

Genome-wide identification, classification, and expression analysis of the *HSF* gene family in pineapple (*Ananas comosus*)

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ABSTRACT

Transcription factors (TFs), such as heat shock transcription factors (HSFs), usually play critical regulatory functions in plant development, growth, and response to environmental cues. However, no HSFs have been characterized in pineapple thus far. Here, we identified 22 *AcHSF* genes from the pineapple genome. Gene structure, motifs, and phylogenetic analysis showed that *AcHSF* families were distinctly grouped into three subfamilies (12 in Group A, seven in Group B, and four in Group C). The *AcHSF* promoters contained various *cis*-elements associated with stress, hormones, and plant development processes, for instance, STRE, WRKY, and ABRE binding sites. The majority of *HSFs* were expressed in diverse pineapple tissues and developmental stages. The expression of *AcHSF-B4b/AcHSF-B4c* and *AcHSF-A7b/AcHSF-A1c* were enriched in the ovules and fruits, respectively. Six genes (*AcHSF-A1a*, *AcHSF-A2*, *AcHSF-A9a*, *AcHSF-B1a*, *AcHSF-B2a*, and *AcHSF-C1a*) were transcriptionally modified by cold, heat, and ABA. Our results provide an overview and lay the foundation for future functional characterization of the pineapple *HSF* gene family.

Subjects Agricultural Science, Bioinformatics, Genomics, Molecular Biology, Plant Science

Keywords *AcHSF*, Phylogenetic analysis, Pineapple, Cold, Heat, ABA

INTRODUCTION

The living environment of plants is faced with many challenges, including cold, heat, drought, and salinity stresses (Hu & Xiong, 2014; Pereira, 2016; Zhu, 2016). Due to global warming, heat stress is becoming a serious agricultural threat for agricultural production and planting areas worldwide (Wahid et al., 2007). Typically, plants face heat stress when the temperature rises 10 to 15 degrees above the optimum growth environment. Heat stress affects plant development and growth and eventually leads to a decrease in crop yield. Consequently, as a defense or signaling mechanism in response to environmental stresses, plants regulate the expression of several genes through different transcription factors (TFs). The heat shock transcription factor family of plants is involved in heat stress response and regulates the expression of several stress-responsive proteins, including heat shock proteins (HSPs), ascorbate peroxidase (APX), and catalase (CAT) (Ohama et

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et al., 2017). Previously, several studies have validated the roles of heat shock transcription factors (HSFs) in plant stress response. For example, the overexpression of *CarHsf-A2* could enhance chickpea stress tolerance without any pleiotropic effects (*Chidambaramathan et al.*, 2018). Besides, *AtHSF-B1* and *AtHsf-B2b* act as expression repressors after heat-stress, and the *AtHSF-A1* is involved in cold acclimation in *Arabidopsis thaliana* (*Ikeda, Mitsuda & Ohme-Takagi*, 2011; *Olate et al.*, 2018). Similarly, *PeuHsf-A2* gets induced by heat stress, increasing desert poplar acclimation (*Zhang et al.*, 2016b).

HSFs contain several evolutionarily conserved functional domains. The canonical HSF protein contains an N-terminal DNA-binding domain (DBD) that binds to HSEs; a hydrophobic amino acid residue (HR-A/B) oligomerization domain (OD) heptad repeat bound to DBD by a flexible linker. In addition, it also includes a region of nuclear localization signal (NLS), a region of nuclear export signal (NES) and a motif of activator AHA located at the C-terminal (*Guo et al.*, 2016; *Nishizawa-Yokoi et al.*, 2011; *Singh et al.*, 2012; *Yabuta*, 2016). Three types of plant HSF have been identified based on the variable linker (usually 15–80 aa) and HR-A/B domain, including nine class A (A1–A9), four class B (B1–B4), and two class C (C1–C2) (*Giesguth et al.*, 2015; *Nishizawa-Yokoi et al.*, 2011; *Shim et al.*, 2009; *Singh et al.*, 2012; *Yabuta*, 2016). Initially, HSFs were identified in yeast (Scharf *et al.*, 1990), and the first HSF plant gene was identified in tomatoes (Sorger & Pelham, 1988). Since then, several plant HSF gene families, including *Arabidopsis thaliana* (*Guo et al.*, 2008), rice (*Oryza sativa* L.) (*Chauhan et al.*, 2011; *Jin, Gho & Jung*, 2013), maize (*Zea mays* L.) (*Lin et al.*, 2014), *Populus trichocarpa* (*Wang et al.*, 2012), wheat (*Triticum aestivum* L.) (*Chauhan et al.*, 2013), soybean (*Glycine max*) (*Chung, Kim & Lee*, 2013), Chinese cabbage (*Brassica rapa ssp. pekinensis*) (*Song et al.*, 2014), cotton (*Gossypium hirsutum*) (*Wang et al.*, 2014), barrel medic (*Medicago truncatula*) (*Lin et al.*, 2014), pepper (*Capsicum annuum* L.) (*Guo et al.*, 2015; *Guo et al.*, 2014), strawberry (*Fragaria vesca*) (*Hu et al.*, 2015) and tea plant (*Camellia sinensis*) (*Liu et al.*, 2016), etc.

Pineapple (*Ananas comosus*) is grown in subtropical and tropical regions and is widely loved worldwide and its genome is sequenced (*Bai et al.*, 2019; *Ming et al.*, 2015). However, many biotic and abiotic environmental stresses, pathogen infection, and degradation of good breeds limit pineapple production (*Barral et al.*, 2019). Therefore, it is essential to identify and characterize genes involved in response to environmental stresses and study the underlying molecular mechanism that could be used for possible genetic breeding applications. HSFs are well known for the co-involvement in different environmental cues, such as cold, heat, and ABA. However, *AcHSFs* and their possible role in pineapples have not been explored. Here, whole-genome identification and expression analysis of *AcHSFs* gene during flower, fruit development, and abiotic stress was conducted to expand the understanding of *AcHSFs* gene and its application in genetic breeding.

METHODS

Identification and Characterization of *AcHSF* genes in pineapple

The amino acid sequences of pineapple HSFs were retrieved using the HSF-type DBD domain (Pfam: PF00447) as a query in *Phytozome* JGI. A total of 30 pineapple HSFs were

obtained from JGI. Only 22 *AcHSFs* left, after filtering out the non-typical HSF-type DBD and repeated sequences or canonical coiled-coil structures by SMART online tool (Letunic, Doerks & Bork, 2012). The information of Chromosome localization, CDS, and AA length for *AcHSFs* was obtained from JGI *Phytozome v12.1*. The biophysical properties of coding *AcHSFs* were calculated using the Expasy ProtParam tool. The subcellular localization of *AcHSFs* was analyzed using BUSCA (<http://busca.biocomp.unibo.it/>).

Chromosome Localization phylogenetic relationships

The information of all pineapple *HSFs*' chromosome localization site was acquired from *Phytozome v12.1*, including chromosome length, chromosome location, and gene start site. The MapChart v2.0 (<https://mapchart.net/>) was adopted to map the chromosomal location. The phylogenetic relationship of different HSF proteins was explored, and the phylogenetic tree was created using the *AcHSF* amino acid sequences and the other three species, *Arabidopsis thaliana*, *A. thaliana*, *Oryza sativa* (*O. sativa*), and *Populus trichocarpa* (*P. trichocarpa*) by the MEGAX with a bootstrap value of 1000. The HSF gene in pineapples was referred to as *AcHSF* genes and classified according to HSFs in the phylogenetic tree classes A, B, and C.

Genetic structure and *cis*-acting elements

The gene structures of *AcHSF*, including exons, introns, and UTR were displayed by the GSDS online tool (Guo *et al.*, 2007). The promoter sequence of *AcHSFs* found in *Phytozome* is located 2kb upstream of the translation initiation site. These sequences were analyzed using a plant *cis*-acting element database New PLACE (Higo *et al.*, 1999), to identify *cis*-elements necessary for gene expression, development, and hormone signaling under abiotic stress.

Conserved domains and motifs analysis of *AcHSFs*

Clustal X 2.0 and DNAMAN software was used to align and edit the DBD domain and HR-A/B regions (OD). Using the cNLS online tool, NLS domains were predicted and the NetNES 1.1 online server identified NES domains in the *AcHSFs*. MEME server (<http://meme-suite.org/>) was applied to define the conserved motifs of *AcHSFs* following the parameters: the number of repetitions = any, the maximum number of motifs = 10, minimum width ≥ 10 , maximum width ≤ 50 , and motifs with an *E*-value < 0.01 were retained.

