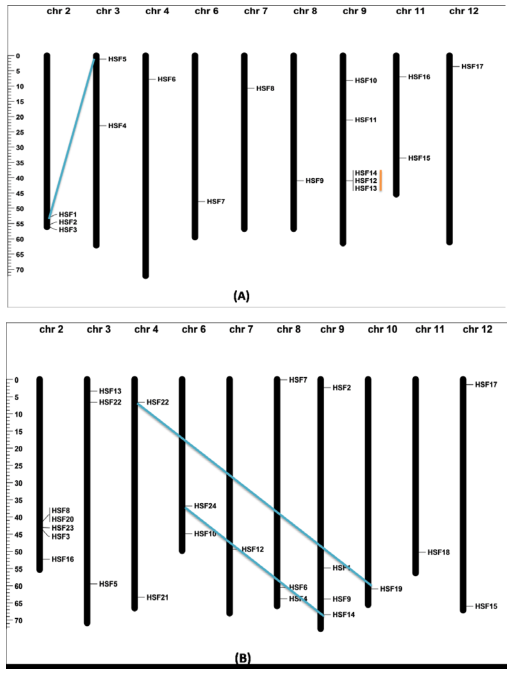

Figure 1 : Distribution of HSF genes on 12 chromosomes of potato and tomato. (A) Percentage of HSF genes on potato. (B) Percentage of HSF genes on tomato. Tandem duplicated genes on particular chromosomes are represented by orange colour and segmental duplications by blue colour.

Gene structure

The exon and intron structure of the HSF genes of potato and tomato was analyzed to understand the structural components of the HSF genes. It was observed that most of the HSF genes in tomato and potato retain only one intron. In potato, out of 17 genes, 15 genes had only one intron while one gene (PGSC0003DMG400019344) was intronless and one gene (PGSC0003DMG402019343) had 2 introns. However, in case of tomato, 91.7 % of genes had only one intron while 9.3 % (Soly06g053960.2, Soly09g065660.2) genes had only 2 introns. Intronless gene represents loss of introns while genes having 2 introns represent gain of introns during evolutionary process. It was depicted from previous studies of cucumber and soybean that mostly HSF genes have only 1 intron. In soybean gene structure analysis it was noticed that soybean HSF genes had one intron only except one gene[3] while in cucumber 2 genes had 2 introns and one gene had 3 introns (Zhou et al. 2013) and in chickpea only three genes had 2 introns (Zafar et al. 2016). The gene structure of Solanum tubersoum and Solanum lycopersicum has been represented in Figure 2.

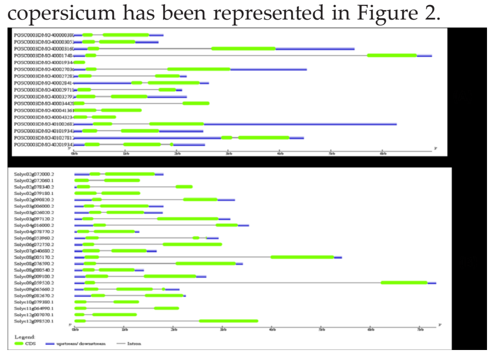

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Figure 2: (A) Gene structure of potato HSF genes (B) Gene structure of tomato HSF genes. Here green colour represents CDS region, blue colour represents upstream or downstream region and grey lines shows introns.

Phylogenetic analysis

To analyze the evolutionary relationship among potato and tomato HSF genes, a phylogenetic tree was developed using 17 genes of potato and 24 genes of tomato. In our phylogenetic tree, four major groups of HSF genes were found with several paralogous as well as orthologous genes. In 1st group, 5 HSF genes were found while in 2nd group we had 11 genes, 3rd had 11 genes and 4th group is the largest group consisting of 19 members as shown in Figure 3. Each group possesses potato as well as tomato genes. In previous study of chickpea, 4 groups were found in phylogenetic tree which is same as our results (Zafar et al. 2016). Similarly in Arabidopsis and rice, 3 groups were found in the phylogenetic tree of HSF genes (Guo et al. 2008).

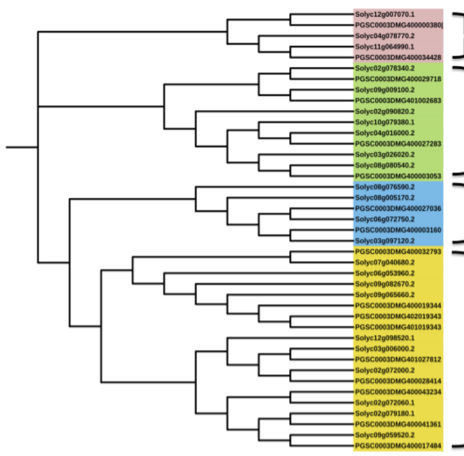

Figure 3: Comparative phylogenetic analysis of Solanum tuberosum and Solanum lycopersicum.

