



Molecular Characterization of *HSF* Genes in *Ricinus Communis* L.: A Foundation for Breeding Stress-Resilient Genotypes

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Received: 24 September 2025 / Accepted: 12 January 2026 / Published online: 18 February 2026
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Abstract

Ricinus communis L., known as castor, is an industrial crop known for its globally high demand and valuable ricinoleic acid oil. It faces challenges as it is commonly cultivated globally under harsh environmental conditions in of arid/semi-arid regions. In Brazil, it is mainly cultivated by smallholder farmers in the northeastern semi-arid region, which has a relevant socioeconomic impact. Heat shock transcription factors (HSFs) represent an important class of heat shock protein (HSP) regulators in plants adapting to abiotic stress conditions. We characterized the *RcHSF* gene family expression under abiotic stress conditions in different tissues and stages of germination and seedling development, as the most critical phases for crop establishment and productivity. We identified 18 *RcHSF* genes (3 classes, 10 subclasses) distributed across 7 chromosomes containing cis-elements responsive to abiotic stresses (ABRE, MeJARE, GARE, SARE). Abiotic stresses (mannitol, saline, heat) did not affect total germination but compromised seedling vigor. *RcHSFs* responded to high and low temperatures during germination and early seedling. *RcHSF08* (Class-A) was highly expressed under stresses in the early seedling stage, while *RcHSF10* (Class-C) was induced during early seedling stages in control and all stresses compared to dry seeds. This study highlights *RcHSF* genes involved in abiotic stress tolerance across different *Ricinus communis* tissues during germination and early seedling growth, providing a foundation for future functional characterization of HSF genes in *Arabidopsis thaliana* and identifying promising candidates for *R. communis* breeding programs.

Keywords *R. communis* · Abiotic stresses · Heat shock factor · Gene expression

Isabela Dias Santos and Jair Lucas Oliveira Júnior contributed equally to this work as first authors. Valdir Gomes Neto and Renato Delmondez de Castro are designated as the corresponding authors.

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Introduction

Plants have developed biochemical and physiological mechanisms to respond to environmental stressors such as drought, high temperatures, and saline conditions. These mechanisms include the activation of stress-responsive genes regulated by a network of transcription factors (TF), including heat shock transcription factors (HSF) (Nishad & Nandi 2021). *HSF* genes regulate the expression of heat shock proteins (HSPs) and other stress-responsive genes, which act as molecular chaperones, preventing the formation of deleterious protein conformations (Andrási et al. 2021; Zhao et al. 2021) to maintain or re-establish cellular homeostasis under harsh environmental conditions.

Expanding knowledge on HSFs appears critical as alternative molecular markers in crop breeding strategies aiming for *R. communis* genotypes with enhanced abiotic stress tolerance, especially during the early stages of germination and seedling establishment, i.e., the most critical

phase in plants' life cycle (Kermode 2008; Nonogaki et al. 2010; Pan et al. 2023), as demonstrated in other plant species (Li et al. 2013; Song et al. 2016; Guo et al. 2020, 2023; Liao et al. 2024).

HSFs have a conserved modular structure and are classified into three classes (A, B, and C) based on phylogenetic analysis, properties, and structural distinctions within their oligomerization domains (Döring et al. 2000; Tripp et al. 2009; Guo et al. 2016). The N-terminal DNA-binding domain (DBD) features a central helix-turn-helix motif that specifically recognizes heat shock elements (HSEs, 5'-nGAAnnTTCn-3') in target promoters, facilitating the activation of stress-inducible genes (Baniwal et al. 2004; Sakurai & Enoki 2010). The oligomerization domain (OD or HR-A/B), located proximal to the DBD, mediates protein-protein interactions and trimerization during transcriptional activation (Andrási et al. 2021). The C-terminal activation domains of plant HSFs are distinguished by short peptide motifs known as AHA motifs, comprising large acidic residues and hydrophobic aromatic amino acids. These motifs are unique to the HSF class A and are absent in HSF classes B or C (Nover et al. 2001; Döring et al. 2000). Members of the HSF class B mainly exhibit a conserved tetrapeptide LFGV motif within the C-terminal domain, functioning as a repression domains (RD) (Czarnecka-Verner et al. 2000; Fragkostefanakis et al. 2019). Although the precise functions of HSF class C members remain unexplored, studies suggest they may have specialized roles in monocotyledons (Scharf et al. 2012; Guo et al. 2016). The subcellular localization of HSFs involves dynamic trafficking between the cytoplasm and the nucleus, regulated by nuclear localization signals (NLS) and nuclear export signals (NES) located at the C-terminus (Baniwal et al. 2004; Andrási et al. 2021).

The number of *HSF* genes varies among different species, playing a crucial role in conferring resistance to various abiotic stresses by regulating stress-responsive genes (Busch et al. 2005; Guo et al. 2008, 2016; Scharf et al. 2012; Li et al. 2013). This regulation is influenced by various abiotic stresses and phytohormones, as demonstrated in several species (Hu et al. 2015; Huang et al. 2015; Zhang et al. 2015; Fragkostefanakis et al. 2019). For example, overexpression of the *BcHSFA1* gene from *Brassica campestris* in *Nicotiana tabacum* improved enzymatic antioxidant activity, chlorophyll content, and heat tolerance (Zhu et al. 2018). In *Arabidopsis*, overexpression of *HSFA2* and *HSFA3* induced key enzymes in the biosynthesis of oligosaccharides that act as antioxidants (Nishizawa et al. 2008; Song et al. 2016), whereas overexpression of *SIHSFA3*, *ZmHSF01*, and *ZmHSF04* conferred increased thermotolerance (Li et al. 2013; Zhang et al. 2020a; Guo et al. 2020). Conversely, suppression of *HSFB1*, which acts as a transcriptional repressor, significantly increased the induction of HSPs due to the greater activity of other HSFs, resulting

in greater thermotolerance compared to the wild type (Fragkostefanakis et al. 2019).

Ricinus communis L. (Euphorbiaceae), commonly known as castor bean, is widely distributed mainly across semi-arid tropical, subtropical, and warm temperate regions. This non-edible oilseed crop holds substantial importance due to its ricinoleic acid (12-hydroxy-cis-9-octadecenoic acid), a unique, highly demanded valuable oil in a wide range of (bio)industrial applications, including chemical, pharmaceutical, and biodiesel production (Ogunniyi 2006; Kumar & Sharma 2011; Bibin et al. 2020). However, the growth and development of *R. communis* are significantly hindered by high temperatures and possibly also other abiotic stresses prevalent mainly in global-wide tropical semi-arid regions where it is traditionally cultivated (Severino et al. 2012; Ribeiro et al. 2014a; Esmaeilian et al. 2023). Therefore, comprehensive research efforts are imperative to enhance and expand the genetic foundations of *R. communis*, with the ultimate goal of increasing productivity in commercial production systems (Bergmann, 2013). Notably, while various studies have illuminated the characterization and significance of HSFs in different plant species, these investigations have yet to be conducted in *R. communis*. Hence, there is a pressing need to unravel the regulatory mechanisms governing abiotic stress-responsive *HSF* genes in *Ricinus communis* by combining bioinformatics and functional approaches to enable the identification, characterization, and functional prediction of genes that influence important crop traits such as stress tolerance, yield, and disease tolerance (Ribeiro 2021). This will improve the understanding of molecular and biochemical mechanisms, aiding the selection of useful HSF genes for breeding abiotic stresses in this oilseed species, especially during germination and initial seedling development when typically cultivated in the tropical arid/semi-arid regions, as in Brazil, where drought and heat limit *Ricinus communis* crop stand establishment and productivity.

Materials and Methods

Identification, Physicochemical Characterization, and Chromosomal Position of *RcHSF*

The amino acid sequences of *Arabidopsis thaliana* heat shock factors (AtHSFs) (Guo et al. 2008) were used as queries for a BLAST nucleotide search on the Phytozome platform (<https://phytozome-next.jgi.doe.gov/blast-search>) against the genome of *R. communis*. BLAST searches were conducted using the following parameters: an *E*-value cutoff of -1 , the BLOSUM62 scoring matrix, and default word length settings. The output was configured to display up to 100 alignments. Gap opening and extension were permitted, and query sequences were filtered using the default BLAST

filtering options. Subsequently, the amino acid sequences of the predicted RcHSFs were retrieved, and the InterPro database (<https://www.ebi.ac.uk/interpro/>) was used to identify conserved structural domains of HSFs. The domain (HSF_DNA-bind—PF00447) was confirmed in the identified RcHSF proteins. Subcellular localization prediction was analyzed using CELLO (<http://cello.life.nctu.edu.tw/>) and the ProtParam tool (<https://web.expasy.org/protparam/>) to predict protein physicochemical characteristics. Nuclear localization signal (NLS) prediction of coding sequences (CDS) was analyzed in the NLS Mapper database (https://nls-mapper.iab.keio.ac.jp/cgi-bin/NLS_Mapper_form.cgi). Chromosome lengths and gene locations of *RcHSF* were obtained (Yu et al. 2019a). Visualization of gene locations was accomplished using TBtools (v1.098726), available at <https://github.com/CJ-Chen/TBtools/releases>.

Phylogenetic Relationship

Amino acid sequences from *Arabidopsis thaliana*, *Oryza sativa*, and *Populus trichocarpa* were aligned with the predicted *R. communis* (RcHSFs) sequences using the MUSCLE algorithm. Subsequently, a phylogenetic analysis was performed employing the Neighbor-Joining (NJ) method within the MEGA 7.0 software and a phylogenetic tree was constructed using ITool (<https://itol.embl.de/>), reliably organizing orthologs within highly conserved HSF gene families. The robustness of the phylogenetic tree was evaluated through 1000 bootstrap replicates. The phylogenetic tree was manually edited using the iTOL server (<https://itol.embl.de/>).

Gene Structure, Motif Identification, and Multiple Sequence Alignment

The complete set of genomic sequences, coding sequences (CDS), and amino acid sequences for RcHSF was acquired from the Phytozome database (<https://phytozome.jgi.doe.gov/pz/portal.html#>). Gene structure analysis was conducted using the Gene Structure Display Server 2.0 (<http://gsds.cbi.pku.edu.cn/>). The Multiple Em for Motif Elicitation (MEME Suite 5.0.1) (<http://meme-suite.org/>) was employed to identify conserved motifs. The parameters utilized for motif analysis included a minimum motif width of 6, a maximum motif width of 50, and a maximum number of motifs to identify set at 10. For multiple sequence alignment of DNA-binding domains and the HR-A/B regions (OD), amino acid sequences were aligned using the Muscle algorithm and subsequently visualized using GeneDoc software (<https://genedoc.software.informer.com/2.7/>). The identification of conserved secondary structures was confirmed through the JPred server (<https://www.compbio.dundee.ac.uk/jpred/>).

Prediction of Potential Cis-regulatory Elements

The prediction of the biological function of *RcHSF* genes was based on 1500 bp upstream sequences from the initiation sites of *RcHSF* genes, sourced from the Phytozome database. Subsequently, an analysis was conducted utilizing the PlantCARE database (<https://bioinformatics.psb.ugent.be/webtools/plantcare/html/>). The outcomes of this analysis were then visualized by employing TBtools. The CDS sequences of *RcHSF* genes were uploaded and analyzed against the *R. communis* miRNA database (63 published miRNAs) using psRNATarget (<http://plantgrn.noble.org/psRNATarget/>). The miRNA data were sourced from Xu et al. (2013).

Seed Germination and Seedling Performance Under Distinct Abiotic Stresses

Ricinus communis seeds cv. Energia were incubated for imbibition and germination under control (water/25 °C) and under distinct abiotic stress conditions: water restriction (drought) by mannitol (−0.34 MPa/25 °C) and saline water restriction by NaCl (−0.34 MPa/25 °C), and heat stress (water/35 °C, as previously determined by Ribeiro et al. (2018). Seeds were sown on sterile “Germitest” type germination paper moistened with aqueous solutions at a rate of three times the weight of the dry paper substrate, followed by incubation in germination chambers—around 60% humidity and continuously darkness. The papers and aqueous solutions were carefully replaced every 3 days. Three replicates of 20 seeds per treatment were used, and germinability was monitored for 170 h (approximately 7 days), after which seedlings were classified as normal or abnormal (Brasil, 2009). The length of the hypocotyl-root axis of normal seedlings was measured to calculate the average length. Fresh and dry mass (after drying at 80 °C for 48 h) were also recorded. Germination parameters were analyzed using the GERMINATOR software (Joosen et al. 2010), i.e., maximum or total germination percentage (Gmax), germination speed (time to reach 50% germination, T50), germination uniformity (U84-16, defined as the time interval between 84 and 16% germination), and the overall integrated germination performance index after 170 h (AUC170). Seedling parameters were hypocotyl-root axis length and dry mass (calculated dry mass per number of normal seedlings). Statistical analyses were conducted using the Scott-Knott test ($p \leq 0.05$). Standard deviations were also calculated and are presented.

Transcriptomic Analysis of *RcHSF* Genes in Different Tissues

The RNA-Seq dataset utilized was obtained from the European Nucleotide Archive (ENA), accessible online

(<http://www.ebi.ac.uk/ena>), under the accession number ERA047687 (Brown et al. 2012). Data from 5 paired-end reads corresponding to five different tissue types were analyzed: developing seeds at the early oil accumulation stage (Endosperm II/III—ERR044267) and rapid oil accumulation stage (Endosperm V/VI—ERR044268), germinating seeds (Seed—ERR044269), leaves (Leaf—ERR044270), and male flowers (Flower—ERR044271). The method of pseudo-alignment was used (Chung et al. 2021; Costa-Silva et al. 2017). The sequencing data was initially cleaned and prepared using the Trimmomatic software, and data quality was assessed with FASTQC. Transcript quantification from RNA-Seq data was performed using Salmon, generating TPM values that were subsequently used as input for differential expression analysis in DESeq2. Genes with significant changes in expression (adjusted *p*-value below 0.05) were identified. Expression data were represented in heat maps using a Log2 scale with relative values. All analyses were conducted using R-4.4.1 (<https://www.r-project.org/>).

Modulation of *RcHSF* Genes During Germination and Early Seedling Growth Under Heat Stress

Gene expression data for the *RcHSF* genes were obtained by our group in a previous microarray analysis of *R. communis* samples collected at various seed imbibition and germination stages, i.e., dry seeds, initial imbibition, radicle protrusion (germination per se), and seedlings with 2 cm roots, under three distinct temperatures (20, 25, and 35 °C) (Ribeiro et al. 2018). The microarray analysis involved the utilization of three independent biological replicates, each consisting of 20 seeds without the seed coat, in which 20 °C was a suboptimal low temperature, 25 °C the optimal control temperature, and 35 °C as critical heat stress.