Expression patterns analysis

The transcriptomic data generated from different organs and developmental stages of pineapple have been described previously (Ming *et al.*, 2015; Wang *et al.*, 2020). Briefly, the different organs include 3 stages of petal tissues, 4 stages of sepal tissues, 6 stages of stamen tissues, 7 stages of ovule tissues, 7 stages of gynoecium tissues, and root, mixed flower, leaf, and 6 stages of fruit were used to generate heatmap using the pheatmap package of R software.

Stress treatments

One-month-old uniform tissue-cultured seedlings in a rooting medium were used for the stress treatment analyses (Priyadarshani *et al.*, 2018). The stress treatments of pineapple seedling as follows: cold (4 ° C), heat (45 ° C), and ABA (100 mM). Leaves were collected from three independent plants at 12 h, 24 h and 48 h after treatment and immediately stored in liquid nitrogen before RNA extraction. Untreated pineapple seedlings of the same size were used as controls.

RNA extraction and qRT-PCR

Total RNA of pineapple leaf tissues was obtained using the RNeasy Plant Mini Kit (50) (Qiagen). For cDNA synthesis, a total of 1 µg RNA per sample was used with cDNA Synthesis SuperMix (Transgen, Beijing, China). qPCR was conducted by the Bio-Rad CFX manager machine using TransStart[®] Top Green qPCR SuperMix (Transgen, Beijing, China). Pineapple *Actin2* is used as the reference gene for qPCR (Wang *et al.*, 2020). There are total of three biological replicates for each sample, and the results are shown as the mean ± standard deviations.

RESULTS

Genome-wide identification of HSF genes in pineapple

HSF-type DBD domains (Pfam: PF00447) amino acid sequences were submitted into *Ananas comosus* v3 *Phytozome* database v12.1. A total of 30 putative pineapple HSF sequences were acquired. And then, checked by the *SMART* online tool and *Pfam* database, 1 pineapple HSF sequence was rejected due to the absence of typical HSF-DBD domains, and 7 HSF sequences were abandoned due to the absence of coiled-coil structure. As a result, 22 non-redundant pineapple *HSFs* were identified (Table 1). The comprehensive information of these 22 *AcHSF* genes, including gene name, gene ID, CDS and protein length, isoelectric points, molecular weights, predicted subcellular location, and other features are presented in Table 1. The gene with the longest amino acid length is *ACHSF-A5*, which contains 601 amino acids and also has the largest molecular weight of 65.81 KDa; and the gene with the shortest amino acid length was *AcHSF-A9b*, which contains 129 amino acids and also has the smallest molecular weight of 13.77 KDa. Prediction of protein isoelectric points (pI) can aid in the purification and isolation of proteins. The predicted isoelectric points (pI) of *AcHSFs* ranged from 4.68 (*AcHSF-B2b*) to 9.63 (*AcHSF-B4c*). Detailed information on other parameters has been given in Table 1.

According to the detailed gene information, 19 *AcHSF* genes were mapped to the 11 pineapple chromosomes and 3 *AcHSF* genes located in the scaffold (Table 1). The number of pineapple HSF genes for each chromosome varied significantly, and there is no discernible pattern in the location of these genes on chromosomes. For example, three *AcHSF* genes were located in chromosome 5, whereas only one was present in chromosomes 2, 6, 17, and 18 respectively (Fig. 1).

Phylogenetic analysis of *AcHSFs* gene family

A phylogenetic analysis of 31 *Populus trichocarpa* HSFs, 25 rice HSFs, and 21 *Arabidopsis* HSFs was performed to classify the phylogenetic relationships (Guo *et al.*, 2016), together

Table 1 Protein information of pineapple (*Ananas comosus*) heat shock transcription factors (AcHSFs). Including protein name, sequenced ID, subfamily, Chromosome Localization, CDS and amino acid (AA) length, molecular weight (MW), isoelectric point (pI), and predicted subcellular location.

NO	Protein name	Gene ID	Subfamily	Chromosome Localization	CDS length	AA length	pI	Mw (kDa)	Predicted subcellular location	
1	AcHSF-A1a	Aco027746.1	A1	Chr 5	11287848-11300490	1752	583	5.08	63.91	nucleus
2	AcHSF-A1b	Aco020850.1	A1	Chr 9	8088573-8101648	1725	574	4.86	62.37	nucleus
3	AcHSF-A1c	Aco016980.1	A1	Chr 8	13365954-13368969	1338	445	4.89	48.59	nucleus
4	AcHSF-A2	Aco008819.1	A2	Chr 9	2742350-2746933	1098	365	5.44	41.31	nucleus
5	AcHSF-A3	Aco016689.1	A3	Chr 17	159803-163500	1671	556	5.03	61.08	nucleus
6	AcHSF-A4	Aco005592.1	A4	Chr 11	11506920-11509712	1359	452	5.28	51.19	nucleus
7	AcHSF-A5	Aco016114.1	A5	Chr 21	1487000-1489970	1806	601	7.68	65.81	nucleus
8	AcHSF-A6	Aco009685.1	A6	Chr 1	380459-383347	1116	371	4.78	41.94	nucleus
9	AcHSF-A7a	Aco005862.1	A7	Chr 16	10841121-10844044	1089	362	5.8	41.47	nucleus
10	AcHSF-A7b	Aco015210.1	A7	Chr 5	1225625-1227632	999	332	4.8	36.96	nucleus
11	AcHSF-A9a	Aco021879.1	A9	Chr 21	5356086-5390683	1395	464	5.17	51.49	nucleus
12	AcHSF-A9b	Aco022474.1	A9	Chr 1	5983677-5984066	390	129	5.18	13.77	nucleus
14	AcHSF-B1a	Aco002688.1	B1	Chr 6	10679048-10683679	948	315	8.54	34.28	nucleus
13	AcHSF-B1b	Aco030273.1	B1	scaffold_1756	6908-10841	930	309	8.72	33.57	nucleus
15	AcHSF-B1c	Aco001320.1	B1	Chr 2	12375558-12379157	930	309	8.88	33.76	nucleus
16	AcHSF-B2a	Aco013873.1	B2	Chr 8	9295304-9296338	906	301	6.06	32.77	nucleus
17	AcHSF-B2b	Aco027680.1	B2	scaffold_382	177006-178278	1173	390	4.68	40.94	nucleus
18	AcHSF-B4b	Aco017163.1	B4	Chr 18	739089-740863	957	318	5.7	36.55	nucleus
19	AcHSF-B4c	Aco031324.1	B4	scaffold_2094	3028-3903	876	291	9.63	31.26	extracellular space
20	AcHSF-C1a	Aco006046.1	C1	Chr 16	9444927-9446130	888	295	6.35	33.69	nucleus
21	AcHSF-C1b	Aco005573.1	C1	Chr 11	11371568-11373537	696	231	8.52	26.42	nucleus
22	AcHSF-C2	Aco027352.1	C2	Chr 5	1814813-1816318	1113	370	8.3	39.62	nucleus

with those of *AcHSFs* by generating a neighbor-joining phylogenetic tree. The HSFs were grouped into three clusters, A, B, and C, according to the difference between the amino acid sequences of the DBD domain, the HR-A/B region, and the linker between them (Guo et al., 2016; Scharf et al., 2012). Class A consisted of 10 subclusters, designated A1 to A10. Class B contained B1 to B4, and Class C comprises C1 and C2 sub-clusters. In pineapple (*Ananas comosus*), according to their phylogenetic relationship, 12 *AcHSFs* out of 22 proteins belong to class A, followed by seven *AcHSFs* belonging to class B, and three copies of class C (Fig. 2). As a monocot, the pineapple was more similar to rice, rather than the dicot *Arabidopsis* and *Populus trichocarpa*. However, none of the *AcHSFs* were found in the subclass A8 and B3, which was reported to only exist in the monocots (Li et al., 2014). It is strange that the pineapple and rice subclass A7 HSFs showed higher similarity to A2 rather than the *Arabidopsis* and *Populus trichocarpa* subclass A7, and the *AtHSF-A6a* also shows abnormal clustering (Fig. 2).