Motif Prediction

To predict the motifs conserved among HSF genes, we used MEME software. In potato as well as in tomato we find 5 different types of motifs. In potato HSF genes, all these 5 motifs were highly conserved (Table 2). On N –terminal of potato HSF genes, motif 2 is conserved while on C-terminal motif was present on all the HSF sequences and Central position was occupied by motif 1, 4 and 5 respectively. In case of tomato, out of 5 identified motifs, 4 motifs were highly conserved among all the tomato HSF genes while motif 3 was available only in 16 HSF genes of tomato instead of 24 genes. Previous study on soybean also depicted 5 conserved motifs among Soybean HSF proteins (Li et al. 2014).

Table 2: Conserved motifs in HSF transcription factors through MEME suite.

S.No	Motif width	Best possible match
1	36	NSFVVWDPPEFARDLLPKYFKHNNF-SSFVRQLNTYG
2	29	HGNCPPPFLTKTYEMVDDPSTDHIVS-WNR
3	50	LMMELVRLRQQQQATDHYMQT-MTQRLQAMEQRQQQMMS-FLAKAMQNPGFV
4	15	FRKIDPDRWEFANEC
5	21	FLRGQKHLLCNIHRRKPWHSH

Synteny analysis

To find synteny among the potato and tomato, potato HSF sequences were used as query against the tomato database while performing BLASTN for identification of orthologs with more than 80% silmilarity and E-value less than 1e-05. To confirm the potential orthologs, reciprocal BLAST was performed. A total of 16 genes of tomato showed syntenic relationship with potato HSF genes. Solanum tuberosum shows maximum synteny with chromosome 9 of solanum lycopersicum followed by chromosome 2 and chromosome 3.

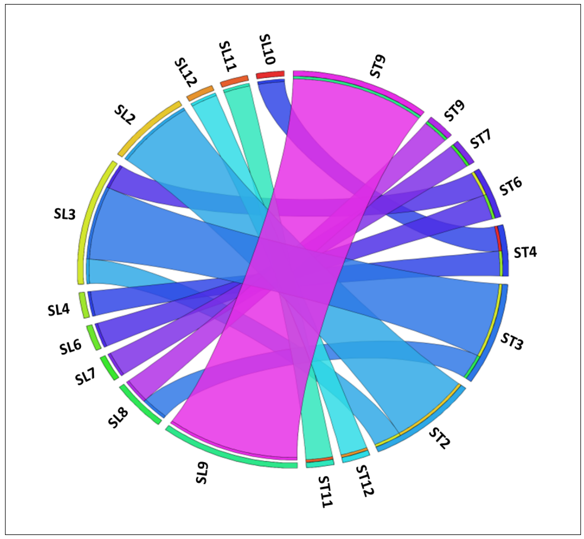

Figure 4: Comparative map showing orthologous gene positions of HSF genes among different chromosomes of Solanum tuberosum and Solanum lycopersicum.

Cis-regulatory elements

1kb upstream region of promoter sequences was used for the analysis of cis-regulatory elements through PlantCare database. In our investigation, potato HSF genes have 75 different types of cis-regulatory elements while in tomato non-repetitive 107 types of cis-elements were found. Out of these total cis-elements, 60 different cis-elements were identified as common among potato and tomato HSF genes which showed that most of the HSF genes among different crops have same types of cis-regulatory elements. These cis-elements were involved in different abiotic stresses like high temperature, drought, light etc. which some cis-regulatory elements were also involved in hormone and development. Among abiotic stress related cis-regulatory elements, important elements that were found in promoter region of HSF genes were ARE (anaerobic induction element), MBS, TC-rich repeats and HSE etc. In stress related cis-regulatory elements, gibberellins responsive element, salicylic, abscisic acid and auxin responsive elements were found while in developmental category, circadian and meristem related cis-acting elements were found. The presence of HSE cis element is often correlated with the expression of respective Hsp and Hsf genes under heat stress as shown in the microarray analysis of Arabidopsis, rice and maize (Lin et al. 2011, Mittal et al. 2009). These findings will help to get the concepts of responses of HSF in stress condition and the whole genomic information of HSF family. This data will facilitate selecting candidate genes for stress condition and further functional and comparative characterization.

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