Expression of *RcHSF* Genes in Seedlings Under Abiotic Stresses Conditions by qPCR

The total RNA was extracted from approximately 100 mg samples of embryos (cotyledons and radicles) of *Ricinus communis* seeds cv. Energia using the column kit method (PureLink Life Technologies). cDNA was synthesized using the GoScript™ Reverse Transcription System Kit (Promega) using 1 µg of total RNA. The qPCR reaction contained 10 µL of SYBR Green Master Mix Reagent (Applied Biosystems, USA), 5.0 µL of cDNA sample, and 1 µL of gene-specific primer (10 nM) in a final volume of 20 µL. Each pair of primers was designed using Primer3Plus software (<https://www.primer3plus.com/index.html>), expecting an amplicon size of 100–150 bp (Supplementary Table S1). The qRT-PCR program was set with denaturation at 95 °C for 15 s and extension at 60 °C for 60 s for 40 cycles. The $2^{-\Delta\Delta C_t}$ method was used to analyze the data. Actin and PP2AA1

were employed as reference genes, previously validated for expression stability under the stress conditions applied in this study (Cassol et al. 2016; Ribeiro et al. 2014b; Gomes Neto et al. 2018; Neto et al. 2020). The expression level of *RcHSF* genes in the treatment was compared to that in the dry seeds. Three biological replicates were included for each experimental group, and three technical replicates were included for each biological sample.

Results

Identification, Protein Physicochemical Properties, NLS Signals, and Chromosomal Position of *RcHSF* Genes

Eighteen *HSF* genes were retrieved in *R. communis* (*RcHSF01* to *RcHSF18*) (Table 1) from the *Phytozome* database. The length of the *RcHSF* proteins varied between 84 amino acids for *RcHSF15* and 521 amino acids for *RcHSF02*, whereas their molecular weights ranged from 9.61 to 58.65 kDa, respectively. The 10 members of group A (excluding *RcHSF15*) have proteins with the highest numbers of amino acids and molecular weights, with averages of 436 amino acids and 49.38 kDa. On the other hand, the 6 members of group B have averages of 280 amino acids and 31.71 kDa. The only member of group C, *RcHSF10*, has 337 amino acids and 37.32 kDa.

The isoelectric points (pI) show that the majority have an acidic nature (with pH ranging from 4, 5, and 6), except for 3 proteins from class B, where *RcHSF07* had the highest pH of 9.76, *RcHSF12* with 7.01, and *RcHSF17* with 7.72. Group A has the lowest pH values (all between 4 and 5), with *RcHSF11* being the lowest at 4.68.

Calculations of the instability index indicated that most (94%) of the *HSF* proteins were predicted to be unstable in vitro, with *RcHSF15* constituting the exception (Table 1). Besides that, the Aliphatic index values ranged from 64.14 (*RcHSF04*) to 76.93 (*RcHSF16*), suggesting minor variations in thermal stability among the proteins.

Subcellular localization predictions consistently indicated nuclear localization for all predicted *HSF* proteins (Table 1). Only *RcHSF01* and *RcHSF15* may have extracellular localization, in addition to nuclear localization. The presence of nuclear localization signals (NLS) in the predicted proteins of *RcHSF* was confirmed using NLS Mapper, with approximately 11 out of the 18 *R. communis* *HSFs* exhibiting these signals. The position, type (monopartite and bipartite), and score of the NLS were also predicted (Supplementary Table 2). Furthermore, of the 11 NLS sequences present in *RcHSF*, 8 sequences, whether monopartite or bipartite, were identified in group A, 2 in group B, and 1 in the only member of group C (*RcHSF10*). The highest NLS scores

Table 1 The physicochemical properties and basic information of RcHSF proteins

ID	Name	Group	Number of amino acids	Molecular weight (Da)	pI	Instability index*	Aliphatic index	Subcellular localization
29968.m000635	RcHSF11	A	409	46,934.69	4.68	45.86 (U)	73.20	Nuclear
30054.m000802	RcHSF14	A1	510	55,981.19	4.83	60.65 (U)	66.71	Nuclear
30073.m002276	RcHSF15	A1	84	9605.82	5.59	24.47 (S)	77.50	Nuclear, Extracellular
29739.m003565	RcHSF05	A3	371	41,674.19	4.76	56.60 (U)	74.10	Nuclear
29844.m003222	RcHSF06	A3	359	41,213.29	5.24	56.87 (U)	75.40	Nuclear
29883.m001955	RcHSF08	A3	360	41,024.04	5.73	52.43 (U)	66.11	Nuclear
29912.m005397	RcHSF09	A3	478	54,185.36	4.92	51.76 (U)	68.68	Nuclear
29092.m000454	RcHSF02	A4	521	58,650.62	4.89	63.14 (U)	69.60	Nuclear
29629.m001435	RcHSF03	A4	491	54,951.90	5.72	50.46 (U)	67.01	Nuclear
29636.m000750	RcHSF04	A4	464	52,555.78	5.99	55.94 (U)	64.14	Nuclear
30026.m001484	RcHSF13	A4	402	46,226.40	5.15	58.78 (U)	62.59	Nuclear
29851.m002407	RcHSF07	B1	218	24,931.39	9.76	59.95 (U)	74.72	Nuclear
30147.m014282	RcHSF16	B2	323	35,794.88	5.25	59.36 (U)	76.93	Nuclear
30190.m011156	RcHSF18	B2	337	37,054.13	5.31	58.40 (U)	70.09	Nuclear
30006.m000287	RcHSF12	B3	248	28,738.22	7.01	51.32 (U)	62.54	Nuclear
28312.m000133	RcHSF01	B4	191	22,144.06	6.80	51.61 (U)	74.45	Nuclear, Extracellular
30170.m013859	RcHSF17	B4	362	40,499.59	7.72	51.36 (U)	70.80	Nuclear
29912.m005526	RcHSF10	C	337	37,322.87	5.16	58.95 (U)	69.14	Nuclear

*U, unstable; S, stable

(ranging between 9 and 10) were also observed in group A proteins (*RcHSF03*, *RcHSF05*, *RcHSF06*, *RcHSF08*, and *RcHSF13*), all of which are of the monopartite NLS type (Supplementary Table 2).

The *RcHSF* genes were physically mapped onto the 10 chromosomes of *R. communis* (Yu et al. 2019a, b). The distribution revealed considerable variation in the number of HSFs across different chromosomes, with some chromosomes containing notably more *HSF*s than others. The highest number of *HSF* genes was observed on chromosome 6 (6 genes), followed by chromosome 1 (3 genes). Chromosomes 2, 3, and 7 contained two gene copies each, while a single *HSF* was located on chromosomes 4 and 9. Notably, chromosomes 5, 8, and 10 were devoid of *HSF* genes (Fig. 1).

Phylogenetic Tree

The evolutionary relationship among *Oryza sativa* (monocot), *Arabidopsis thaliana*, *Populus trichocarpa*, and *Ricinus communis* (eudicots) *HSF* genes showed the division between HSF groups and subgroups (Fig. 2) and the relationship of orthologs among the species (Supplementary Table 4).

The different identified and classified subgroups were based on their orthologs in the phylogenetic tree generated in the present study, following the classification previously described for *A. thaliana*, *O. sativa*, and *P. trichocarpa*

genes (Guo et al. 2008; Zhang et al. 2015). Group A was subdivided into A, A1, A2, A3, and A4, and group B was subdivided into B1, B2, B3, and B4, and group C did not show any subdivisions (Fig. 2).

R. communis contains 11 genes belonging to group A, 6 genes from group B, and only 1 gene from group C (red balls). This pattern was consistent across all species, with the highest number of genes in group A, followed by groups B and C (Supplementary Table 3). A total of 18 HSF genes were identified in *R. communis*, the lowest number compared to the other species.

Gene Structure, Motifs Analysis, and Multiple Sequence Alignment of *RcHSF*

To further elucidate the structural relationships among the *RcHSF* genes, the intron-exon organization of all targeted *RcHSF* genes was analyzed using the Gene Structure Display Server 2.0 (<http://gsds.gao-lab.org/>). In general, the numbers of introns and exons were highly similar across the genes, although differences in intron number and length were observed among phylogenetically related members (Fig. 3a). Most of the *RcHSF* genes exhibited two exons across the three groups, suggesting a highly conserved exon-intron splicing pattern. However, two genes contained three exons, i.e., *RcHSF04* from group A and *RcHSF01* from group B.

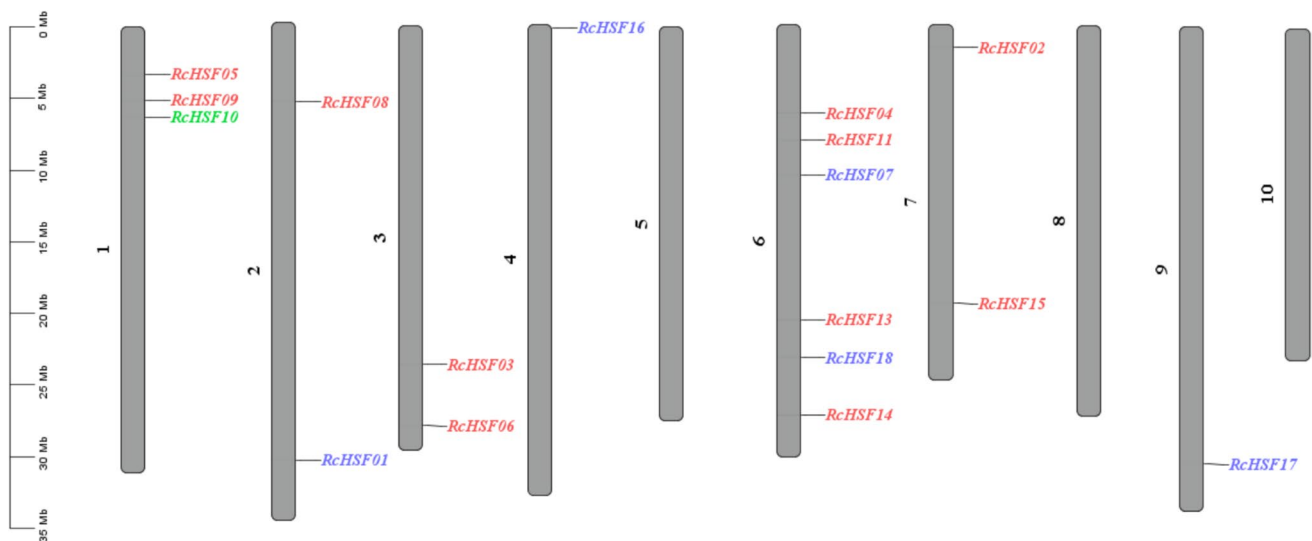


Fig. 1 The distribution of *RcHSF* genes on the *R. communis* chromosome scaffolds. The scale on the left side represents megabases (Mb). The chromosome number is indicated in the middle of each bar

Motif 1 (WSSDGNSFVWBPPEFARDLLPKYFKHN-NFSSFVRQLNTY) was confirmed in the InterPro database under the code Pf00447, corresponding to the structure of the dimeric DNA-binding domain (DBD—Supplementary Table 5). The sequence of this conserved protein is further detailed in Supplementary Figure 1, alongside the localization of the predicted NLS for each protein (Supplementary Table 2).

In total, 10 motifs, designated as motifs 1–10, were identified across the *RcHSF* proteins (Fig. 3b and Supplementary Table 5). All *RcHSF* proteins contained motifs 1 and 3, and the relative positions of these motifs were consistent in most sequences. Motif 2 was present in all HSF proteins, except for *RcHSF15* (Class-A). Motifs 5, 7, 9 and 10 were exclusive to the HSF A, while motif 6 was unique to the HSF B group, except for *RcHSF01*. Motif 4 was found in the HSF A and HSF C groups.

The multiple alignments revealed the highly structured domains of the DNA-binding domain (DBD) and the oligomerization domain (OD), specifically within the HR-A/B regions (Fig. 4). The DBD was present in the region of motif 1 (Pf00447) (Supplementary Table 5), being highly conserved among the 18 *RcHSF* genes, with approximately 90 amino acids (Supplementary Figure 1). The protein secondary structure with the highest level of conservation among all 18 genes, according to the results from the Jpred server (<https://www.compbio.dundee.ac.uk/jpred/>—Supplementary Table 6), was predicted to contain three α -helix bundles ($\alpha 1$ – $\alpha 3$) and three reverse-parallel β -folds ($\beta 1$ – $\beta 3$), in the form of $\alpha 1$ – $\beta 1$ – $\beta 2$ – $\alpha 2$ – $\alpha 3$ – $\beta 3$ – $\beta 4$.

Predicted Plant Hormones of Cis-acting Elements and miRNA Prediction of *RcHSFs*

The promoter region of *RcHSF* gene family members harbors diverse elements associated with growth, development, hormonal reactions, and responses to stress. The most common elements in the promoters of *RcHSF* genes were the abscisic acid element (ABRE) and anaerobic induction; both were identified 27 times (Fig. 5). Also, the jasmonic acid element (MeJARE) was found 21 times; the circadian control elements and the salicylic acid-responsive elements (SARE) were both found 10 times; the cis-acting element involved in defense and stress was found 9 times, and the gibberellic acid response element (GARE) and the low temperature were both found 8 times in *RcHSF* genes. The promoters of 13 *RcHSFs* genes contained the ABA-responsive element (ABRE) and 12 *RcHSF* genes contained the anaerobic induction, being the two elements present in greater numbers of *RcHSF* genes (Supplementary Table 8). Furthermore, 8 *RcHSF* gene promoters contained MeJARE, 8 GARE, 7 defense and stress, 7 circadian control, 6 SARE and 5 low temperatures (Supplementary Table 8).

The gene with the highest number of cis-acting elements was *RcHSF17* (group B), with 12 elements, then *RcHSF02* (group A) with 10 elements, then *RcHSF03* and *RcHSF06* (both from group A) with 9 elements, and *RcHSF16* and *RcHSF18* (both from group B) with 8 elements. *RcHSF10* was the only member of group C, having 5 elements, i.e., 2 binding sites for ABRE, 3 for anaerobic induction, and 1 for low temperature.

R. communis contains 11 genes belonging to group A, 6 genes from group B, and only 1 gene from group C (red balls). This pattern was consistent across all species, with the highest number of genes in group A, followed by groups B and C (Supplementary Table 3). A total of 18 HSF genes were identified in *R. communis*, the lowest number compared to the other species.