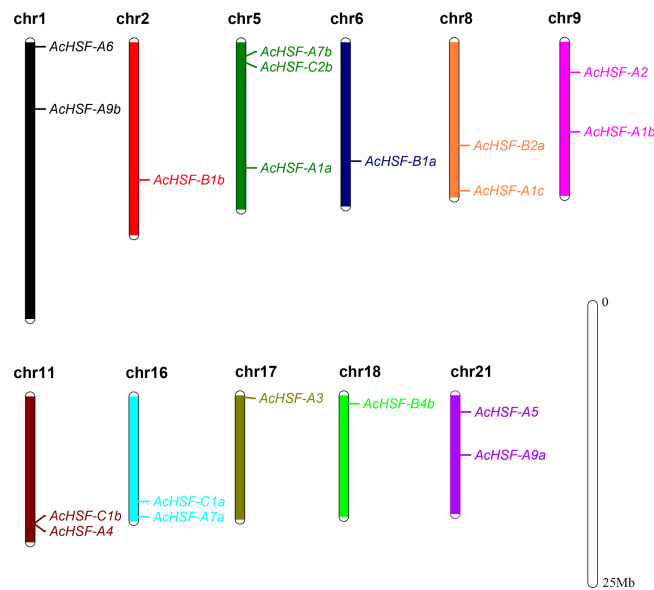


Figure 1 Distribution of AcHSF genes in pineapple genome. The different color bars represent the different chromosomes and the chromosome numbers are showed on the top of the bars. Length of the bars are related with the size of the chromosomes. AcHSF genes distribute on the 11 chromosomes. The white bar represent the scale bar 25 Mb.

Full-size DOI: 10.7717/peerj.11329/fig-1

Gene structures and *cis*-acting elements analysis of *AcHSFs*

Intron, exon, 5' UTR, and 3' UTR structures were analyzed using Gene Structure Display Server (GSDS) v2.0.0 to reveal the gene structural features of *AcHSFs*. The number of exons for *AcHSFs* ranged from 1 to 5 (Fig. 3), while only in the longest *AcHSF-A9a* (genomic sequence 34,598 bp.) was found 5 exons. The 5' UTR, and 3' UTR sequence of the *AcHSF* genes are incomplete, 8 out of 22 *AcHSFs* (*AcHSF-A3*, *AcHSF-A7b*, *AcHSF-B1c*, *AcHSF-B2a*, *AcHSF-B2b*, *AcHSF-B4c*, *AcHSF-C1a*, and *AcHSF-C1b*) do not have 5' UTR and 3' UTR sequences, 3 *AcHSFs* (*AcHSF-A1b*, *AcHSF-A9a*, and *AcHSF-B4b*) have only 5'UTR sequences, 6 *AcHSFs* (*AcHSF-A4*, *AcHSF-A5*, *AcHSF-A7*, *AcHSF-B1a*, *AcHSF-B1b*, and *AcHSF-C2*) have only 3'UTR sequences, and 5 *AcHSFs* (*AcHSF-A1a*, *AcHSF-A1c*, *AcHSF-A2*, *AcHSF-A6*, and *AcHSF-A9*) both have 5' UTR and 3' UTR sequences.

Stress responses elements of the dehydration-responsive element (DRE), ABA-responsive element (ABRE), low-temperature responsive element (LTRE), MYB, MYC, and WRKY elements has been reported to play important roles in drought, salt, cold, ABA, and GA responses (Chai *et al.*, 2020; Li *et al.*, 2012; Zhang *et al.*, 2009). The 2kb sequences upstream of *AcHSFs* gene were selected for analysis. The *cis*-acting elements analysis of *AcHSFs* promoter demonstrated that every pineapple *HSF* contains at least 2 MYB, MYC, and WRKY elements, except for *AcHSF-B4c* (Table 2). But for the *AcHSF-B4c*, only 110bp promoter sequence can be found in the upstream area, among the 110bp promoter sequence, the main core component of the promoter TATA-box and CAAT-box, the light regulatory element (RYREPEATBNNAPA), and root-specific expression related elements (ROOTMOTIFTAPOX1) can be found. We also detected the ABRE, DRE, and LTRE in

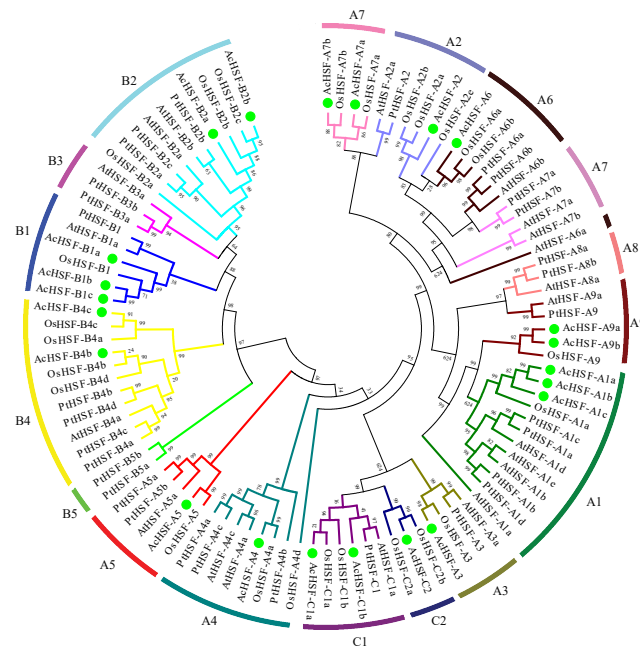


Figure 2 The phylogenetic tree of HSF proteins. The phylogenetic tree of HSF proteins in pineapple and other plant species was generated by MEGA 5 using the neighbor-joining method and the bootstrap values were set at 1000. AcHSFs were divided into three classes and 13 subclasses (A1, A2, A3, A4, A5, A6, A7, A9, B1, B2, B4, C1 and C2) and separated by green dots. Ac, *Ananas comosus*; At, *Arabidopsis thaliana*; Os, *Oryza sativa* and Pt, *Populus trichocarpa*.

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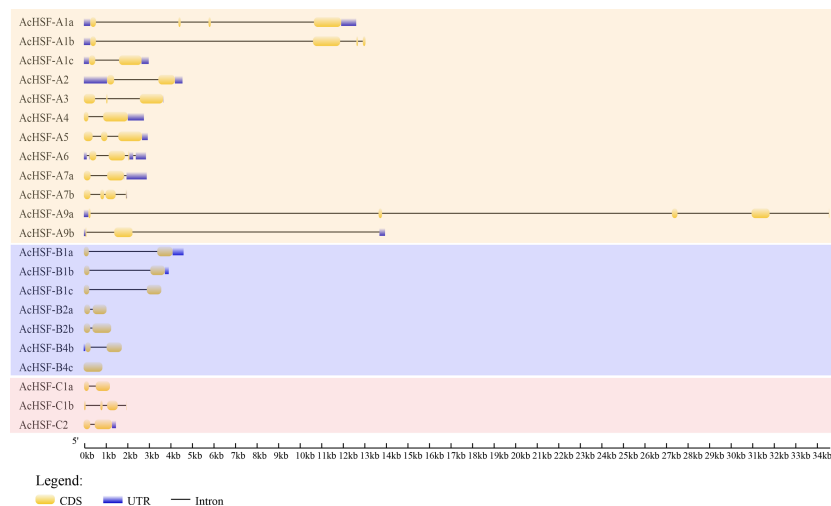


Figure 3 Exon–intron organization of AcHSF genes. The exons, introns and untranslated regions (UTRs) were indicated by the yellow boxes, black lines and blue boxes, respectively.

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Table 2 Distribution of ABRE, DRE, LTRE, MYB, MYC and WRKY cis-acting elements in pineapple HSF promoters.

Gene	ABRE	DRE	LTRE	MYB	MYC	WRKY
AcHSF-A1a	0	1	5	10	2	10
AcHSF-A1b	1	1	5	7	2	8
AcHSF-A1c	14	2	2	2	2	2
AcHSF-A2	12	4	6	17	4	7
AcHSF-A3	11	4	6	22	10	6
AcHSF-A4	0	2	4	18	12	10
AcHSF-A5	8	1	4	19	20	7
AcHSF-A6	1	0	2	15	8	3
AcHSF-A7a	2	1	5	24	22	7
AcHSF-A7b	12	2	3	20	8	11
AcHSF-A9a	0	0	0	59	28	11
AcHSF-A9b	2	1	3	25	12	7
AcHSF-B1a	7	2	3	18	12	7
AcHSF-B1b	6	5	4	18	12	10
AcHSF-B1c	6	5	4	18	12	10
AcHSF-B2a	6	1	4	18	10	10
AcHSF-B2b	1	2	1	22	10	6
AcHSF-B4b	0	0	0	14	16	11
AcHSF-B4c	0	0	0	0	0	0
AcHSF-C1a	3	1	3	31	28	8
AcHSF-C1b	11	0	0	11	16	9
AcHSF-C2	25	5	7	18	10	6

the *AcHSFs* promoter area. The result showed that the *AcHSF-A1a* and *AcHSF-A4* lacked ABRE, the *AcHSF-A6* and *AcHSF-C1b* lacked DRE, the *AcHSF-C1b* lacked LTRE, and the *AcHSF-A9a*, *AcHSF-B4b*, and *AcHSF-B4c* did not have these three stress response elements (Table 2). The cis-element studies in the promoters indicate that HSFs are highly related to the response to stress.