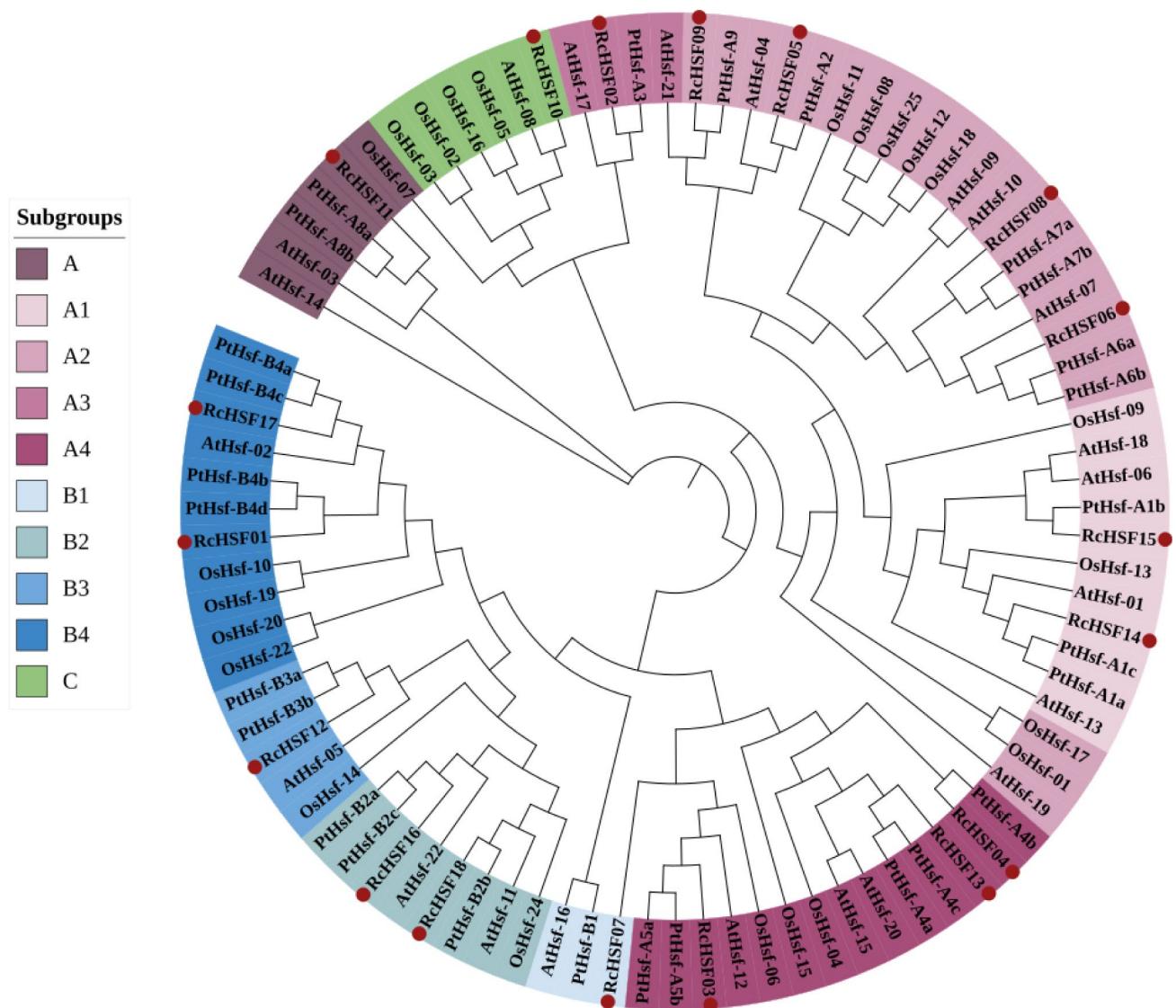


Fig. 2 Phylogenetic analysis of *R. communis*, *A. thaliana*, *O. sativa*, and *P. trichocarpa*. MEGA 7.0 was used to build a multiple sequence alignment and a Neighbor-Joining (NJ) phylogenetic tree with 1000 bootstrap replicates

ABA and anaerobic induction elements are found in all *RcHSF* gene groups (A, B, and C). About 70% of group A genes (*RcHSF02*, *RcHSF03*, *RcHSF06*, *RcHSF09*, *RcHSF11*, *RcHSF13*, *RcHSF14*, *RcHSF15*) contain ABA elements, with a maximum of 3 binding sites per gene, while *RcHSF17* in group B has 5 binding sites. One gene in

each group has a maximum of 3 binding sites for anaerobic induction (group A *RcHSF08*; group B *RcHSF18*; group C *RcHSF10*). The prevalent presence of these elements suggests that the *RcHSF* gene family may be broadly implicated in ABA signaling, hypoxia, and other abiotic stress responses.

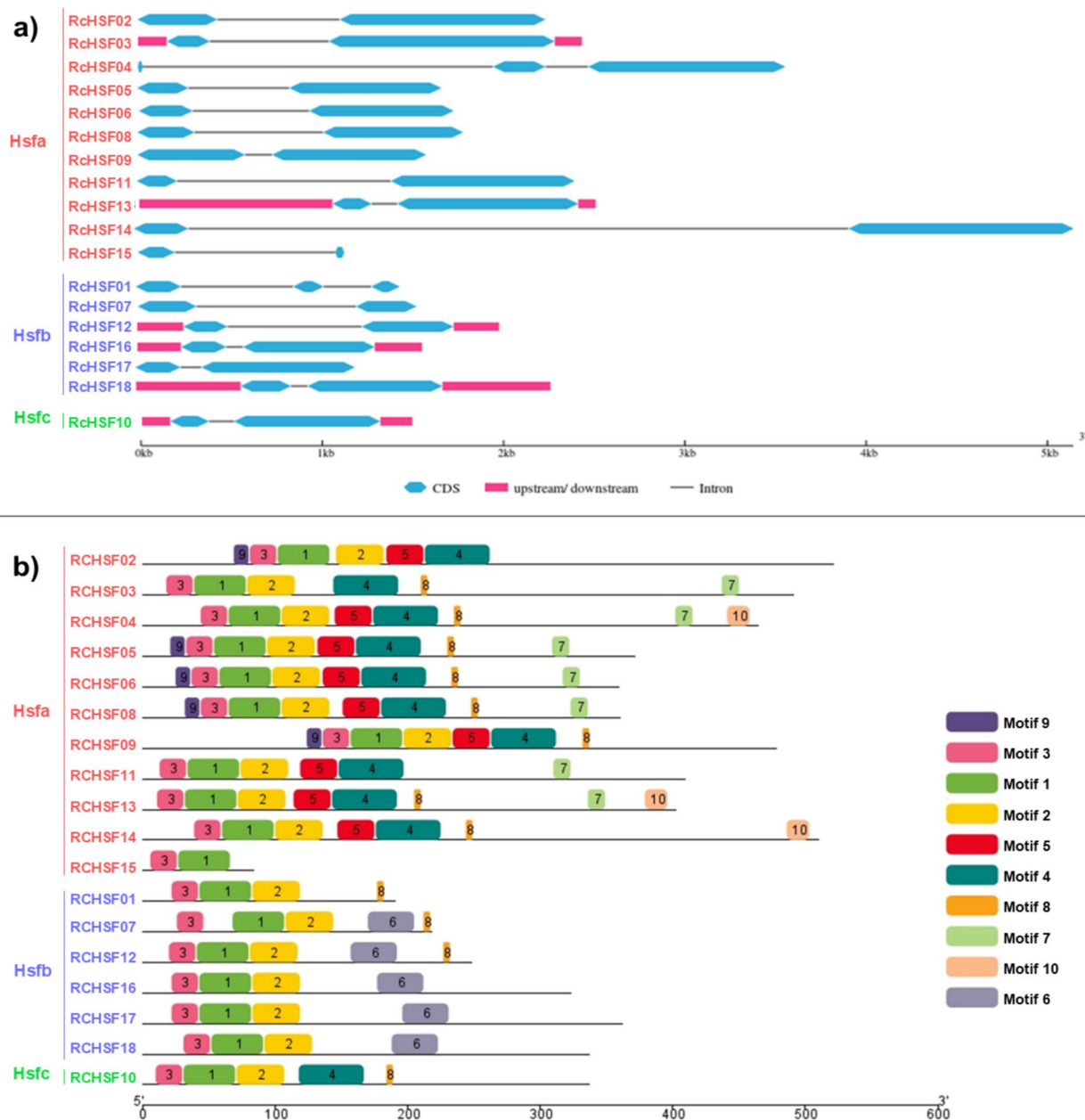


Fig. 3 **a** Intron-exon structure of the *HSF* genes in *R. communis*. Blue boxes represent exons (CDS); black lines represent introns, and pink boxes represent 5' and 3' untranslated regions. **b** Conserved motifs of

RcHSF proteins. Bottom scale bar indicate the protein lengths, and sequence logos for each conserved motif are shown on the right

Conversely, methyl jasmonate (MeJA) is crucial for promoting plant growth and development and activating antioxidant systems (Yu et al. 2019b). *RcHSF13* and *RcHSF17* have four MeJA binding sites each, and *RcHSF16* has six. Salicylic acid (SA) enhances plant immunity and systemic acquired resistance (Peng et al. 2021). SA-responsive elements are prominent in the group A genes (*RcHSF02*, *RcHSF05*, *RcHSF13*, *RcHSF14*, *RcHSF15*), suggesting a

role in stress response regulation. Moreover, gibberellins (GAs) promote cell elongation and division, which are essential for growth (Miceli et al. 2019). GA-responsive elements are present in eight *RcHSF* genes (*RcHSF06*, *RcHSF07*, *RcHSF09*, *RcHSF11*, *RcHSF12*, *RcHSF13*, *RcHSF14*, *RcHSF17*).

The miRNA prediction indicates that four *RcHSFs* genes are possibly regulated by miRNAs, i.e., *RcHSF04* is

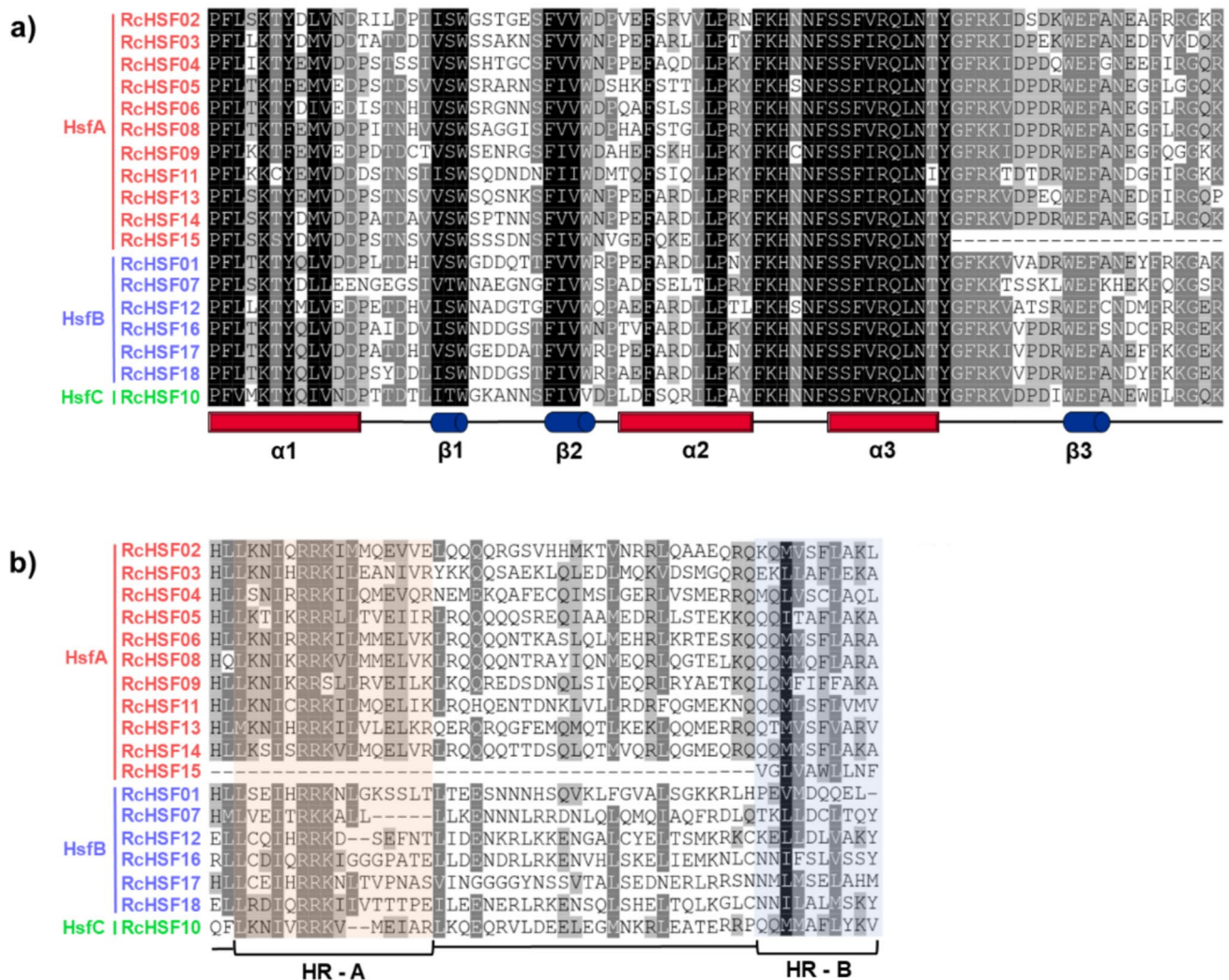


Fig. 4 Multiple sequence alignment of DNA-binding domains and the HR-A/B regions (OD) of RcHSF proteins. **a** DBD domain sequences of RcHSFs identified by InterPro database were aligned by Muscle. The helix-turn-helix motifs of the DBD (α 1- β 1- β 2- α 2- α 3- β 3) are shown at the bottom. Red tubes represent the α -helices, and blue

cylinders represent the β -sheets. **b** Multiple sequence alignment of the HR-A/B regions of the HSF protein family in *R. communis*. The definitions of the HSF numbers correspond to the order of alignment. The scheme below depicts the locations and boundaries of the HR-A core, insert, and HR-B regions within the HR-A/B regions

regulated by *rco*-miR164; *RcHSF06* by *rco*-miR395 and also by *rco*-miR167; *RcHSF08* by *rco*-miR395; and *RcHSF13* by *rco*-miR167 (Supplementary Table 7).

Effects of Abiotic Stresses on Germination and Seedling Development

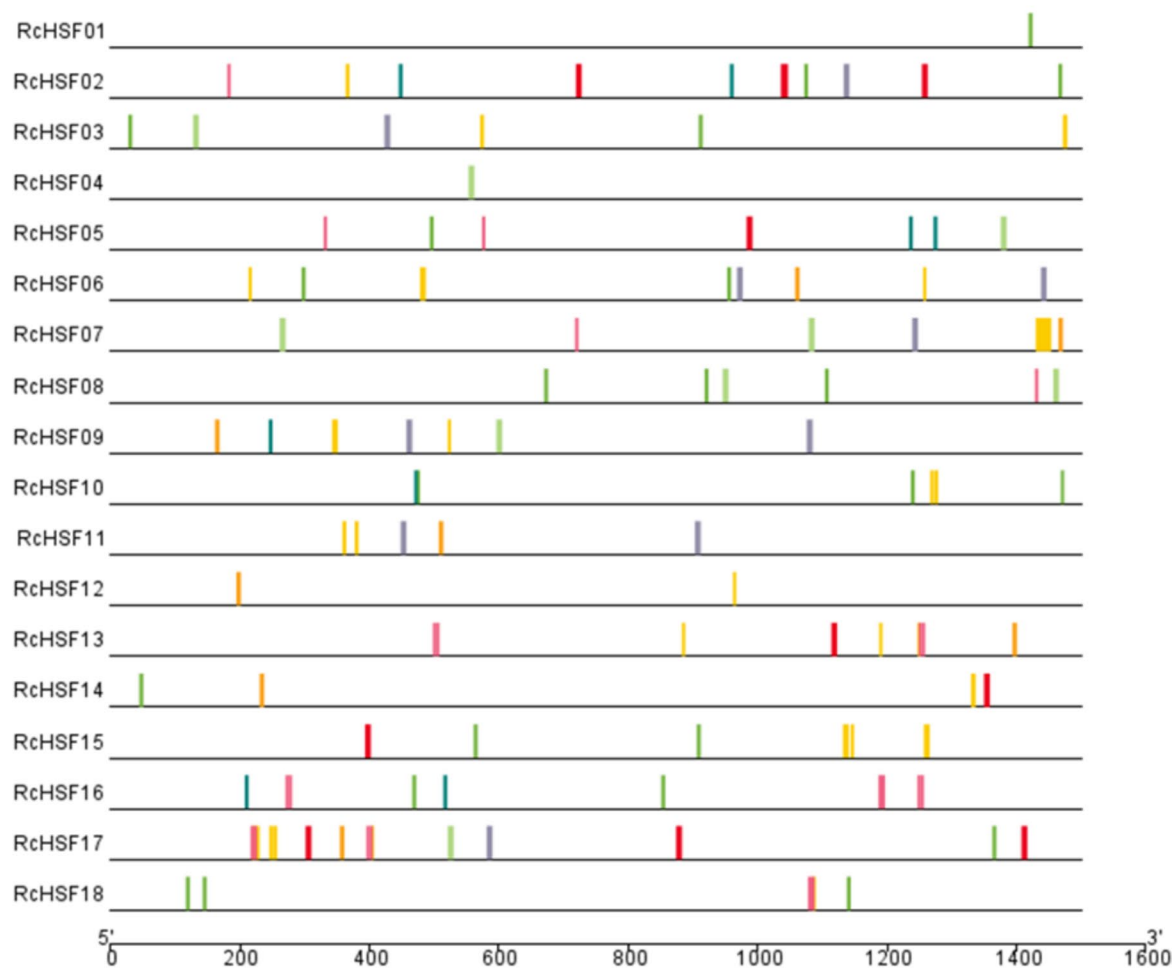
The germinability and seedling development parameters of *Ricinus communis* under distinct abiotic stress conditions are shown in Fig. 6 and Supplementary Figure 2.

The germination speed, measured as the time to reach 50% germination (T50), was lowest at 35 °C, indicating that the increased temperature accelerated germination, making this treatment the first to reach 50% germination, followed by

the control conditions (Fig. 6a). Thus, the germination performance over time, assessed by the AUC170 index, further indicates that this accelerated pattern was maintained throughout the total germination until 170 h or 100% germination (Fig. 6b). Besides that, growth at 35 °C increased dry mass and resulted in longer hypocotyls, thereby enhancing overall seedling vigor (Fig. 6c and d). In contrast, osmotic stress (mannitol −0.34 MPa/25 °C) had the strongest negative effect on germination speed, followed by salt stress (NaCl −0.34 MPa/25 °C) (Fig. 6a). Both osmotic and salt stress resulted in lower AUC values and reduced hypocotyl-root axis length. All parameters were compared to the control (water/25 °C).

The different stress treatments did not significantly affect the percentage of fully germinated seedlings (Gmax)

a)



b)

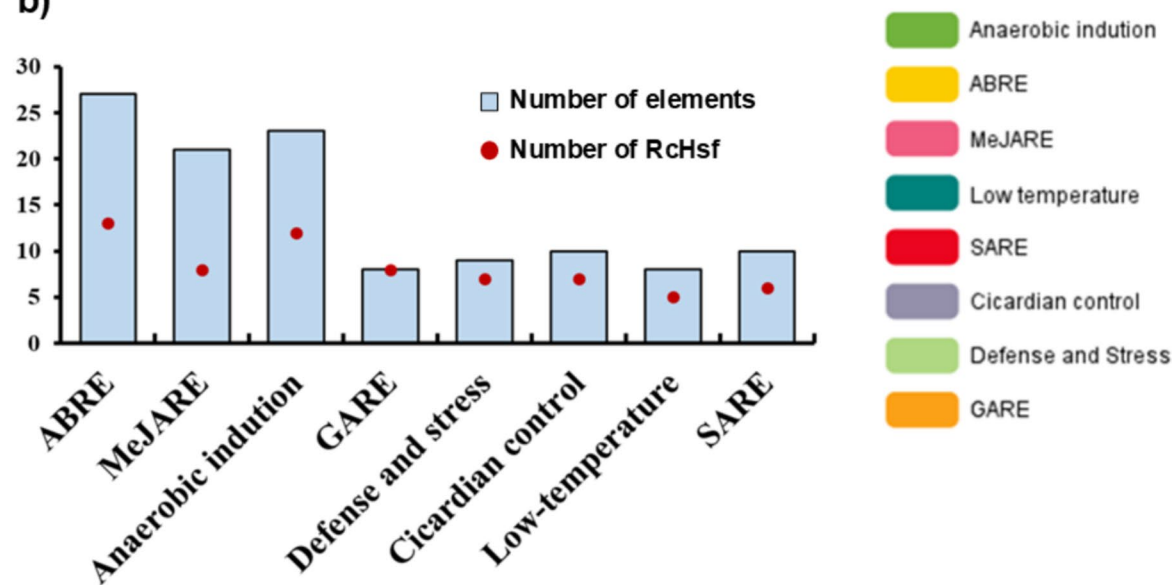


Fig. 5 **a** Diagram of cis-acting elements identified in the promoter regions of the *RcHSF* genes. Rectangular boxes of different colors represent individual cis-acting elements, and some elements overlap. **b** The column indicates the number of elements, and the red dots are the quantities of *RcHSF* for each element

(Supplementary Figure 2a). Despite that, the germination uniformity represented by the interval between 84 and 16% germination (U84–16) was greater under control and heat-stress conditions (i.e., lower values indicating faster and more synchronized germination). In contrast, osmotic and salt stress showed the lowest uniformity values (Supplementary Figure 2b). Furthermore, while both the control and heat-stress treatments showed a high percentage of normal seedlings, osmotic and salt stress resulted in a reduction in the total number of normal seedlings during germination. Finally, it is important to highlight that although no statistical differences were detected between the control and heat-stress treatments, the latter showed a noticeable reduction in the total number of normal seedlings compared with the control, suggesting that this temperature may begin to affect germination and early seedling development.

Expression Pattern of *RcHSF* Genes in Different Tissues

We accessed the expression data from Brown et al. (2012) to investigate the expression patterns of *R. communis* *RcHSF* genes in different tissues and conditions to obtain insights into their potential functions. The *RcHSF* genes displayed distinct expression patterns across various tissues and organs (Fig. 7), with each gene exhibiting a tissue-specific expression profile. This suggests that *RcHSF* genes are differentially expressed in various tissues and during multiple stages of development. Specifically, *RcHSF05*, *RcHSF09*, *RcHSF07*, *RcHSF16*, *RcHSF02*, *RcHSF13*, and *RcHSF03* were predominantly expressed in the flowers, while four genes, i.e., *RcHSF18*, *RcHSF11*, *RcHSF01*, and *RcHSF17*, showed high expression in the Endosperm V/VI. Additionally, *RcHSF12*, *RcHSF10*, and *RcHSF08* were highly expressed in the cotyledons of germinated seeds, whereas *RcHSF04*, *RcHSF06*, *RcHSF14*, and *RcHSF15* exhibited higher expression levels in the seedling leaves. In contrast, *RcHSF* genes showed lower expression levels in the Endosperm II/II compared to other tissues.

RcHSF08, *RcHSF10*, and *RcHSF12* were underexpressed during seed development but were highly expressed during germination, whereas *RcHSF04* and *RcHSF02* were repressed in the transition from early (Endo II/III) to late (Endo V/VI) endosperm formation (Brown et al. 2012) but were induced during germination (Fig. 7). On the other hand, *RcHSF01*, *RcHSF11*, *RcHSF17*, and *RcHSF18* were expressed at low levels in the early developmental (Endo II/

III) stage but were strongly induced during later endosperm development (Endo II/III) (Fig. 7), suggesting that these genes may be involved in the seed maturation phase.

Expression Pattern of *RcHSF* Genes Detected by Transcriptome Data

The analysis of the previous transcriptome data from our research group (Ribeiro et al. 2018) exhibited different expression patterns of *RcHSF* genes during early imbibition, radicle protrusion (germination per se), and early seedling stage (2 cm roots) under varying temperature conditions (cold stress 20 °C, normal/control 25 °C, and heat stress 35 °C). The number of upregulated *RcHSF* genes in dry seeds and at the beginning of imbibition was higher than during germination (Fig. 8). Six *RcHSF* genes (*RcHSF05*, *RcHSF08*, *RcHSF09*, *RcHSF10*, *RcHSF13*, and *RcHSF18*) presented high expression in dry seeds compared to the three stages of germination in the control (water/25 °C), suggesting their involvement in seed maturation and desiccation. All *RcHSF* genes, except *RcHSF04*, showed high expression under at least one stress condition during early imbibition and germination, indicating their involvement in the germinative process. Only three *HSF* genes (*RcHSF04*, *RcHSF09*, *RcHSF12*) were not regulated by heat stress.

Figure 8 also shows that heat stress (water/35 °C) induced high expression of *RcHSF01*, *RcHSF02*, *RcHSF05*, *RcHSF06*, *RcHSF08*, *RcHSF11*, *RcHSF14*, *RcHSF16*, *RcHSF17*, and *RcHSF18* in radicle protrusion and of *RcHSF02*, *RcHSF03*, *RcHSF06*, *RcHSF07*, *RcHSF09*, *RcHSF11*, *RcHSF13*, *RcHSF16*, and *RcHSF17* genes in young seedlings, compared with the control (water/25 °C). The genes induced by heat stress in dry seeds were present on chromosomes 1, 2, 4, and 6, while those in seedlings were concentrated on chromosomes 3, 6, and 7.

Overall, 11 *RcHSF* (*RcHSF02*, *RcHSF03*, *RcHSF04*, *RcHSF05*, *RcHSF08*, *RcHSF09*, *RcHSF11*, *RcHSF13*, *RcHSF14*, *RcHSF16*, and *RcHSF18*) genes showed higher expression in all temperatures, whereas heat stress (water/35 °C) also induced *RcHSF07* and *RcHSF10* in early imbibition, compared to radicle protrusion and 2 cm seedlings. By contrast, *RcHSF01* and *RcHSF12* were downregulated in all temperatures at early imbibition and showed higher expression in radicle protrusion and seedlings, indicating that the expression of individual *RcHSF* genes differs between the germination stages.

Gene Expression Differentiation Among *RcHSF* Classes A, B, and C During Abiotic Stress Induction

Genomic expression analyses across various plant species have shown that the expression levels of specific *HSF* genes are modulated in response to different abiotic stresses (Li