Conserved domains and motifs of pineapple HSFs

The modular structure of the HSFs contains 5 typical conserved domains: DBD, OD, NLS, NES, and AHA domains from N to C-terminal (Table 3). The most conserved DBD domain composed of approximately 100 amino acids, containing three α -helices and a four-stranded antiparallel β -sheet ($\alpha 1$ - $\beta 1$ - $\beta 2$ - $\alpha 2$ - $\alpha 3$ - $\beta 3$ - $\beta 4$) (Fig. 4A). In addition to the DBD domain, the HR-A/B next to the DBD domain is also important and plays a crucial role in HSF-HSF interaction (Scharf et al., 2012). Besides, HR-A/B also presents in all *AcHSFs* (Table 3, Fig. 4B). According to the previous studies, HSFs were artificially divided into A, B, and C classes by the distinction between the HR-A and HR-B motifs (Cheng et al., 2015; Giesguth et al., 2015; Singh et al., 2012). In general, the variable length of the flexible linker between parts A and B of the HR-A/B motif of classes A and C HSFs is approximately 15 to 80 amino acids, while the HR-A/B region is tightly connected without the embedded sequence in the middle in class B members. But strangely, the insert lengths between the

Table 3 Functional domains of AcHSFs.

Gene	Subgroup	DBD	HR-A/B	NLS	NES	AHA motif
AcHSF-A1a	A1	27-195	235-277	(302)KKRR	nd	(519-526)DTFWEQFL
AcHSF-A1b	A1	26-119	159-201	(226)KKRR	nd	(443-450)DTFWEQFL
AcHSF-A1c	A1	37-130	170-212	(238)KKRR	nd	(403-410)DSFWEQFL
AcHSF-A2	A2	49-142	180-222	(248)KKRR	(292-299)LDLETAL	(319-326)DEFWEELL
AcHSF-A3	A3	113-228	263-305	(319) KEQKRIALPRPKRKFLK	nd	nd
AcHSF-A4	A4	11-104	141-183	(202)KKRR	(136-142)HEKGLLI	(388-395)DVFWWEQFL
AcHSF-A5	A5	174-267	301-343	(371)KKRR	(327-331)LDMEQ	(558-565)DVFWWEQFL
AcHSF-A6	A6	44-137	174-216	(242)KKRR	(279-284)LDSLAL	(311-318)DGFWEELL
AcHSF-A7a	A7	44-137	174-216	(131) KNIKRRR	nd	(305-312)EVVWEELL
AcHSF-A7b	A7	42-130	159-197	(223)KRRR	(216-218)LLL	(271-278)DMIWEELL
AcHSF-A9a	A9	117-210	253-295	(321)KKRR	(254-262)MQELVKLRL	(424-432)DDDFSEQD
AcHSF-A9b	A9	5-49	92-134	(160)KKRR	(93-101)MQELVKLRL	(263-271)DDDFSEQD
AcHSF-B1a	B1	17-110	165-207	(108) RRK	(252-254)VIL	nd
AcHSF-B1b	B1	24-117	167-209	(264)DRKKGDGRKRGR	(210-219)LDVNKLDLTL	nd
AcHSF-B1c	B1	24-117	167-209	(264)DRKKGDGRKRGR	(210-219)LDVNKLDLAL	nd
AcHSF-B2a	B2	28-121	164-201	nd	nd	nd
AcHSF-B2b	B2	39-132	202-244	nd	nd	nd
AcHSF-B4b	B4	23-116	178-220	nd	(289)L	nd
AcHSF-B4c	B4	1-47	123-165	(186)GLVDQRR	(274-279)LENEDL	nd
AcHSF-C1a	C1	15-108	138-180	(202)KRRR	(190-192)LIL	nd
AcHSF-C1b	C1	14-95	110-152	(166)KKKQRPGSEHKKP	(117-124)LRKEQKAL	nd
AcHSF-C2	C2	43-136	193-235	(281)KRAR	nd	nd

Notes.

nd, not detected.

HR-A and HR-B have almost no difference in pineapple HSFs (Fig. 4B). And the length of the total HR-A/B domain is about 42 amino acids almost the same in pineapple classes A, B, and C HSFs, while the length of classes A and C HSFs is about 50 amino acids and 29 amino acids of class B HSFs in *Arabidopsis*, rice and soybean (Chauhan et al., 2011; Guo et al., 2008; Jin, Gho & Jung, 2013; Li et al., 2014).

The nuclear localization signals (NLS) and nuclear export signals (NES) are necessary for proteins to import and export the nucleus. The intracellular distribution of HSFs varies dynamically between the nucleus and the cytoplasm, depending on nuclear import and export balance. (Heerklotz et al., 2001; Scharf et al., 1998). After detecting, almost all the HSFs contained NLS sequences rich in basic amino acid residues (K/R), except for AcHSF-B2a, AcHSF-B2b and AcHSF-B4b. However, a total of 8 AcHSFs did not find the NES motifs. As reported in other plants, the transcription activator AHA motif was only located in class A AcHSFs, but the difference is AcHSF-A3 lacks the AHA motif (Table 3).

In addition to the typical conserved domains of HSF, we also detected the putative motifs by Multiple Em for Motif Elicitation (MEME). A total of 10 different motifs were identified in AcHSFs with lengths ranging from 20 to 50 aa (Fig. 5). The motif composition of the same group members is similar, but there are great differences among different group members. The conserved motifs in HSFs indicated that all AcHSFs contained motif

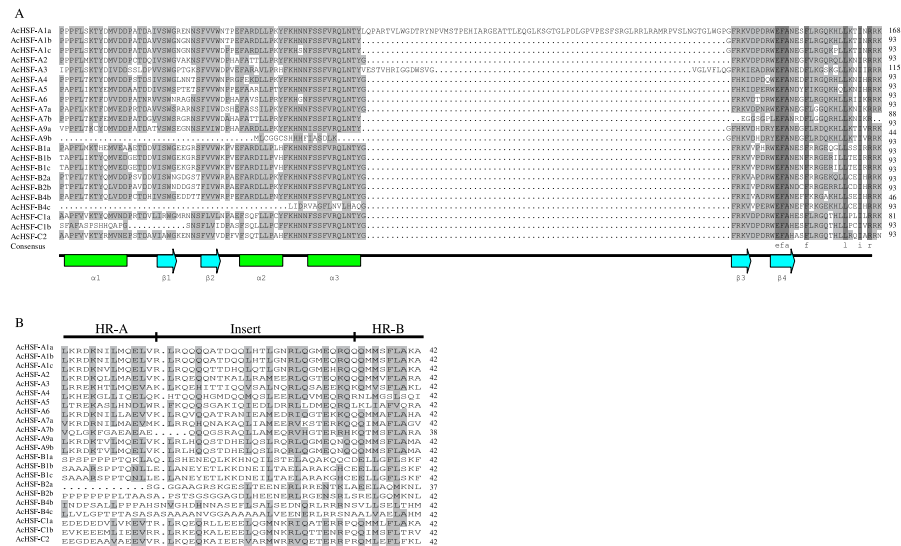


Figure 4 Multiple sequence alignment of DNA binding domains and the HR-A/B regions (OD) of pineapple HSFs. (A) DBD domain sequences of AcHSFs identified by Pfam database were aligned by Clustal X 2.0 software and edited by DNAMAN (v 9.0) software. The black and gray backgrounds indicate entire conservative residues and 75% conservative residues respectively. The helix-turn-helix motifs of DBD ($\alpha 1$ - $\beta 1$ - $\beta 2$ - $\alpha 2$ - $\alpha 3$ - $\beta 3$ - $\beta 4$) are shown at the bottom. Green tubes represent the α -helices and blue arrows represent the β -sheets. (B) The HR-A/B region sequences identified by SMART online tool were aligned by Clustal X 2.0 software and edited by DNAMAN (v 9.0) software. The black backgrounds indicate the 50–75% conservative residues respectively. The three line segments at the top divide HR-A core, insert and HR-B regions orderly.

Full-size [DOI: 10.7717/peerj.11329/fig-4](https://doi.org/10.7717/peerj.11329/fig-4)

1, motif 2, except for AcHSF-A9a and AcHSF-B4c lack of motif 1. Motif 3 only exists in class A and C HSFs, not in class B. However, motif 7 only present in class A HSFs, and motif 5 only presents in class B HSFs. Additionally, some motifs were only discovered in a certain subfamily of AcHSFs, for example, motif 9 was present in the B1 subclass (Fig. 5).