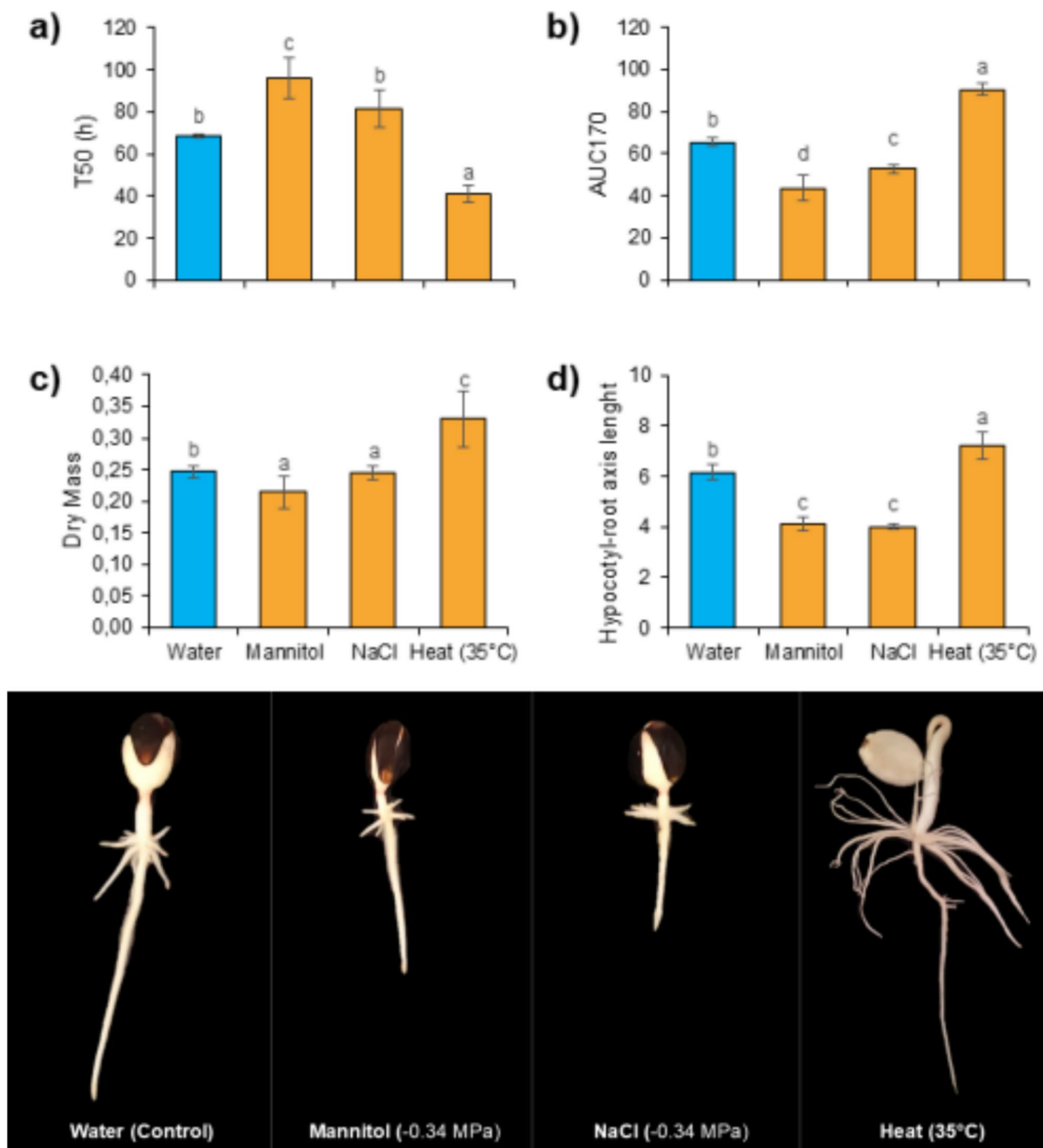


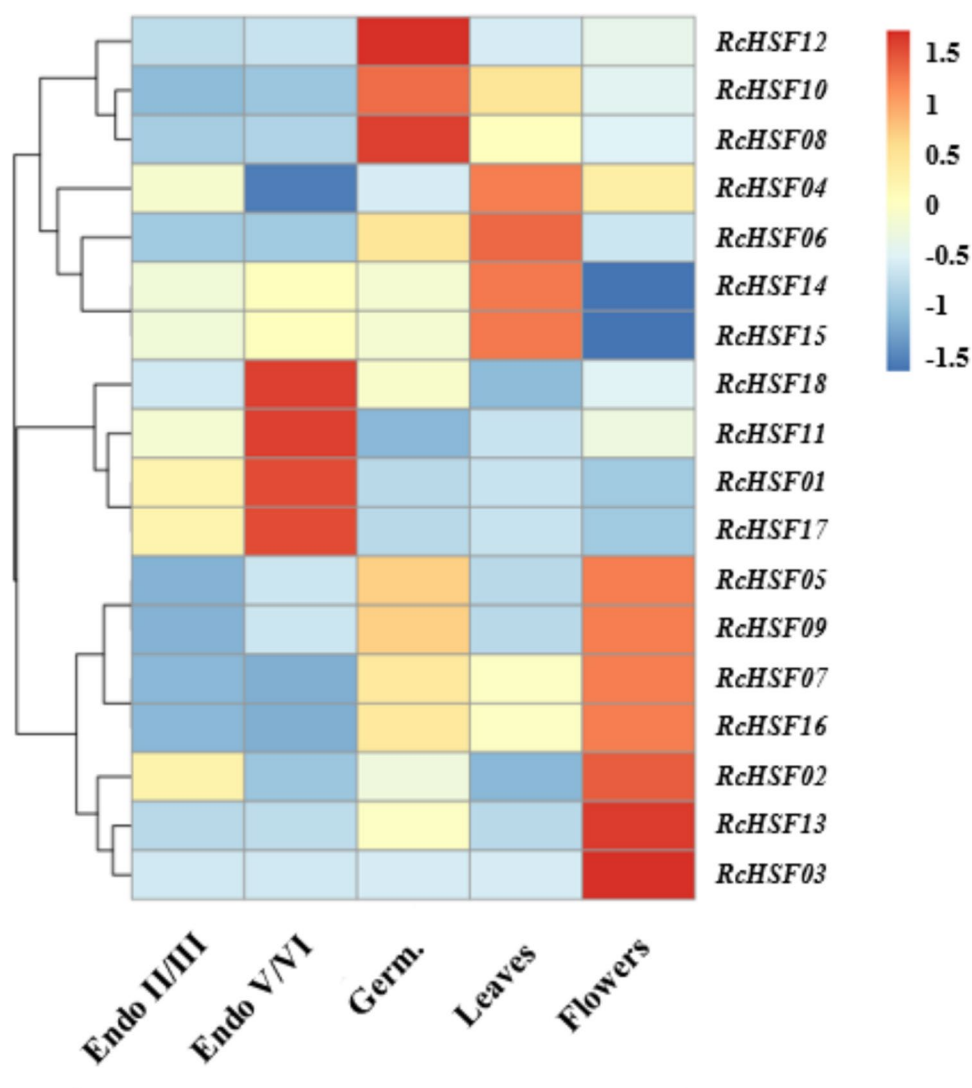
Fig. 6 Germination and seedling performance of *R. communis* cv. Energia imbibed for 170 h under control (water, 25 °C) and abiotic stress conditions: water restriction by mannitol (−0.34 MPa, 25 °C) and by NaCl salinity (−0.34 MPa, 25 °C), and heat (water, 35 °C). **a** Germination speed measured as the time to 50% germination (T50). **b**

Area under the germination curve index at 170 h (AUC170). **c** Seedling dry mass. **d** Hypocotyl-root axis length. **e** Seedling phenotypes at 170 h. Different letters indicate statistically significant differences according to the Scott-Knott test ($p \leq 0.05$). Error bars represent standard deviation

et al. 2013; Dossa et al. 2016; Wu et al. 2018). To investigate the expression of these predicted genes from different classes in *Ricinus communis* and to confirm their responsiveness to

abiotic stress, we selected the genes *RcHSF06*, *RcHSF07*, *RcHSF08*, and *RcHSF10* with the aim of encompassing representatives of the different classes of HSFs. Furthermore,

Fig. 7 Heatmap illustrates the expression patterns of 18 *RcHSF* genes across various tissues and developmental stages in *R. communis* (Brown et al. 2012). These stages include initial endosperm (Endo II/III), late endosperm (Endo V/VI) development, cotyledons of germinating seeds (Germ.), seedling leaves, and male flowers. The color scale, based on log₂ TPM expression values, transitions from blue (low expression) to red (high expression), with intermediate values represented in green



Brown et al., 2012

these genes exhibit high levels of expression in germinated seeds and throughout different stages of germination, suggesting that they may play distinct and complementary roles in the early stages of development. We performed quantitative real-time polymerase chain reaction (RT-qPCR) on dry seeds (control) and seeds with 2 cm roots subjected to water (control/25 °C), water restriction (drought) by mannitol and by NaCl salinity (−0.34 MPa/25 °C), and heat stress (water/35 °C). The results revealed differential expressions of these genes in seeds under the distinct stress conditions (Fig. 9).

From group A, the *RcHSF06* gene exhibited higher expression in dry seeds and control conditions but showed lower expression under stress treatments during this stage of development. In contrast, the *RcHSF08* gene displayed high expression levels across all three types of stress. In group B,

the *RcHSF07* gene was active in all germination treatments, with particularly high expression under salt stress, indicating a potential role in the salt stress response during germination. Additionally, the *RcHSF10* gene, which belongs to group C, showed low expression in dry seeds but elevated expression under both normal and stress conditions.

Discussion

We identified 18 *HSF* genes in *R. communis*, a number lower than that observed in *Oryza sativa* (25 *HSF* genes, Guo et al. 2008), *Arabidopsis thaliana* (22 *HSF* genes, Guo et al. 2008), and *Populus trichocarpa* (28 *HSF* genes, Zhang et al. 2015), but slightly higher than in *Jatropha curcas* (17 *HSF* genes, Zhang et al. 2020b). The

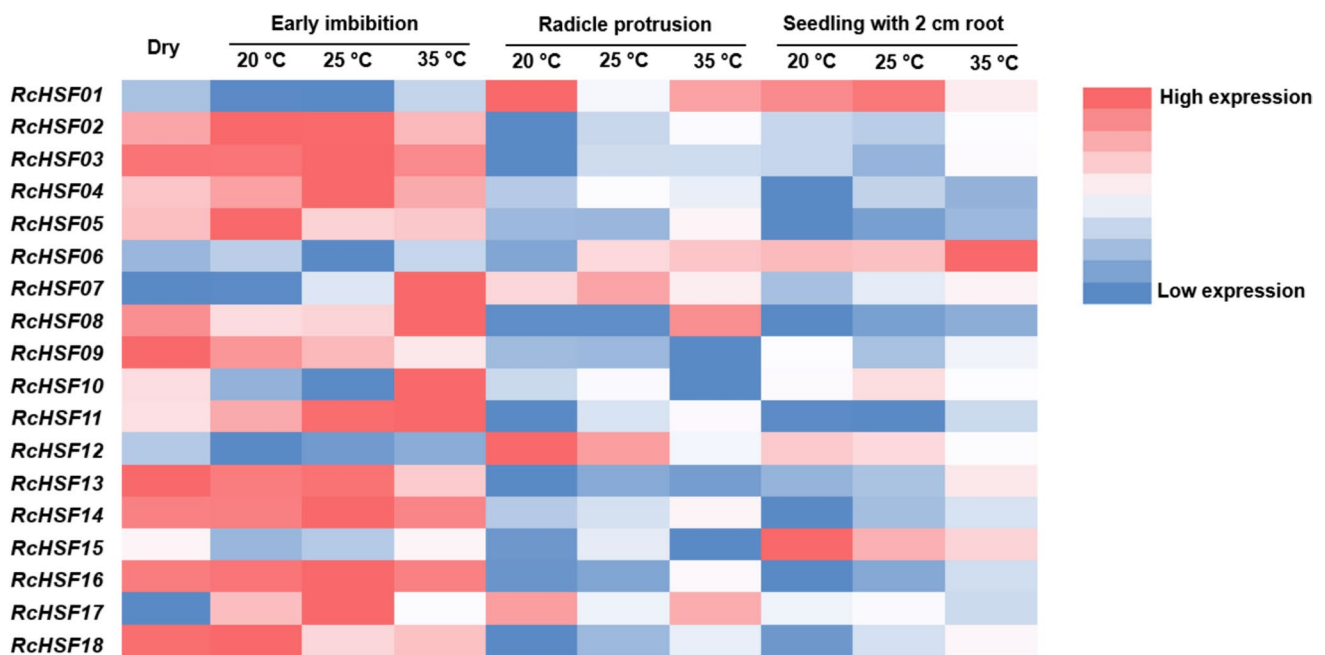


Fig. 8 Heatmap representation of *RcHSF* expression profiles during early imbibition, radicle protrusion, and seedlings with a 2 cm root (Ribeiro et al. 2018). Blue represents low expression, and red repre-

sents high expression. At each stage and temperature, three independent biological replicates were used, each consisting of 20 seeds

distribution of *HSF* genes among classes A, B, and C varies among species, with most genes typically belonging to class A. *R. communis* has 11 *HSF*-A, 6 *HSF*-B, and 1 *HSF*-C (Supplementary Table 3). Also, *RcHSF* genes generally have orthologs in at least one of the species evaluated and are present across all three *HSF* classes (A, B, and C; Supplementary Table 4). The *HSF* gene family underwent significant expansion and independent subfamily evolution during plant evolution. While the entire family expanded dramatically, phylogenetic analyses indicate that its subfamilies diverged and evolved independently, a pattern especially pronounced in angiosperms. Furthermore, evolutionary dynamics differed between major lineages. The *HSFB* and *HSFC* groups are more ancient and under stronger purifying selection than *HSFA*, suggesting they perform essential, conserved biological functions. In contrast, the *HSFA* group shows greater lineage-specific diversification. This is evident between monocots and eudicots, which differ in subfamily composition, gene copy number, and selection pressures. These patterns also extend to specific species comparisons. For instance, within the *Euphorbiaceae*, *Ricinus communis* and *Jatropha curcas* possess a similar, conserved number of *HSF* genes. However, *R. communis* generally has fewer *HSFs* than *Arabidopsis thaliana* or *Populus trichocarpa*, often possessing a single gene where the latter species have two (e.g., *RcHSF11* and *RcHSF13* have two orthologs

in *A. thaliana* and *P. trichocarpa*) (Liao et al. 2022; Wang et al. 2018).

The physicochemical characterization of the 18 *RcHSF* proteins provides insights into their potential functional behavior in *R. communis*. Members of group A, which possess the highest molecular weights and protein lengths, likely reflect a more complex domain architecture, consistent with their typical role as strong transcriptional activators in other plant species. The predominance of acidic isoelectric points within this group further suggests its preferential activity in nuclear environments, where acidic TFs commonly interact with co-activators and DNA. In contrast, several class B members exhibit neutral to basic pI values, which may indicate distinct interaction partners or regulatory mechanisms, aligning with the known repressor-like functions described for some *HSFB* proteins in model plants. The single class C member, *RcHSF10*, displays intermediate physicochemical parameters, supporting its previously proposed functional resemblance to class A proteins. Although the instability index predicted most *RcHSF* proteins as unstable in vitro, this is characteristic of many eukaryotic transcription factors, which often rely on intrinsically disordered regions to enable rapid turnover and flexible DNA-binding dynamics under fluctuating stress conditions. Meanwhile, the relatively narrow range of aliphatic index values implies overall conserved thermal stability across the family, which may be relevant given the central role of *HSFs* in heat stress responses.

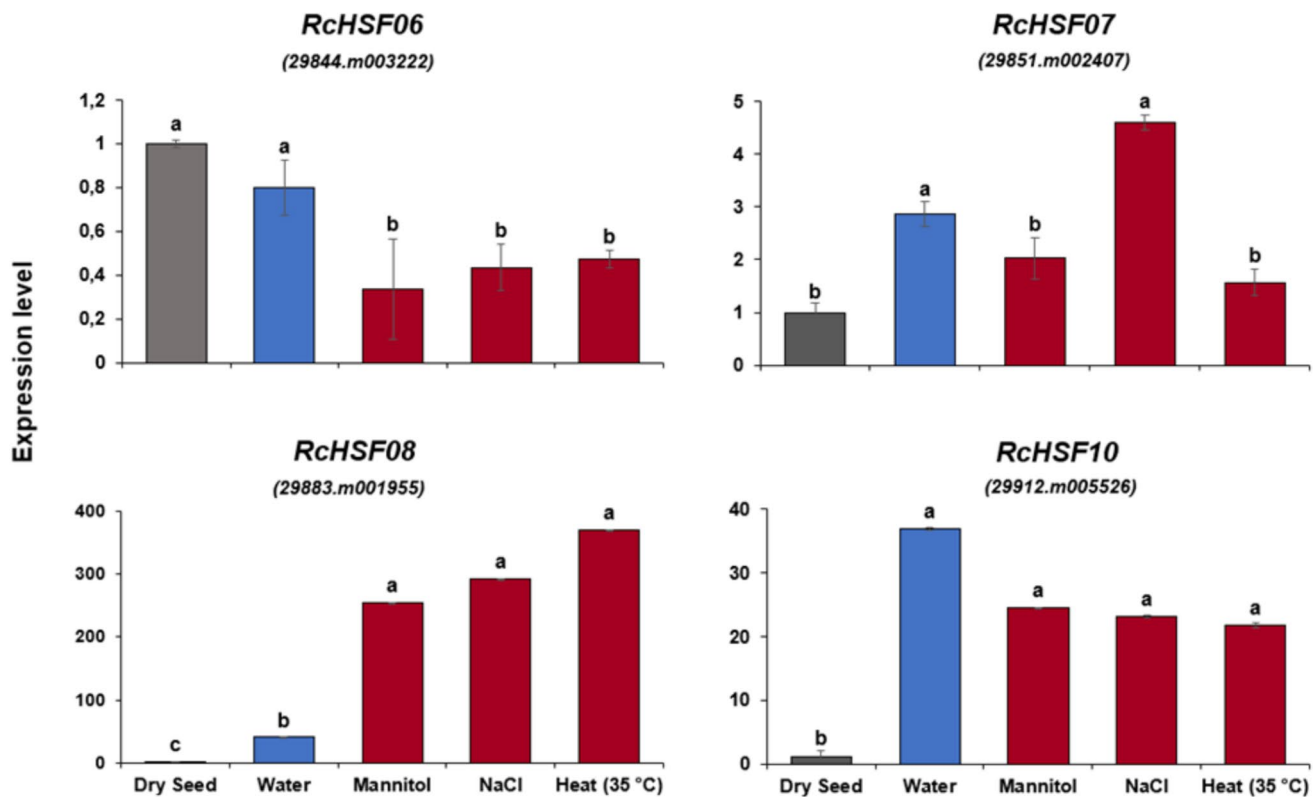


Fig. 9 Relative quantitative expression levels of *RcHSF* genes in dry seeds (control) and germination in water (control) and under water restriction by mannitol and by NaCl salinity (both at -0.34 MPa/25 °C) and heat (water/35 °C) stress treatments. The mean values were obtained from three biological and three technical replicates (error

bars indicate standard deviation). Bars of the same accession showing the same letter are not significantly different at a 5% probability level. Different letters indicate significant differences between data points as evaluated by the Scott-Knott test. The error bars represent the standard error

Together, these physicochemical traits offer preliminary clues into the specialization, regulatory mechanisms, and stress-responsive functions of *RcHSF* proteins (Alvarez-Ponce et al. 2018; Liu et al. 2006; Long et al. 2025).