Expression analysis of *AcHSFs* in different tissues

Gene expression profiles are related to their functions (Su et al., 2017). To better understand the functions of 22 pineapple *AcHSF* genes, the tissue-specific expression patterns were detected by 36 different tissues transcriptome sequencing, including flower (mixed stage), leaf, root, fruit S1, S2, S3, S4, S5, and S7, Se1-4, Petal 1-3, Ov 1-7, St 1-6, and Gy 1-7 from Wang et al. (2020).

The results showed some genes are highly expressed in certain tissues, while others are expressed gradually with the development of tissues (Fig. 6). For example, *AcHSF-A1c* and *AcHSF-A7b* have high expression levels in 7 fruit tissues, the expression of *AcHSF-A9a* gradually increased in petal development and have the highest expression value in the P3 development stage. The *AcHSF-B4b* and *AcHSF-B4c* are highly expressed in the 7 ovule development stages, which illustrate their important roles in the pineapple ovule development process. We also found that some genes showed tissue-specific expression patterns, such as the *AcHSF-B2a* was mainly expressed in the fruit S7 stage, *AcHSF-A2* and *AcHSF-A6* are highly expressed in leaf and flower tissues. In addition, the expression

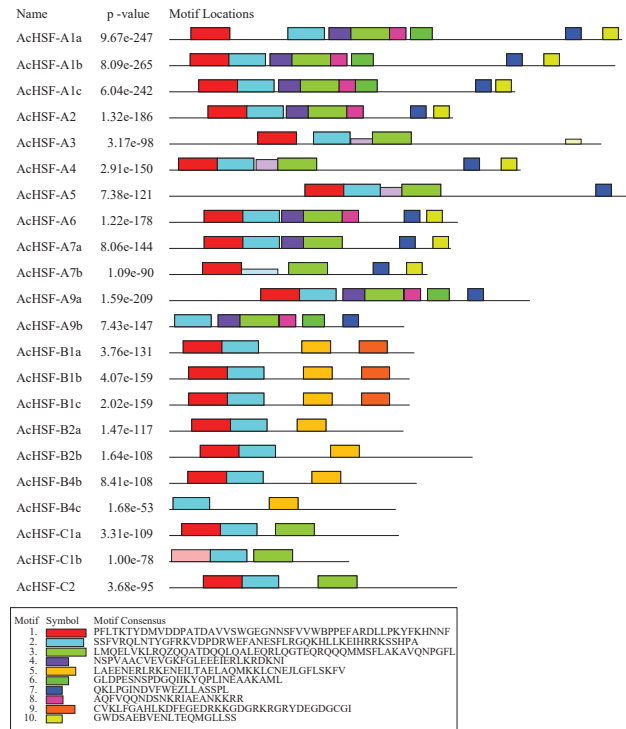


Figure 5 The conserved motif analysis of 22 AcHSFs. A total of 10 conserved motifs were identified using Multiple Em for Motif Elicitation (MEME). This is the combined match p -value. The combined match p -value is defined as the probability that a random sequence (with the same length and conforming to the background) would have position p -values such that the product is smaller or equal to the value calculated for the sequence under test.

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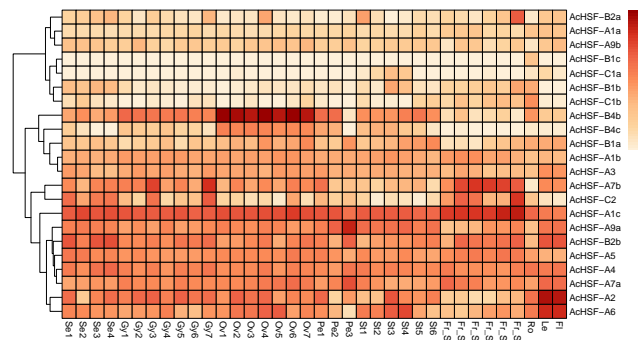


Figure 6 Expression pattern of AcHSFs genes in pineapple flower and vegetative tissues. Log₂(FPKM+1) values were used for the heatmap. Se: Sepal, Pe, Petal; Ov, Ovule; Gy, Gynoecium; St, Stamen; Fr, fruit; Ro, Root; Le, Leave, Fl, Flower.

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profiles of the genes in the same class are significantly different. For instance, three members of *AcHSF-A1* have different expression patterns in all detected tissues and development stages.

Expression profiles of *AcHSFs* response to various stresses

To extend our understanding of *AcHSFs* in response to stresses, we performed qRT-PCR to investigate the expression patterns of 6 randomly selected *AcHSF* genes (*AcHSF-A1a*, *AcHSF-A2a*, *AcHSF-A9a*, *AcHSF-B2a*, *AcHSF-B4a*, and *AcHSF-C1a*) in heat, cold and ABA stresses. The results illustrated that almost all of the selected *AcHSFs* showed similar expression patterns under the same stress conditions.

Cold stress drastically affects plant growth and development, and leads to a significant reduction in crop yield (Cai *et al.*, 2015); therefore, plants must respond quickly to cold stress. As shown in the results, under the cold stress treatment, the expression of all the 6 *AcHSFs* increased rapidly from 0 h to 24 h and then reduced at 48 h (Fig. 7A). These may indicate that *AcHSFs* are commonly up-regulated within a short-timer by cold stresses, and then the expression is down-regulated rapidly. Heat shock transcription factors play crucial roles in response to heat shock induction. The result showed that the expression of 6 *AcHSFs* continues to increase from 0 h to 48 h in heat stress treatment (Fig. 7B). After ABA treatment, the expression of most selected *AcHSF* genes increased from 0 h to 12 h, and then decreased after 12 h, while the expression of *AcHSF-A2a* continued to increase (Fig. 7C). This result implies that the expressions of *AcHSFs* were suppressed under the longtime ABA treatment and might play crucial roles in different stress (cold and heat, etc.) response pathways.

DISCUSSION

During its growth and developmental stages, the pineapple is severely destroyed by various abiotic stresses (cold, heat, drought, etc.) and biotic stresses (especially fungal pathogen infection). HSFs are among the critical regulatory components of various abiotic and biotic stresses in plants. This research identified and characterized, for the first time, a systematic genome-wide review of the *AcHSF* family. Consequently, from the pineapple genome, a total of 30 *AcHSF* genes were identified. The widely accepted model of *HSFs* defines the necessity of HSF-type DBD and OD characterized by a coiled-coil structure. Thus, due to the absence of HSF-type DBD domains and/or coiled-coil structures, 8 of them were discarded. Meanwhile, pineapple HSF has a similar subfamily distribution compared with the monocots plant *O. sativa*, but is different from dicots plants *A. thaliana* and *P. trichocarpa*. Some genes are unique to monocots or dicots. For example, the subclasses *AcHSF-A8* and *AcHSF-B3* are confined to dicots, while *AcHSF-A9* and *AcHSF-C2* are characteristic of monocots, suggesting that different evolutionary events of HSF genes occurred in dicots and monocots (Fig. 2, Table 1).

Research on gene expression regulation mediated by introns has made significant progress (Le Hir, Nott & Moore, 2003; Li *et al.*, 2019; Rose, 2008; Shaul, 2017). Therefore the study of gene structure is beneficial to elucidate the gene function. Analysis of *AcHSFs* gene structures revealed that most of the classes A *AcHSFs* contain more than one intron, and several *AcHSFs* have 3 or 4 introns, such as *AcHSF-A1a*, *AcHSF-A1b*, and *AcHSF-A9a*. However, the genes in the class B and C only contain 1 intron, except for *AcHSF-C1b* (Fig. 3). This particular intron structure may be due to the specific functions of the *AcHSF* genes.