In the conserved motif analysis, although motifs 1 and 3 were present in all proteins—with motif 1 corresponding to the DBD domain (PF00447), which is conserved across all heat shock factors—the arrangement of the remaining motifs suggests functional divergence and specialization among the different *RcHSF* classes. Class A contained four class-specific motifs (5, 7, 9, and 10), with motif 5 present in all proteins except *RcHSF03*, which instead carries motif 7. Class A also shared motif 4 with the only class C member, *RcHSF10*, reinforcing the greater structural—and potentially functional—similarity between these two classes. As noted previously, motif 6 was found exclusively in class B proteins, with the exception of *RcHSF01*. Furthermore, the predicted nuclear localization signals (NLS) with the highest scores—identified in group A proteins *RcHSF03*, *RcHSF05*, *RcHSF06*, *RcHSF08*, and *RcHSF13*—are predominantly located farther from motif 1 (PF00447) and, therefore, from the N-terminal region, with the exception of *RcHSF05*.

Moreover, motifs 1, 2, 3, 4, 5, and 9 are located closer to the N-terminal, while motifs 6, 7, 8, and 10 are nearer the C-terminal. Besides that, the gene structure analysis showed that most *RcHSF* genes have a conserved exon/intron structure, typically composed of two exons. This pattern is consistent across species such as *Hevea brasiliensis*, *P. trichocarpa*, *J. curcas*, and *O. sativa* (Li et al. 2019; Zhang et al. 2015, 2020b; Shamshad et al. 2023). Two exceptions in *R. communis*—*RcHSF04* (class A) and *RcHSF01* (class B)—possess three exons, suggesting possible evolutionary events such as alternative splicing or gene fragment insertions (Staiger & Brown 2013). This exception is also observed in orthologs such as *PtHsf-A2* in *P. trichocarpa* and several genes in *O. sativa* and *H. brasiliensis*, indicating it may be a conserved event among HSF families.

The presence of *RcHSFs* on seven of the ten *R. communis* chromosomes indicates a broad genomic dispersion, a feature that may act as an evolutionary safeguard. By having members of the gene family positioned on different chromosomes, the loss or disruption of a single genomic region is less likely to eliminate an entire functional group, thereby supporting long-term gene retention. This distribution also

enables diversified regulatory control, which is particularly relevant for plant development and responses to abiotic stresses. This interpretation is reinforced by the observation that orthologous gene pairs such as *RcHSF05/RcHSF09* and *RcHSF04/RcHSF13* occur on the same chromosome and consistently cluster together in phylogenetic analyses, similar to patterns reported in other angiosperms. Such organization may contribute to transcriptional robustness by buffering against local chromosomal mutations or cis-regulatory modifications. In addition, the presence of these orthologs on the same chromosome aligns with expectations from past whole-genome duplication (WGD) events that occurred during angiosperm diversification. For example, in *A. thaliana*, the *HSFA9* gene originated from *HSFA2* following ancestral duplication events (Liao et al. 2022), and in *R. communis*, their orthologs *RcHSF05* and *RcHSF09* are likewise collocated and phylogenetically grouped. A similar pattern is observed for *RcHSF04* and *RcHSF13* (orthologs of *HSFA4*), consistent with syntenic relationships described in *A. thaliana*, *P. trichocarpa*, *Solanum lycopersicum*, and *Zea mays* (Scharf et al. 2012; Liao et al. 2022).

HSF genes are described as being regulated by alternative splicing under heat stress in plants. In higher plants, including Arabidopsis, rice, tomato, and maize, alternative splicing of HSF genes has been observed. In Arabidopsis, temperature influences the production of different HSF isoforms with different functions. In tomato, wild varieties that accumulated *HSFA2-II* demonstrated heat acclimation, while *HSFA2-I* reduced heat tolerance. In rice, *HSFA2d* has one isoform that is active under heat stress and one that is inactive. In maize, a truncated alternative isoform of the subclass A2 gene *ZmHSF17*, which lacks transcriptional activity, accumulates under heat stress, while the active non-truncated isoform can enhance thermotolerance in Arabidopsis. These findings demonstrate the importance of alternative splicing in the regulation of HSF genes in plants, allowing for adjustment to stress responses (Ling et al. 2021; Zhang et al. 2022).

The distribution of cis-acting elements in *RcHSF* promoter regions suggests functional diversification in stress response regulation. Absciscic acid (ABA), a key hormone in plant stress responses—particularly in drought and temperature regulation (Aslam et al. 2022; Lata & Prasad 2011; Chen et al. 2020)—was associated with a higher number of responsive elements in group A genes. Likewise, the abundance of elements responsive to methyl jasmonate and salicylic acid reinforces the interaction between the signaling pathways of these hormones and the stress response networks in plants mediated by the *RcHSF* genes (Rivas-San Vicente & Plasencia 2011). These results suggest that the expression of *RcHSF* genes may be regulated by cis-elements associated with plant developmental processes, hormones, and stress responses. Furthermore, microRNAs

(miRNAs) regulate plant growth, hormonal signaling, and stress responses at both transcriptional and post-transcriptional levels (Zhang 2015; Kushawaha et al. 2021). miR167, for instance, regulates osmotic stress via IAR3-mediated auxin release, crucial for root development (Kinoshita et al. 2012). In this study, miR167 may target *RcHSF13*, which is expressed in flowers and during heat-stressed germination (Supplementary Table 7; Fig. 7). miR164, miR390, and miR395—known to be modulated under drought and heat stress (Hivrale et al. 2015)—were predicted to target *RcHSF04*, *RcHSF06*, and *RcHSF08*, respectively (Supplementary Table 7). *RcHSF06* and *RcHSF08*, although both from the A3 subgroup with similar structures and motifs (Fig. 3), are located on different chromosomes and show divergent expression patterns, implying functional specialization (Figs. 1, 7, and 8).

RNA-Seq analysis showed that *RcHSF* genes exhibit diverse expression profiles across all tissues and high expression in at least one developmental stage of *R. communis* (Fig. 7). Notably, *RcHSF05* (homologous to *OsHSF12*) is highly expressed during flower development, while *RcHSF08* (similar to *OsHSF01*) is seed-specific (Chauhan et al. 2011; Guo et al. 2016). Most HSFs showed low expression in early seed development (Endosperm II/III) but were upregulated during rapid oil accumulation (Endosperm V/VI), with *RcHSF11* (class A), *RcHSF18* (B2), *RcHSF01*, and *RcHSF17* (both B4) being prominent, suggesting key roles in late endosperm and cotyledon development. During germination, active expressions were observed for one gene in each class: *RcHSF08* (A3), *RcHSF12* (B3), and *RcHSF10* (C), indicating potential class crosstalk during the critical germination period of *R. communis*. In seedling leaves, group A genes predominated—particularly *RcHSF14* and *RcHSF15* (A1), *RcHSF04* (A4), and *RcHSF06* (A3). Flower-specific expression included members of A3 (*RcHSF02*, *RcHSF05*, *RcHSF09*), A4 (*RcHSF03*, *RcHSF13*), and B (*RcHSF07*—B1, *RcHSF16*—B2). *RcHSF05*'s expression profile is similar to *SIHSFA2* in tomato (Frank et al. 2009; Giorno et al. 2010). The genes regulated by miRNAs do not appear to have inhibited their activities in tissue specialization. Chromosomal localization supports this tissue-specific expression. For example, A3 subgroup genes—*RcHSF08* (germination), *RcHSF06* (leaves), *RcHSF05*, and *RcHSF09* (flowers)—are found on different chromosomes (2, 3, 1, and 1, respectively), providing a possible regulatory safeguard. Similar spatial separation is seen in B2 members *RcHSF16* (flower; chromosome 4) and *RcHSF18* (endosperm; chromosome 6). On the other hand, microarray data show that all *RcHSF* genes except *RcHSF04* were upregulated in at least one stress condition (only *RcHSF04*, *RcHSF09*, and *RcHSF12* not regulated by heat stress) (Fig. 8). Notably, most HSFs were induced in dry seeds and early imbibition, likely contributing to desiccation tolerance through

activation of LEAs and HSPs (Neto et al. 2020; Hundertmark & Hinch 2008). Besides that, six genes (*RcHSF01*, *RcHSF06*, *RcHSF07*, *RcHSF12*, *RcHSF15*, and *RcHSF17*) showed low expression in dry seeds but were activated during radicle protrusion and seedling development, suggesting stress-responsive activation.

Seedling establishment is a critical and vulnerable phase in plant development, as young plants are not yet fully formed. During this stage, abiotic stresses including drought, salinity, and heat can significantly compromise plant vigor and future productivity (Ghosh et al. 2025). Interestingly, castor (*R. communis*) exhibits a unique response to heat stress: seeds germinate more rapidly at 35 °C than at lower temperatures, such as 25 °C, and often produce vigorous seedlings under these conditions. Ribeiro et al. (2014a, b, 2015a, b) noted that while castor seeds achieve high germination percentages and faster germination rates at 35 °C compared to normal temperature conditions (25 °C), seedling establishment may still be compromised. Furthermore, this positive growth response to elevated temperature is genotype-dependent, highlighting the importance of genetic variation in thermal adaptation. The physiological results demonstrated that *R. communis* seeds maintained their germination capacity under abiotic stress conditions, particularly under heat stress (35 °C), where germination performance and early seedling development were only mildly affected. In contrast, drought caused by the water restriction effects of mannitol and by the combined salinity-water restriction stress of NaCl (both at 0.34 MPa) significantly impaired germination speed, uniformity, and initial seedling growth. These findings corroborate our previous study (Ribeiro et al. 2018) and are consistent with the anticipated role of the heat shock factors (HSFs) characterized in this study, supporting their importance in protecting cellular processes during such abiotic challenges—at elevated temperatures, misfolded proteins accumulate and bind to HSPs, releasing heat stress transcription factors (HSFs). These factors activate the heat stress response, which is essential for the induction of HSF and HSP genes and for increasing heat tolerance (Scharf et al. 2012). Also, the maintenance of germination and seedling vigor under heat stress suggests a functional activation of HSF-regulated pathways, which likely contribute to enhanced stress tolerance during the critical early stages of plant development and crop stand establishment. Thus, the physiological resilience observed under heat stress provides further evidence for the essential role of HSFs in the adaptation of *Ricinus communis* to environmental stresses.

Importantly, this temperature-responsive behavior in the *R. communis* physiological results is consistent with the strong enrichment of abscisic acid (ABA)–responsive cis-elements identified in *RcHSF* promoter regions, given that ABA accumulation is known to be triggered by temperature

fluctuations. The predominance of ABA-related motifs therefore supports the hypothesis that *RcHSFs* contribute to the thermotolerance and enhanced early development observed at higher temperatures. In plants, abiotic stresses frequently induce ABA accumulation, which regulates key physiological processes and promotes the synthesis of protective proteins such as heat shock proteins (HSPs), whose expression is controlled by heat shock factors (HSFs) (Sezgin Muslu et al. 2021). Additionally, anaerobic response elements (AREs) may participate in broader abiotic stress regulatory networks, and plant responses to anaerobic conditions can also involve ABA-mediated signaling. Nevertheless, the interaction between hormone-driven pathways and ARE-mediated transcriptional regulation remains insufficiently understood and represents an important knowledge gap in plant stress biology (Sadeghnezhad et al. 2014). Furthermore, internal oxygen levels in plants depend on atmospheric diffusion, photosynthetic oxygen production, and respiratory consumption. Even under normal environmental conditions, hypoxic pockets can form when gas diffusion is restricted or respiration rates are high, and such hypoxic niches have been reported in growing tissues like the shoot apex, lateral root primordia, developing fruits, and hormone-induced calli. Moreover, recent observations show cyclic hypoxia in young *Arabidopsis* leaves during day/night cycles, indicating that hypoxic metabolism can be activated even in well-aerated plants (van Veen et al. 2025). Therefore, the presence of anaerobic induction elements in the promoter regions of *RcHSF08* and *RcHSF10* may be related to their activity during the seedling stage in the qPCR analysis. Thus, the presence of an abscisic acid–responsive element may mediate *RcHSF10* activity under anaerobic conditions, whereas the methyl jasmonate–responsive element in the *RcHSF08* promoter may induce this gene more specifically in stress situations, consistent with the qPCR results.