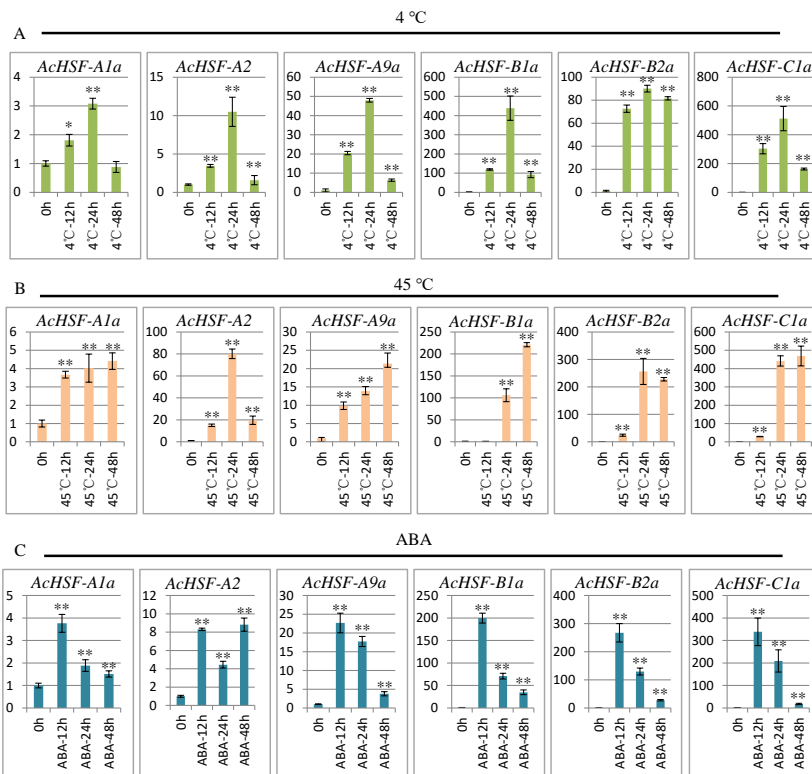


Figure 7 qRT-PCR expression analysis of 6 selected AcHSF genes in response to different abiotic stress treatments. (A) Cold stress treatment (4 °C) (B) Heat stress treatment (45 °C); (C) 100 nM ABA treatment. Mean expression value was calculated from three independent replicates. Error bars indicate \pm SD ($n = 3$). Asterisks on top of the bars indicating statistically significant differences between the stress and counterpart controls (* $P < 0.05$, ** $P < 0.01$).

Full-size DOI: 10.7717/peerj.11329/fig-7

The required DBD domain and unique protein domains (HR-A/B, NLS, NES, RD, and AHA) are found in all 22 AcHSF proteins (Table 3, Fig. 4), which provide the structural basis for their conserved function (Giorno *et al.*, 2012). The HSF DBD domain contains approximately 100 amino acids and is strongly preserved in various plant-to-animal species; we also found the same conserved domain in pineapple (Fig. 4A). As reported previously in other plants, the transcription activator AHA motif was only located in class A AcHSFs, but AcHSF-A3 lacks the AHA motif (Table 3). The HSFs that lack AHA domains might contribute to the activator's function differently or form hetero-oligomers by binding to other HSF-As (Guo *et al.*, 2008).

The expression patterns analysis of different AcHSFs showed that AcHSF-B4b and AcHSF-B4c are highly expressed in 7 ovule development stages, indicating the potential functions in pineapple ovule development. The high expression levels of AcHSF-A7b, AcHSF-C2, and AcHSF-A1c in fruit development stages uncovered their important roles in fruit development (Fig. 6). Furthermore, we found that the expression of AcHSF-A9a gradually increased throughout the development stage and reached the highest expression level in the third stage of petal development. AcHSF-A2 and AcHSF-A6 have high expression

levels in leave and mixed flower tissues (Fig. 6). These data suggest that *AcHSFs* may regulate several developmental processes. The stress response is very significant for plant growth and development. Previous studies have shown that the *HSF* genes are involved in several abiotic stress response, including heat, cold, drought, and salt stress responses in different plants such as *Arabidopsis*, tomato, apple, *Populus euphratica*, and *Phyllostachys edulis* (Fragkostefanakis et al., 2015; Giorno et al., 2012; Ikeda, Mitsuda & Ohme-Takagi, 2011; Xie et al., 2019; Xue et al., 2014; Zhang et al., 2016a). In our study, most of the selected *AcHSFs* showed similar expression patterns under the same stress conditions. Under the cold stress (4 °C) treatment, the expressions of *AcHSFs* were induced from 0 h to 12 h and then inhibited after 12 h (Fig. 7A). The same expression pattern was also observed in the 100 mM ABA treatment, but the difference was that the *AcHSFs* were more sensitive to ABA treatment (Fig. 7C). The continuous increase in the expression pattern of *AcHSFs* was observed at 45 °C treatment, indicating that heat stress-induced the expression of *AcHSFs* (Fig. 7C).

Taken together, this study is the first to identify the *AcHSF* family genes, their properties as well as their expression profiles. This information could be used to utilize them as potential candidates in a breeding program of pineapple. However, gene expression and function analysis are complicated biological mechanisms, and additional studies are necessary to interpret the regulatory process.

CONCLUSIONS

In the present study, 22 *AcHSF* genes were identified in pineapple (*Ananas comosus*) and generated detailed information on the gene and protein structures. The expression profiles of various tissues and developmental stages were analyzed by the RNA-seq data, which may help to study their functions in different developmental processes or regulatory pathways. We also showed that some *AcHSF* genes participate in various biotic and abiotic stresses (heat, cold, and ABA), which may help develop new pineapple varieties with desired agronomic traits stress tolerance.

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ADDITIONAL INFORMATION AND DECLARATIONS

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Competing Interests

The authors declare there are no competing interests.

Author Contributions

- Lulu Wang conceived and designed the experiments, performed the experiments, analyzed the data, prepared figures and/or tables, authored or reviewed drafts of the paper, and approved the final draft.
- Yanhui Liu performed the experiments, prepared figures and/or tables, and approved the final draft.
- Mengnan Chai and Huihuang Chen analyzed the data, prepared figures and/or tables, and approved the final draft.
- Mohammad Aslam, Yuan Qin and Hanyang Cai conceived and designed the experiments, authored or reviewed drafts of the paper, and approved the final draft.
- Xiaoping Niu analyzed the data, prepared figures and/or tables, authored or reviewed drafts of the paper, and approved the final draft.

DNA Deposition

The following information was supplied regarding the deposition of DNA sequences:

The RNA-seq data of ovule, stamen, petal, Gynoecium, and Sepal were downloaded in the European Nucleotide Archive (ENA): [PRJEB38680](https://www.ebi.ac.uk/ena/record/PRJEB38680).

The RNA-seq datasets of Root, Leaf, Flower, and Fruit were downloaded from <http://pineapple.angiosperms.org/pineapple/html/download.html>.

Data Availability

The following information was supplied regarding data availability:

The raw measurements showing all the analysis processes and results are available in the [Supplementary Files](#).

Supplemental Information

Supplemental information for this article can be found online at <http://dx.doi.org/10.7717/peerj.11329#supplemental-information>.

REFERENCES

- Bai YH, Dai XZ, Li Y, Wang LL, Li WM, Liu YH, Cheng Y, Qin Y. 2019. Identification and characterization of pineapple leaf lncRNAs in crassulacean acid metabolism (CAM) photosynthesis pathway. *Scientific Reports* 9: ARTN 6658 DOI 10.1038/s41598-019-43088-8.
- Barral B, Chillet M, Lechaudel M, Lartaud M, Verdeil JL, Conejero G, Schorr-Galindo S. 2019. An imaging approach to identify mechanisms of resistance to pineapple fruitlet core rot. *Frontiers in Plant Science* 10:1065 DOI 10.3389/fpls.2019.01065.