The HSF class A is crucial in the response to stresses such as water restriction (drought) by mannitol and NaCl, and heat, regulating the expression of DREB2A and hormonal signaling in crops like *Z. mays*, *T. aestivum*, and *A. thaliana* (Li et al. 2010; Guo et al. 2020). Therefore, RNA-Seq and qPCR analyses confirmed that *RcHSF08* is upregulated during germination and under abiotic stress in seedlings, respectively. Orthologous genes of *RcHSF08* in *Arabidopsis* and tomato also respond to abiotic stress (Mesihovic et al. 2022; Zang et al. 2019; Liu et al. 2013), suggesting its role in heat, saline, and drought tolerance in *Ricinus communis*. Conversely, *RcHSF06* showed leaf-specific expression and lower stress-induced expression during germination, indicating potential functional divergence despite their structural similarities (Figs. 7 and 9). The similarity structure between *RcHSF06* and *RcHSF08* is consistent with the fact that both genes are orthologs of *AtHSF07*, suggesting that they may have originated from a

relatively recent duplication event followed by functional divergence. Despite this, the main distinction between the two genes lies in their promoter composition—in addition to possibly being targeted by different miRNAs (Fig. 5a; Supplementary Table 7). *RcHSF06* contains cis-elements associated with abscisic acid (ABA) signaling, gibberellins, and circadian regulation, whereas *RcHSF08* carries methyl jasmonate-responsive elements, anaerobic induction motifs, and stress and defense-related elements. These differences indicate that *RcHSF08* is likely integrated into early stress-response pathways—including jasmonate and hypoxia signaling—while *RcHSF06* may participate in later or more hormonally regulated phases of the response. In addition, RNA-Seq and microarray datasets support this interpretation: *RcHSF08* is strongly induced during early developmental stages, whereas *RcHSF06* shows a delayed or repressed expression pattern, suggesting a more downstream or regulatory role. Together, these promoter signatures and expression profiles provide plausible hypotheses for their opposing stress-response behaviors. On the other hand, classes AtHSF B and AtHSF C do not directly activate the transcription of heat shock genes but may act as co-regulators or repressors, increasing thermotolerance (Li et al. 2010; Kotak et al. 2007; von Koskull-Döring et al. 2007). *RcHSF07*, a class B gene, was significantly regulated under saline stress and water control. This suggests that its lower expression in heat stress may reflect a response to the positive regulation of other HSF genes under these stresses. On the other hand, RNA-Seq data suggest that *RcHSF07* has limited involvement in germination but shows high expression specifically in flowers. In contrast, *RcHSF10*, a class C gene, showed expression during germination and under stress, confirmed by RNA-Seq and microarray data (early imbibition at 35 °C), similar to other studies with *HSF* genes of *O. sativa*, *L. perenne*, *F. arundinacea*, and *P. vulgaris* (Schmidt et al. 2012; Zhuang et al. 2018; Sun et al. 2022; Guo et al. 2023). This behavioral pattern indicates more similarity between class C and class A in *Ricinus communis*, suggesting that class C may also play a role in positive regulation among *HSF*, aiding the plant in combating abiotic stresses through the activation of heat shock proteins (HSPs).

Conclusions

This study identified and characterized 18 heat shock transcription factor (*RcHSF*) genes in *R. communis*, analyzing their structure, properties, and promoter elements. It also examined their chromosomal locations and phylogenetic relationships with other species. The *RcHSF* genes showed diverse expression patterns across tissues, developmental stages, and under various abiotic stresses, suggesting their

role in stress responses during seed germination and initial seedling development. The superior germination and seedling vigor at 35 °C may be linked to *RcHSF* activity, consistent with the predominance of ABA-responsive promoter elements that support their role in temperature-dependent regulation and thermotolerance. *RcHSFs* aid not only in heat stress response but also in osmotic water restriction by mannitol and NaCl and in low-temperature stress. Their expression across tissues and stages, even without stress, implies their role in both stress prevention and healthy plant development. These findings highlight the importance of *RcHSF* genes in stress tolerance mechanisms during seed development, germination, early seedling growth, and crop stand establishment. Functional characterization of HSF genes in *Arabidopsis thaliana* can provide good candidates for *Ricinus communis* breeding.

Supplementary Information The online version contains supplementary material available at <https://doi.org/10.1007/s11105-026-01675-w>.

Acknowledgements We acknowledge the Laboratory of Biochemistry, Biotechnology and Bioproducts (LBBB) of the Federal University of Bahia (UFBA), coordinated by Prof. Luzimar G. Fernandez, where a significant part of the study was conducted until it was destroyed by occurrence of a fire in June 2022.

Author Contributions The experimental plan was designed by IDS, JLOJ, VGN, PRR, and RDC. Phylogenetic and in silico analyses were performed by IDS, JLOJ, PHOWS, and VGN. Samples were collected by JLOJ, IDS, and PHOWS. Expression data were performed and analyzed by IDS, JLOJ, PHOWS, PRR and VGN. The manuscript was written by IDS, JLOJ, VGN, RDC, and reviewed by all authors.

Funding The Article Processing Charge (APC) for the publication of this research was funded by the Coordenação de Aperfeiçoamento de Pessoal de Nível Superior - Brasil (CAPES) (ROR identifier: 00x0ma614). Bahia State Research Support Foundation (FAPESB-Brazil), Trainee fellowship, Trainee fellowship, Postgraduate Program in Biotechnology at the Federal University of Bahia (PPGBiotec/UFBA), Financial support, National Council for Scientific and Technological Development (CNPq), 314835/2020-2; 442783/2023-0

Data Availability All accessions and transcriptomic data presented in this work can be found in the supplementary data retrieved from public databases detailed in the Materials and Methods section.

Declarations

Competing interests The authors declare no competing interests.

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References

- Alvarez-Ponce D, Ruiz-González MX, Vera-Sirera F, Feyertag F, Perez-Amador MA, Fares MA (2018) Arabidopsis heat stress-induced proteins are enriched in electrostatically charged amino acids and intrinsically disordered regions. *Int J Mol Sci* 19(8):2276. <https://doi.org/10.3390/ijms19082276>
- Andrási N, Pettkó-Szandtner A, Szabados L (2021) Diversity of plant heat shock factors: regulation, interactions, and functions. *J Exp Bot* 72:1558–1575. <https://doi.org/10.1093/jxb/eraa576>
- Aslam M, Fakher B, Ashraf MA, Cheng Y, Wang B, Qin Y (2022) Plant low-temperature stress: signaling and response. *Agronomy* 12(3):702. <https://doi.org/10.3390/agronomy12030702>
- Baniwal SK, Bharti K, Chan KY, Fauth M, Ganguli A, Kotak S, Mishra SK, Nover L, Port M, Scharf KD, Tripp J, Weber C, Zielinski D, von Koskull-Döring P (2004) Heat stress response in plants: a complex game with chaperones and more than twenty heat stress transcription factors. *J Biosci* 29(4):471–487. <https://doi.org/10.1007/BF02712120>
- Bergmann JC, Tupinambá DD, Costa OYA, Almeida JRM, Barreto CC, Quirino BF (2013) Biodiesel production in Brazil and alternative biomass feedstocks. *Renew Sustain Energy Rev* 21:411–420. <https://doi.org/10.1016/j.rser.2012.12.058>
- Bibin C, Gopinath S, Aravindraj R, Devaraj A, Gokula Krishnan S, Jeevaanathan JKS (2020) The production of biodiesel from castor oil as a potential feedstock and its usage in compression ignition engine: a comprehensive review. *Mater Today Proc* 33:84–92. <https://doi.org/10.1016/j.matpr.2020.03.205>
- Brown AP, Kroon JTM, Swarbreck D, Febrer M, Larson TR, Graham IA, Caccamo M, Slabas AR (2012) Tissue-specific whole transcriptome sequencing in castor, directed at understanding triacylglycerol lipid biosynthetic pathways. *PLoS ONE* 7(2):e30100. <https://doi.org/10.1371/journal.pone.0030100>
- Busch W, Wunderlich M, Schöffl F (2005) Identification of novel heat shock factor-dependent genes and biochemical pathways in *Arabidopsis thaliana*. *Plant J* 41:1–14. <https://doi.org/10.1111/j.1365-3113.2004.02272.x>
- Cassol D, Cruz FP, Espindola K, Mangeon A, Müller C, Loureiro ME, Corrêa RL, Sachetto-Martins G (2016) Identification of reference genes for quantitative RT-PCR analysis of microRNAs and mRNAs in castor bean (*Ricinus communis* L.) under drought stress. *Plant Physiol Biochem* 106:101–107. <https://doi.org/10.1016/j.plaphy.2016.02.024>
- 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. *Mol Genet Genomics* 286:171–187. <https://doi.org/10.1007/s00438-011-0638-8>
- Chen K, Li GJ, Bressan RA, Song CP, Zhu JK, Zhao Y (2020) Abscissic acid dynamics, signaling, and functions in plants. *J Integr Plant Biol* 62:25–54. <https://doi.org/10.1111/jipb.12899>
- Chung M, Bruno VM, Rasko DA, Cuomo CA, Muñoz JF, Livny J, Shetty AC, Mahurkar A, Dunning Hotopp JC (2021) Best practices on the differential expression analysis of multi-species RNA-seq. *Genome Biol* 22:121. <https://doi.org/10.1186/s13059-021-02337-8>
- Costa-Silva J, Domingues D, Lopes FM (2017) RNA-seq differential expression analysis: an extended review and a software tool. *PLoS One* 12(12):e0190152. <https://doi.org/10.1371/journal.pone.0190152>
- Czarnecka-Verner E, Yuan C-X, Scharf K-D, Englich G, Gurley WB (2000) Plants contain a novel multi-member class of heat shock factors without transcriptional activator potential. *Plant Mol Biol* 43:459–471. <https://doi.org/10.1023/a:1006448607740>
- Döring P, Treuter E, Kistner C, Lyck R, Chen A, Nover L (2000) The role of AHA motifs in the activator function of tomato heat stress transcription factors HSFA1 and HSFA2. *Plant Cell* 12:265–278. <https://doi.org/10.1105/tpc.12.2.265>
- Dossa K, Diouf D, Cissé N (2016) Genome-wide investigation of *HSF* genes in sesame reveals their segmental duplication expansion and their active role in drought stress response. *Front Plant Sci* 7:1522. <https://doi.org/10.3389/fpls.2016.01522>
- Esmailian Y, Babaeian M, Caballero-Calvo A (2023) Optimization of castor bean (*Ricinus communis* L.) cultivation methods using biostimulants in an arid climate. *Euro Mediterr J Environ Integr* 8:823–834. <https://doi.org/10.1007/s41207-023-00406-x>
- Fragkostefanakis S, Simm S, El-Shershaby A, Hu Y, Bublak D, Mesihovic A, Darm K, Mishra SK, Tschiersch B, Theres K, Scharf C, Schleiff E, Scharf KD (2019) The repressor and co-activator HSFB1 regulates the major heat stress transcription factors in tomato. *Plant Cell Environ* 42:874–890. <https://doi.org/10.1111/pce.13434>
- Frank G, Pressman E, Ophir R, Althan L, Shaked R, Freedman M, Shen S, Firon N (2009) Transcriptional profiling of maturing tomato (*Solanum lycopersicum* L.) microspores reveals the involvement of heat shock proteins, ROS scavengers, hormones, and sugars in the heat stress response. *J Exp Bot* 60:3891–3908. <https://doi.org/10.1093/jxb/erp234>
- Ghosh T, Atta K, Mondal S, Bandyopadhyay S, Singh AP, Jha UC, Kumar K, Gujjar RS (2025) Hormonal signaling at seed germination and seedling stage of plants under salinity stress. *Plant Growth Regul* 105:583–600. <https://doi.org/10.1007/s10725-025-01305-7>
- Giorno F, Wolters-Arts M, Grillo S, Scharf KD, Vriezen WH, Mariani C (2010) Developmental and heat stress-regulated expression of HSFA2 and small heat shock proteins in tomato anthers. *J Exp Bot* 61:453–462. <https://doi.org/10.1093/jxb/erp316>
- 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. *J Genet Genomics* 35:105–118. [https://doi.org/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. *Front Plant Sci* 7:114. <https://doi.org/10.3389/fpls.2016.00114>
- Guo XL, Yuan SN, Zhang HN, Zhang YY, Zhang YJ, Wang GY, Li YQ, Li GL (2020) Heat-response patterns of the heat shock transcription factor family in advanced development stages of wheat (*Triticum aestivum* L.) and thermotolerance-regulation by TaHSFA2-10. *BMC Plant Biol* 20:364. <https://doi.org/10.1186/s12870-020-02555-5>
- Guo Q, Wei R, Xu M, Yao W, Jiang J, Ma X, Qu G, Jiang T (2023) Genome-wide analysis of HSF family and overexpression of PsnHSF21 confers salt tolerance in *Populus simonii* × *P. nigra*. *Front Plant Sci* 14:1160102. <https://doi.org/10.3389/fpls.2023.1160102>
- Hivrale V, Zheng Y, Puli COR, Jagadeeswaran G, Gowdu K, Kakani VG, Barakat A, Sunkar R (2015) Characterization of drought- and heat-responsive microRNAs in switchgrass. *Plant Sci* 242:214–223. <https://doi.org/10.1016/j.plantsci.2015.07.018>
- 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*. *Front Plant Sci* 6:736. <https://doi.org/10.3389/fpls.2015.00736>
- Huang XY, Tao P, Li BY, Wang WH, Yue ZC, Lei JL, Zhong XM (2015) Genome-wide identification, classification, and analysis of heat shock transcription factor family in Chinese cabbage

- (*Brassica rapa pekinensis*). Genet Mol Res 14:2189–2204. <https://doi.org/10.4238/2015.March.27.5>
- Hundertmark M, Hinch DK (2008) LEA (late embryogenesis abundant) proteins and their encoding genes in *Arabidopsis thaliana*. BMC Genomics. <https://doi.org/10.1186/1471-2164-9-118>
- Joosen RVL, Kodde J, Willems LAJ, Ligterink W, van der Plas LHW, Hilhorst HWM (2010) Germinator: a software package for high-throughput scoring and curve fitting of *Arabidopsis* seed germination. Plant J 62(1):148–159. <https://doi.org/10.1111/j.1365-313X.2009.04116.x>
- Kermode AR (2008) Regulatory mechanisms involved in the transition from seed development to germination. Crit Rev Plant Sci 9(2):155–195. <https://doi.org/10.1080/07352689009382286>
- Kinoshita N, Wang H, Kasahara H, Liu J, MacPherson C, Machida Y, Kamiya Y, Hannah MA, Chuah NH (2012) IAA-ala resistant3, an evolutionarily conserved target of miR167, mediates *Arabidopsis* root architecture changes during high osmotic stress. Plant Cell 24:3590–3602. <https://doi.org/10.1105/tpc.112.097006>
- Kotak S, Vierling E, Bäumlein H, Von Koskull-Döring P (2007) A novel transcriptional cascade regulating expression of heat stress proteins during seed development of *Arabidopsis*. Plant Cell 19:182–195. <https://doi.org/10.1105/tpc.106.048165>
- Kumar A, Sharma S (2011) Potential non-edible oil resources as biodiesel feedstock: an Indian perspective. Renew Sustain Energy Rev 15:1791–1800. <https://doi.org/10.1016/j.rser.2010.11.020>
- Kushawaha AK, Khan A, Sopory SK, Sanan-Mishra N (2021) Priming by high temperature stress induces MicroRNA regulated heat shock modules indicating their involvement in thermopriming response in rice. Life 11:291. <https://doi.org/10.3390/life11040291>
- Lata C, Prasad M (2011) Role of DREBs in regulation of abiotic stress responses in plants. J Exp Bot 62:4731–4748. <https://doi.org/10.1093/jxb/err210>
- Li M, Berendzen KW, Schöffl F (2010) Promoter specificity and interactions between early and late *Arabidopsis* heat shock factors. Plant Mol Biol 73:559–567. <https://doi.org/10.1007/s11103-010-9643-2>
- Li Z, Zhang L, Wang A, Xu X, Li J (2013) Ectopic overexpression of SIHSFA3, a heat stress transcription factor from tomato, confers increased thermotolerance and salt hypersensitivity in germination in transgenic *Arabidopsis*. PLoS ONE 8(1):e54880. <https://doi.org/10.1371/journal.pone.0054880>
- Li Y, Yu W, Chen Y, Yang S, Wu S, Chao J, Wang X, Tian WM (2019) Genome-wide identification and characterization of heat-shock transcription factors in rubber tree. Forests 10:1157. <https://doi.org/10.3390/f10121157>
- Liao W, Tang X, Li J et al (2024) Genome wide investigation of Hsf gene family in *Phoebe bournei*: identification, evolution, and expression after abiotic stresses. J For Res 35:11. <https://doi.org/10.1007/s11676-023-01661-y>
- Liao Y, Liu Z, Gichira AW, Yang M, Mbichi RW, Meng L, Wan T (2022) Deep evaluation of the evolutionary history of the heat shock factor (HSF) gene family and its expansion pattern in seed plants. PeerJ 10:e13603. <https://doi.org/10.7717/peerj.13603>
- Ling Y, Mahfouz MM, Zhou S (2021) Pre-mRNA alternative splicing as a modulator for heat stress response in plants. Trends Plant Sci 26(11):1153–1170. <https://doi.org/10.1016/j.tplants.2021.07.008>
- Liu HC, Chang YY (2013) Common and distinct functions of *Arabidopsis* class A1 and A2 heat shock factors in diverse abiotic stress responses and development. Plant Physiol 163:276–290. <https://doi.org/10.1104/pp.113.221168>
- Liu J, Perumal NB, Oldfield CJ, Su EW, Uversky VN, Dunker AK (2006) Intrinsic disorder in transcription factors. Biochemistry 45(22):6873–6888. <https://doi.org/10.1021/bi0602718>
- Long W, Zhao L, Yang H, Yang X, Bai Y, Xue X, Wang D, Han S (2025) Genome-wide characterization of wholly disordered proteins in *Arabidopsis*. Int J Mol Sci 26(3):1117. <https://doi.org/10.3390/ijms26031117>
- Mesihovic A, Ullrich S, Rosenkranz RRE, Gebhardt P, Bublak D, Eich H, Weber D, Berberich T, Scharf KD, Schleiff E, Fragkostefanakis S (2022) HSFA7 coordinates the transition from mild to strong heat stress response by controlling the activity of the master regulator HSFA1a in tomato. Cell Rep 38:110224. <https://doi.org/10.1016/j.celrep.2021.110224>
- Miceli A, Moncada A, Sabatino L, Vetrano F (2019) Effect of gibberellic acid on growth, yield, and quality of leaf lettuce and rocket grown in a floating system. Agronomy 9:382. <https://doi.org/10.3390/agronomy9070382>
- Neto VG, Ribeiro PR, Del-Bem LE, Bernal DT, Cunha Lima ST, Ligterink W, Fernandez LG, de Castro RD (2018) Characterization of the superoxide dismutase gene family in seeds of two *Ricinus communis* L. genotypes submitted to germination under water restriction conditions. Environ Exp Bot 155:453–463. <https://doi.org/10.1016/j.envexpbot.2018.08.001>
- Neto VG, Barbosa RR, Carosio MGA, Ferreira AG, Fernandez LG, de Castro RD, Ligterink W, Hilhorst H, Ribeiro PR (2020) Sequence analysis of *Ricinus communis* small heat-shock protein (sHSP) subfamily and its role in abiotic stress responses. Ind Crops Prod 152:112541. <https://doi.org/10.1016/j.indcrop.2020.112541>
- Nishad A, Nandi AK (2021) Recent advances in plant thermomemory. Plant Cell Rep 40:19–27. <https://doi.org/10.1007/s00299-020-02604-1>
- Nishizawa A, Yabuta Y, Shigeoka S (2008) Galactinol and raffinose constitute a novel function to protect plants from oxidative damage. Plant Physiol 147:1251–1263. <https://doi.org/10.1104/pp.108.122465>
- Nonogaki H, Bassel GW, Bewley D (2010) Germination - still a mystery. Plant Sci 179(6):574–581. <https://doi.org/10.1016/j.plantsci.2010.02.010>
- Nover L, Bharti K, Döring P, Mishra SK, Ganguli A, Scharf KD (2001) *Arabidopsis* and the heat stress transcription factor world: how many heat stress transcription factors do we need? Cell Stress Chaperones 6:177–189. [https://doi.org/10.1379/1466-1268\(2001\)006%3c0177:aathst%3e2.0.co;2](https://doi.org/10.1379/1466-1268(2001)006%3c0177:aathst%3e2.0.co;2)
- Ogunniyi DS (2006) Castor oil: a vital industrial raw material. Biore-sour Technol 97:1086–1091. <https://doi.org/10.1016/j.biortech.2005.03.028>
- Pan T, Fan X, Sun H (2023) Juvenile phase: an important phase of the life cycle in plants. Ornamental Plant Res 3:18. <https://doi.org/10.48130/OPR-2023-0018>
- Peng Y, Yang J, Li X, Zhang Y (2021) Annual review of plant biology salicylic acid: biosynthesis and signaling. Annu Rev Plant Biol 72:761–791. <https://doi.org/10.1146/annurev-arpla-nt-081320-09285>
- Ribeiro PR (2021) Translating genomic studies into agronomic applications: where do we stand? Agron J 113:5635–5637. <https://doi.org/10.1002/agj2.20808>
- Ribeiro PR, Dekkers BJW, Fernandez LG, De Castro RD, Ligterink W, Hilhorst HWM (2014) Identification of reference genes for gene expression studies during seed germination and seedling establishment in *Ricinus communis* L. Seed Sci Res 24:341–352. <https://doi.org/10.1017/S0960258514000294>
- Ribeiro PR, Fernandez LG, de Castro RD, Ligterink W, Hilhorst HWM (2014) Physiological and biochemical responses of *Ricinus communis* seedlings to different temperatures: a metabolomics approach. BMC Plant Biol 14:223. <https://doi.org/10.1186/s12870-014-0223-5>
- Ribeiro PR, Willems LAJ, Mutimawurugo MC, Fernandez LG, de Castro RD, Ligterink W, Hilhorst HWM (2015) Metabolite profiling of *Ricinus communis* germination at different temperatures

- provides new insights into thermo-mediated requirements for successful seedling establishment. *Plant Sci* 239:180–191. <https://doi.org/10.1016/j.plantsci.2015.08.002>
- Ribeiro PR, Zanotti RF, Deflers C, Fernandez LG, de Castro RD, Ligerink W, Hilhorst HWM (2015b) Effect of temperature on biomass allocation in seedlings of two contrasting genotypes of the oilseed crop *Ricinus communis*. *J Plant Physiol* 185:31–39
- Ribeiro PR, Willems LAJ, Silva AT, Fernandez LG, de Castro RD, Bucher J, Snoek BL, Hilhorst HWM, Ligerink W (2018) Transcriptome profiling of *Ricinus communis* L. provides new insights underlying the mechanisms towards thermotolerance during seed imbibition and germination. *Ind Crops Prod* 126:380–393
- Rivas-San Vicente M, Plasencia J (2011) Salicylic acid beyond defence: its role in plant growth and development. *J Exp Bot* 62:3321–3338. <https://doi.org/10.1093/jxb/err031>
- Sadeghnezhad E, Askari H, Soltani S, Honarvar F (2014) Identification and distribution of anaerobic responsive elements (AREs) in genes functional categorization of *Arabidopsis thaliana*. *J Appl Biotechnol Rep* 1(4):135–141
- Sakurai H, Enoki Y (2010) Novel aspects of heat shock factors: DNA recognition, chromatin modulation and gene expression. *FEBS J* 277:4140–4149. <https://doi.org/10.1111/j.1742-4658.2010.07829.x>
- Scharf KD, Berberich T, Ebersberger I, Nover L (2012) The plant heat stress transcription factor (HSF) family: structure, function and evolution. *Biochim Biophys Acta* 1819:104–119. <https://doi.org/10.1016/j.bbagr.2011.10.002>
- Schmidt R, Schippers JHM, Welker A, Mieulet D, Guiderdoni E, Mueller-Roeber B (2012) Transcription factor oshsf1b regulates salt tolerance and development in *Oryza sativa* ssp. *japonica*. *AoB Plants* 12:pls011
- Severino LS, Auld DL, Baldanzi M, Cândido MJD, Chen G, Crosby W, Tan D, He X, Lakshmmamma P, Lavanya C, Machado OLT, Mielke T, Milani M, Miller TD, Morris JB, Morse SA, Navas AA, Soares DJ, Sofiatti V, Wang ML, Zanotto MD, Zieler H (2012) A review on the challenges for increased production of castor. *Agron J* 104:853–880. <https://doi.org/10.2134/agronj2011.0210>
- Sezgin Muslu A, Kadioğlu A (2021) Role of abscisic acid, osmolytes and heat shock factors in high temperature thermotolerance of *Heliotropium thermophilum*. *Physiol Mol Biol Plants* 27(4):861–871. <https://doi.org/10.1007/s12298-021-00975-7>
- Shamshad A, Rashid M, Zaman Q (2023) In-silico analysis of heat shock transcription factor (OsHSF) gene family in rice (*Oryza sativa* L.). *BMC Plant Biol* 23:395. <https://doi.org/10.1186/s12870-023-04399-1>
- Song C, Chung WS, Lim CO (2016) Overexpression of heat shock factor gene HSFA3 increases galactinol levels and oxidative stress tolerance in *Arabidopsis*. *Mol Cells* 39:477–483. <https://doi.org/10.14348/molcells.2016.0027>
- Staiger D, Brown JWS (2013) Alternative splicing at the intersection of biological timing, development, and stress responses. *Plant Cell* 25:3640–3656. <https://doi.org/10.1105/tpc.113.113803>
- Sun T, Wang W, Hu X, Fang Z, Wang Y, Xiang L, Chan Z (2022) Genome-wide identification of heat shock transcription factor families in perennial ryegrass highlights the role of LpHSFC2b in heat stress response. *Physiol Plant* 174:e13828. <https://doi.org/10.1111/pp1.13828>
- Tripp J, Mishra SK, Scharf KD (2009) Functional dissection of the cytosolic chaperone network in tomato mesophyll protoplasts. *Plant Cell Environ* 32:123–133. <https://doi.org/10.1111/j.1365-3040.2008.01902.x>
- van Veen H, Triozzi PM, Loreti E (2025) Metabolic strategies in hypoxic plants. *Plant Physiol* 197(1):kia564. <https://doi.org/10.1093/plphys/kiae564>
- von Koskull-Döring P, Scharf KD, Nover L (2007) The diversity of plant heat stress transcription factors. *Trends Plant Sci* 12(10):452–457. <https://doi.org/10.1016/j.tplants.2007.08.014>
- Wang X, Shi X, Chen S, Ma C, Xu S (2018) Evolutionary origin, gradual accumulation and functional divergence of heat shock factor gene family with plant evolution. *Front Plant Sci*. <https://doi.org/10.3389/fpls.2018.00071>
- Wu Z, Liang J, Wang C, Zhao X, Zhong X, Cao X, Li G, He J, Yi M (2018) Overexpression of lily *HSFA3* s in *Arabidopsis* confers increased thermotolerance and salt sensitivity via alterations in proline catabolism. *J Exp Bot* 69:2005–2021. <https://doi.org/10.1093/jxb/ery035>
- Xu W, Cui Q, Li F, Liu A (2013) Transcriptome-wide identification and characterization of MicroRNAs from castor bean (*Ricinus communis* L.). *PLoS One* 8(7):e69995. <https://doi.org/10.1371/journal.pone.0069995>
- Yu A, Li F, Xu W, Wang Z, Sun C, Han B, Wang Y, Wang B, Cheng X, Liu A (2019a) Application of a high-resolution genetic map for chromosome-scale genome assembly and fine QTLs mapping of seed size and weight traits in castor bean. *Sci Rep* 9:11950. <https://doi.org/10.1038/s41598-019-48492-8>
- Yu X, Zhang W, Zhang Y, Zhang X, Lang D, Zhang X (2019b) The roles of methyl jasmonate to stress in plants. *Funct Plant Biol* 46:197–212. <https://doi.org/10.1071/FP18106>
- Zang D, Wang J, Zhang X, Liu Z, Wang Y (2019) *Arabidopsis* heat shock transcription factor HSFA7b positively mediates salt stress tolerance by binding to an E-box-like motif to regulate gene expression. *J Exp Bot* 70:5355–5374. <https://doi.org/10.1093/jxb/erz261>
- Zhang B (2015) MicroRNA: a new target for improving plant tolerance to abiotic stress. *J Exp Bot* 66(7):1749–1761. <https://doi.org/10.1093/jxb/erv013>
- Zhang J, Liu B, Li J, Zhang L, Wang Y, Zheng H, Lu M, Chen J (2015) HSF and HSP gene families in populus: genome-wide identification, organization and correlated expression during development and in stress responses. *BMC Genomics* 16:181. <https://doi.org/10.1186/s12864-015-1398-3>
- Zhang H, Li G, Hu D, Zhang Y, Zhang Y, Shao H, Zhao L, Yang R, Guo X (2020a) Functional characterization of maize heat shock transcription factor gene ZmHSF01 in thermotolerance. *PeerJ* 8:e8926. <https://doi.org/10.7717/peerj.8926>
- Zhang L, Chen W, Shi B (2020b) Genome-wide analysis and expression profiling of the heat shock transcription factor gene family in Physic Nut (*Jatropha curcas* L.). *PeerJ* 8:e8467. <https://doi.org/10.7717/peerj.8467>
- Zhang Q, Geng J, Du Y, Zhao Q, Zhang W, Fang Q, Yin Z, Li J, Yuan X, Fan Y, Cheng X, Du J (2022) Heat shock transcription factor (HSF) gene family in common bean (*Phaseolus vulgaris*): genome-wide identification, phylogeny, evolutionary expansion and expression analyses at the sprout stage under abiotic stress. *BMC Plant Biol* 22:33. <https://doi.org/10.1186/s12870-021-03417-4>
- Zhao J, Lu Z, Wang L, Jin B (2021) Plant responses to heat stress: physiology, transcription, noncoding RNAs, and epigenetics. *Int J Mol Sci* 22:1–14. <https://doi.org/10.3390/ijms22010117>
- Zhu X, Wang Y, Liu Y, Zhou W, Yan B, Yang J, Shen Y (2018) Overexpression of BcHSFA1 transcription factor from *Brassica campestris* improved heat tolerance of transgenic tobacco. *PLoS One* 13(11):e0207277. <https://doi.org/10.1371/journal.pone.0207277>
- Zhuang L, Cao W, Wang J, Yu J, Yang Z, Huang B (2018) Characterization and functional analysis of FaHSFC1b from *Festuca arundinacea* conferring heat tolerance in *Arabidopsis*. *Int J Mol Sci* 19:2702. <https://doi.org/10.3390/ijms19092702>