- Cai H, Cheng J, Yan Y, Xiao Z, Li J, Mou S, Qiu A, Lai Y, Guan D, He S. 2015.** Genome-wide identification and expression analysis of calcium-dependent protein kinase and its closely related kinase genes in *Capsicum annuum*. *Frontiers in Plant Science* **6**:737 DOI [10.3389/fpls.2015.00737](https://doi.org/10.3389/fpls.2015.00737).
- Chai M, Cheng H, Yan M, Priyadarshani S, Zhang M, He Q, Huang Y, Chen F, Liu L, Huang X, Lai L, Chen H, Cai H, Qin Y. 2020.** Identification and expression analysis of the DREB transcription factor family in pineapple (*Ananas comosus* (L.) Merr.). *PeerJ* **8**:e9006 DOI [10.7717/peerj.9006](https://doi.org/10.7717/peerj.9006).
- Chauhan H, Khurana N, Agarwal P, Khurana JP, Khurana P. 2013.** A seed preferential heat shock transcription factor from wheat provides abiotic stress tolerance and yield enhancement in transgenic Arabidopsis under heat stress environment. *PLOS ONE* **8**:e79577 DOI [10.1371/journal.pone.0079577](https://doi.org/10.1371/journal.pone.0079577).
- Chauhan H, Khurana N, Agarwal P, Khurana P. 2011.** Heat shock factors in rice (*Oryza sativa* L.): genome-wide expression analysis during reproductive development and abiotic stress. *Molecular Genetics & Genomic Medicine* **286**:171–187 DOI [10.1007/s00438-011-0638-8](https://doi.org/10.1007/s00438-011-0638-8).
- Cheng Q, Zhou Y, Liu Z, Zhang L, Song G, Guo Z, Wang W, Qu X, Zhu Y, Yang D. 2015.** An alternatively spliced heat shock transcription factor, OsHSFA2dI, functions in the heat stress-induced unfolded protein response in rice. *Plant Biology* **17**:419–429 DOI [10.1111/plb.12267](https://doi.org/10.1111/plb.12267).
- Chidambaranathan P, Jagannadham PTK, Satheesh V, Kohli D, Basavarajappa SH, Chellapilla B, Kumar J, Jain PK, Srinivasan R. 2018.** Genome-wide analysis identifies chickpea (*Cicer arietinum*) heat stress transcription factors (Hsfs) responsive to heat stress at the pod development stage. *Journal of Plant Research* **131**:525–542 DOI [10.1007/s10265-017-0948-y](https://doi.org/10.1007/s10265-017-0948-y).
- Chung E, Kim KM, Lee JH. 2013.** Genome-wide analysis and molecular characterization of heat shock transcription factor family in *Glycine max*. *Journal of Genetics and Genomics* **40**:127–135 DOI [10.1016/j.jgg.2012.12.002](https://doi.org/10.1016/j.jgg.2012.12.002).
- Fragkostefanakis S, Roth S, Schleiff E, Scharf KD. 2015.** Prospects of engineering thermotolerance in crops through modulation of heat stress transcription factor and heat shock protein networks. *Plant Cell and Environment* **38**:1881–1895 DOI [10.1111/pce.12396](https://doi.org/10.1111/pce.12396).
- Giesguth M, Sahm A, Simon S, Dietz KJ. 2015.** Redox-dependent translocation of the heat shock transcription factor AtHSFA8 from the cytosol to the nucleus in *Arabidopsis thaliana*. *FEBS Letters* **589**:718–725 DOI [10.1016/j.febslet.2015.01.039](https://doi.org/10.1016/j.febslet.2015.01.039).
- Giorno F, Guerriero G, Baric S, Mariani C. 2012.** Heat shock transcriptional factors in *Malus domestica*: identification, classification and expression analysis. *BMC Genomics* **13**:639 DOI [10.1186/1471-2164-13-639](https://doi.org/10.1186/1471-2164-13-639).
- Guo AY, Zhu QH, Chen X, Luo JC. 2007.** GSDS: a gene structure display server. *Yi Chuan* **29**:1023–1026 DOI [10.1360/yc-007-1023](https://doi.org/10.1360/yc-007-1023).
- Guo J, Wu J, Ji Q, Wang C, Luo L, Yuan Y, Wang Y, Wang J. 2008.** Genome-wide analysis of heat shock transcription factor families in rice and Arabidopsis. *Journal of Genetics and Genomics* **35**:105–118 DOI [10.1016/S1673-8527\(08\)60016-8](https://doi.org/10.1016/S1673-8527(08)60016-8).

- Guo M, Liu JH, Ma X, Luo DX, Gong ZH, Lu MH. 2016.** The plant heat stress transcription factors (HSFs): structure, regulation, and function in response to abiotic stresses. *Frontiers in Plant Science* 7: 114 DOI [10.3389/fpls.2016.00114](https://doi.org/10.3389/fpls.2016.00114).
- Guo M, Lu JP, Zhai YF, Chai WG, Gong ZH, Lu MH. 2015.** Genome-wide analysis, expression profile of heat shock factor gene family (CaHsfs) and characterisation of CaHsfA2 in pepper (*Capsicum annuum* L.). *BMC Plant Biology* 15:151 DOI [10.1186/s12870-015-0512-7](https://doi.org/10.1186/s12870-015-0512-7).
- Guo M, Yin YX, Ji JJ, Ma BP, Lu MH, Gong ZH. 2014.** Cloning and expression analysis of heat-shock transcription factor gene CaHsfA2 from pepper (*Capsicum annuum* L.). *Genetics and Molecular Research* 13:1865–1875 DOI [10.4238/2014.March.17.14](https://doi.org/10.4238/2014.March.17.14).
- Heerklotz D, Doring P, Bonzelius F, Winkelhaus S, Nover L. 2001.** The balance of nuclear import and export determines the intracellular distribution and function of tomato heat stress transcription factor HsfA2. *Molecular and Cellular Biology* 21:1759–1768 DOI [10.1128/MCB.21.5.1759-1768.2001](https://doi.org/10.1128/MCB.21.5.1759-1768.2001).
- Higo K, Ugawa Y, Iwamoto M, Korenaga T. 1999.** Plant cis-acting regulatory DNA elements (PLACE) database: 1999. *Nucleic Acids Research* 27:297–300 DOI [10.1093/nar/27.1.297](https://doi.org/10.1093/nar/27.1.297).
- Hu H, Xiong L. 2014.** Genetic engineering and breeding of drought-resistant crops. *Annual Review of Plant Biology* 65:715–741 DOI [10.1146/annurev-arplant-050213-040000](https://doi.org/10.1146/annurev-arplant-050213-040000).
- Hu Y, Han YT, Wei W, Li YJ, Zhang K, Gao YR, Zhao FL, Feng JY. 2015.** Identification, isolation, and expression analysis of heat shock transcription factors in the diploid woodland strawberry *Fragaria vesca*. *Frontiers in Plant Science* 6:736 DOI [10.3389/fpls.2015.00736](https://doi.org/10.3389/fpls.2015.00736).
- Ikeda M, Mitsuda N, Ohme-Takagi M. 2011.** Arabidopsis HsfB1 and HsfB2b act as repressors of the expression of heat-inducible hsfs but positively regulate the acquired thermotolerance. *Plant Physiology* 157:1243–1254 DOI [10.1104/pp.111.179036](https://doi.org/10.1104/pp.111.179036).
- Jin GH, Gho HJ, Jung KH. 2013.** A systematic view of rice heat shock transcription factor family using phylogenomic analysis. *Journal of Plant Physiology* 170:321–329 DOI [10.1016/j.jplph.2012.09.008](https://doi.org/10.1016/j.jplph.2012.09.008).
- Le Hir H, Nott A, Moore MJ. 2003.** How introns influence and enhance eukaryotic gene expression. *Trends in Biochemical Sciences* 28:215–220 DOI [10.1016/S0968-0004\(03\)00052-5](https://doi.org/10.1016/S0968-0004(03)00052-5).
- Letunic I, Doerks T, Bork P. 2012.** SMART 7: recent updates to the protein domain annotation resource. *Nucleic Acids Research* 40:D302–D305 DOI [10.1093/nar/gkr931](https://doi.org/10.1093/nar/gkr931).
- Li PS, Yu TF, He GH, Chen M, Zhou YB, Chai SC, Xu ZS, Ma YZ. 2014.** Genome-wide analysis of the Hsf family in soybean and functional identification of GmHsf-34 involvement in drought and heat stresses. *BMC Genomics* 15:1009 DOI [10.1186/1471-2164-15-1009](https://doi.org/10.1186/1471-2164-15-1009).
- Li W, Cui X, Meng ZL, Huang XH, Xie Q, Wu H, Jin HL, Zhang DB, Liang WQ. 2012.** Transcriptional regulation of Arabidopsis MIR168a and ARGONAUTE1 homeostasis in abscisic acid and abiotic stress responses. *Plant Physiology* 158:1279–1292 DOI [10.1104/pp.111.188789](https://doi.org/10.1104/pp.111.188789).

- Li YJ, Chen DM, Luo SW, Zhu YY, Jia XJ, Duan YB, Zhou MG. 2019. Intron-mediated regulation of beta-tubulin genes expression affects the sensitivity to carbendazim in *Fusarium graminearum*. *Current Genetics* 65:1057–1069 DOI 10.1007/s00294-019-00960-4.
- Lin Y, Cheng Y, Jin J, Jin X, Jiang H, Yan H, Cheng B. 2014. Genome duplication and gene loss affect the evolution of heat shock transcription factor genes in legumes. *PLOS ONE* 9:e102825 DOI 10.1371/journal.pone.0102825.
- Liu ZW, Wu ZJ, Li XH, Huang Y, Li H, Wang YX, Zhuang J. 2016. Identification, classification, and expression profiles of heat shock transcription factors in tea plant (*Camellia sinensis*) under temperature stress. *Gene* 576:52–59 DOI 10.1016/j.gene.2015.09.076.
- Ming R, VanBuren R, Wai CM, Tang HB, Schatz MC, Bowers JE, Lyons E, Wang ML, Chen J, Biggers E, Zhang JS, Huang LX, Zhang LM, Miao WJ, Zhang J, Ye ZY, Miao CY, Lin ZC, Wang H, Zhou HY, Yim WC, Priest HD, Zheng CF, Woodhouse M, Edger PP, Guyot R, Guo HB, Guo H, Zheng GY, Singh R, Sharma A, Min XJ, Zheng Y, Lee H, Gurtowski J, Sedlazeck FJ, Harkess A, McKain MR, Liao ZY, Fang JP, Liu J, Zhang XD, Zhang Q, Hu WC, Qin Y, Wang K, Chen LY, Shirley N, Lin YR, Liu LY, Hernandez AG, Wright CL, Bulone V, Tuskan GA, Heath K, Zee F, Moore PH, Sunkar R, Leebens-Mack JH, Mockler T, Bennetzen JL, Freeling M, Sankoff D, Paterson AH, Zhu XG, Yang XH, Smith JAC, Cushman JC, Paull RE, Yu QY. 2015. The pineapple genome and the evolution of CAM photosynthesis. *Nature Genetics* 47:1435–1442 DOI 10.1038/ng.3435.
- Nishizawa-Yokoi A, Nosaka R, Hayashi H, Tainaka H, Maruta T, Tamoi M, Ikeda M, Ohme-Takagi M, Yoshimura K, Yabuta Y, Shigeoka S. 2011. HsfA1d and HsfA1e involved in the transcriptional regulation of HsfA2 function as key regulators for the Hsf signaling network in response to environmental stress. *Plant and Cell Physiology* 52:933–945 DOI 10.1093/pcp/pcr045.
- Ohama N, Sato H, Shinozaki K, Yamaguchi-Shinozaki K. 2017. Transcriptional regulatory network of plant heat stress response. *Trends in Plant Science* 22:53–65 DOI 10.1016/j.tplants.2016.08.015.
- Olate E, Jimenez-Gomez JM, Holuigue L, Salinas J. 2018. NPR1 mediates a novel regulatory pathway in cold acclimation by interacting with HSF1 factors. *Nature Plants* 4:811–823 DOI 10.1038/s41477-018-0254-2.
- Pereira A. 2016. Plant abiotic stress challenges from the changing environment. *Frontiers in Plant Science* 7:1123 DOI 10.3389/fpls.2016.01123.
- Priyadarshani S, Hu B, Li W, Ali H, Jia H, Zhao L, Ojolo SP, Azam SM, Xiong J, Yan M, Rahman ZUR, Wu Q, Qin Y. 2018. Simple protoplast isolation system for gene expression and protein interaction studies in pineapple (*Ananas comosus* L.). *Plant Methods* 14:95 DOI 10.1186/s13007-018-0365-9.
- Rose AB. 2008. Intron-mediated regulation of gene expression. *Nuclear Pre-Mrna Processing in Plants* 326:277–290 DOI 10.1007/978-3-540-76776-3_15.

- Scharf KD, Berberich T, Ebersberger I, Nover L. 2012.** The plant heat stress transcription factor (Hsf) family: structure, function and evolution. *Biochimica et Biophysica Acta* **1819**:104–119 DOI [10.1016/j.bbagr.2011.10.002](https://doi.org/10.1016/j.bbagr.2011.10.002).
- Scharf KD, Heider H, Hohfeld I, Lyck R, Schmidt E, Nover L. 1998.** The tomato Hsf system: HsfA2 needs interaction with HsfA1 for efficient nuclear import and may be localized in cytoplasmic heat stress granules. *Molecular and Cellular Biology* **18**:2240–2251 DOI [10.1128/mcb.18.4.2240](https://doi.org/10.1128/mcb.18.4.2240).
- Shaul O. 2017.** How introns enhance gene expression. *International Journal of Biochemistry & Cell Biology* **91**:145–155 DOI [10.1016/j.biocel.2017.06.016](https://doi.org/10.1016/j.biocel.2017.06.016).
- Shim D, Hwang JU, Lee J, Lee S, Choi Y, An G, Martinoia E, Lee Y. 2009.** Orthologs of the class A4 heat shock transcription factor HsfA4a confer cadmium tolerance in wheat and rice. *The Plant Cell* **21**:4031–4043 DOI [10.1105/tpc.109.066902](https://doi.org/10.1105/tpc.109.066902).
- Singh A, Mittal D, Lavania D, Agarwal M, Mishra RC, Grover A. 2012.** OsHsfA2c and OsHsfB4b are involved in the transcriptional regulation of cytoplasmic OsClpB (Hsp100) gene in rice (*Oryza sativa* L.). *Cell Stress & Chaperones* **17**:243–254 DOI [10.1007/s12192-011-0303-5](https://doi.org/10.1007/s12192-011-0303-5).
- Song X, Liu G, Duan W, Liu T, Huang Z, Ren J, Li Y, Hou X. 2014.** Genome-wide identification, classification and expression analysis of the heat shock transcription factor family in Chinese cabbage. *Molecular Genetics & Genomic Medicine* **289**:541–551 DOI [10.1007/s00438-014-0833-5](https://doi.org/10.1007/s00438-014-0833-5).
- Su ZX, Wang LL, Li WM, Zhao LH, Huang XY, Azam SM, Qin Y. 2017.** Genome-wide identification of auxin response factor (ARF) genes family and its tissue-specific prominent expression in pineapple (*Ananas comosus*). *Tropical Plant Biology* **10**:86–96 DOI [10.1007/s12042-017-9187-6](https://doi.org/10.1007/s12042-017-9187-6).
- Wahid A, Gelani S, Ashraf M, Foolad MR. 2007.** Heat tolerance in plants: an overview. *Environmental and Experimental Botany* **61**:199–223 DOI [10.1016/j.envexpbot.2007.05.011](https://doi.org/10.1016/j.envexpbot.2007.05.011).
- Wang F, Dong Q, Jiang H, Zhu S, Chen B, Xiang Y. 2012.** Genome-wide analysis of the heat shock transcription factors in *Populus trichocarpa* and *Medicago truncatula*. *Molecular Biology Reports* **39**:1877–1886 DOI [10.1007/s11033-011-0933-9](https://doi.org/10.1007/s11033-011-0933-9).
- Wang J, Sun N, Deng T, Zhang L, Zuo K. 2014.** Genome-wide cloning, identification, classification and functional analysis of cotton heat shock transcription factors in cotton (*Gossypium hirsutum*). *BMC Genomics* **15**:961 DOI [10.1186/1471-2164-15-961](https://doi.org/10.1186/1471-2164-15-961).
- Wang LL, Li Y, Jin XY, Liu LP, Dai XZ, Liu YH, Zhao LH, Zheng P, Wang XM, Liu YQ, Lin DS, Qin Y. 2020.** Floral transcriptomes reveal gene networks in pineapple floral growth and fruit development. *Communications Biology* **3**:500 DOI [10.1038/s42003-020-01235-2](https://doi.org/10.1038/s42003-020-01235-2).
- Xie LH, Li XY, Hou D, Cheng ZC, Liu J, Li J, Mu SH, Gao J. 2019.** Genome-wide analysis and expression profiling of the heat shock factor gene family in *Phyllostachys edulis* during development and in response to abiotic stresses. *Forests* **10**:100 DOI [10.3390/f10020100](https://doi.org/10.3390/f10020100).

- Xue GP, Sadat S, Drenth J, McIntyre CL. 2014.** The heat shock factor family from *Triticum aestivum* in response to heat and other major abiotic stresses and their role in regulation of heat shock protein genes. *Journal of Experimental Botany* **65**:539–557 DOI [10.1093/jxb/ert399](https://doi.org/10.1093/jxb/ert399).
- Yabuta Y. 2016.** Functions of heat shock transcription factors involved in response to photooxidative stresses in Arabidopsis. *Bioscience Biotechnology and Biochemistry* **80**:1254–1263 DOI [10.1080/09168451.2016.1176515](https://doi.org/10.1080/09168451.2016.1176515).
- Zhang J, Jia H, Li J, Li Y, Lu M, Hu J. 2016a.** Molecular evolution and expression divergence of the *Populus euphratica* Hsf genes provide insight into the stress acclimation of desert poplar. *Scientific Reports* **6**:30050 DOI [10.1038/srep30050](https://doi.org/10.1038/srep30050).
- Zhang J, Jia HX, Li JB, Li Y, Lu MZ, Hu JJ. 2016b.** Molecular evolution and expression divergence of the *Populus euphratica* Hsf genes provide insight into the stress acclimation of desert poplar. *Scientific Reports* **6**:30050 DOI [10.1038/srep30050](https://doi.org/10.1038/srep30050).
- Zhang Y, Chen C, Jin XF, Xiong AS, Peng RH, Hong YH, Yao QH, Chen JM. 2009.** Expression of a rice DREB1 gene, OsDREB1D, enhances cold and high-salt tolerance in transgenic Arabidopsis. *Bmb Reports* **42**:486–492 DOI [10.5483/BMBRep.2009.42.8.486](https://doi.org/10.5483/BMBRep.2009.42.8.486).
- Zhu JK. 2016.** Abiotic stress signaling and responses in plants. *Cell* **167**:313–324 DOI [10.1016/j.cell.2016.08.029](https://doi.org/10.1016/j.cell.2016.08.029).