

Article

FvHsfB1a Gene Improves Thermotolerance in Transgenic *Arabidopsis*

Qian Cao ^{1,†}, Tingting Mao ^{1,†}, Kebang Yang ², Hanxiu Xie ¹, Shan Li ¹ and Hao Xue ^{1,*} 

¹ School of Horticulture, Anhui Agricultural University, Hefei 230036, China; 16634254430@163.com (Q.C.); 15156002326@163.com (T.M.); 19156213306@163.com (H.X.); 19960653711@163.com (S.L.)

² Shanghai Agricultural Technology Extension Service Center, Shanghai 201103, China; 15721351226@163.com

* Correspondence: 15802455673@163.com

† These authors contributed equally to this work.

Abstract

Heat stress transcription factor (Hsf) families play important roles in abiotic stress responses. However, previous studies reported that *HsfBs* genes may play diverse roles in response to heat stress. Here, we conducted functional analysis on a woodland strawberry Class B Hsf gene, *FvHsfB1a*, to improve thermotolerance. The structure of *FvHsfB1a* contains a typical Hsf domain for DNA binding at the N-terminus, and *FvHsfB1a* belongs to the B1 family of Hsfs. The *FvHsfB1a* protein was localized in the nucleus. The *FvHsfB1a* gene was expressed in various strawberry tissues and highly induced by heat treatment. Under heat stress conditions, ectopic expression of *FvHsfB1a* in *Arabidopsis* improves thermotolerance, with higher germination and survival rates, a longer primary root length, higher proline and chlorophyll contents, lower malonaldehyde (MDA) and O^{2−} contents, better enzyme activities, and greater expression of heat-responsive and stress-related genes compared to WT. *FvWRKY75* activates the promoter of the *FvHsfB1a* gene through recognizing the W-box element. Similarly, *FvWRKY75*-OE lines also displayed a heat-tolerant phenotype, exhibiting more proline and chlorophyll contents, lower MDA and O^{2−} contents, and higher enzyme activities under heat stress. Taken together, our study indicates that *FvHsfB1a* is a positive regulator of heat stress.

Keywords: strawberry; *FvHsfB1a*; *FvWRKY75*; heat stress; *Arabidopsis thaliana*



Academic Editor: Vincent G.M. Bus

Received: 27 June 2025

Revised: 29 July 2025

Accepted: 31 July 2025

Published: 2 August 2025

Citation: Cao, Q.; Mao, T.; Yang, K.; Xie, H.; Li, S.; Xue, H. *FvHsfB1a* Gene Improves Thermotolerance in Transgenic *Arabidopsis*. *Plants* **2025**, *14*, 2392. <https://doi.org/10.3390/plants14152392>

Copyright: © 2025 by the authors. Licensee MDPI, Basel, Switzerland. This article is an open access article distributed under the terms and conditions of the Creative Commons Attribution (CC BY) license (<https://creativecommons.org/licenses/by/4.0/>).

1. Introduction

The growth and development of plants require suitable environmental conditions, generally including temperature, light, water, and soil nutrients [1]. Adverse environmental factors, especially heat stress, cause several morphological and physiological changes in plants. This includes protein denaturation, plasma membrane disruption, and reactive oxygen species (ROS) accumulation, and can lead to plant death in severe cases [2–5]. Plants have a series of complex physiological mechanisms and molecular regulatory networks to minimize the damage caused by the external environmental stresses [6,7].

The heat shock transcription factors (Hsfs) family is generally known to play a central role in regulating abiotic stress response [8–10]. Based on the conserved Hsf DNA-binding domain (DBD) and oligomerization domain (OD) regions, Hsfs can be divided into three classes in higher plants, namely HsfA, HsfB, and HsfC [11,12]. In a previous study, HsfAs were found to be master regulators of thermotolerance by regulating the expression of downstream genes in plants [13–16]. In contrast to HsfAs, HsfBs have no transcriptional

activity, because they lack a C-terminal activator domain (CTAD) [17]. Previous studies reported that *HsfBs* are induced by heat stress and involved in the regulation of thermotolerance in many plant species [18,19]. For example, *AtHsfB1* and *AtHsfB2b* act as suppressors of the expression of *Hsfs* and several heat shock protein genes but are necessary for acquiring thermotolerance [20]; *PpHsf5* in peaches and *HsfB1* in grapes were reported to play a positive role in thermotolerance [21,22]. In addition, some *HsfBs* genes, such as *OsHsfB2b* from rice, *CarHsfB2* from chickpeas, *GmHsfB2b* from soybeans, and *ZmHsf08* (Class B gene) from maize, play important roles in drought and salt tolerance responses [23–26]. For *HsfCs* members, it is demonstrated that *HsfC2a* from wheat and *HsfC1b* from tall fescue regulate the heat-responsive genes to increase heat tolerance, and *VaHsfC1* from grapes is involved in multiple abiotic stresses [27–30].

The strawberry is cultivated widely in the world and is threatened by a range of adverse environmental factors. With global warming, the increasing number of extreme temperature events has severely affected the growth, development, productivity, and quality of strawberry, posing a considerable challenge to modern agricultural farming [31,32]. Seventeen *Hsf* genes have been identified in the diploid woodland strawberry (*Fragaria vesca*), and different *FvHsfs* have distinct responses to various biotic and abiotic stresses at the transcriptional level [33]. However, the molecular mechanisms of the *FvHsfs* genes involved in abiotic stress remain unclear. Herein, we cloned the *FvHsfB1a* gene from ‘Ruegen’ strawberry leaves and found that *FvHsfB1a* was induced by heat stress treatment. We then explored its function in heat stress response. The overexpression of the *FvHsfB1a* gene enhances heat stress resistance. Compared with WT plants, heat-responsive and stress-related genes in *FvHsfB1a*-OE lines were rapidly and strongly induced by heat stress treatment. Furthermore, the *FvHsfB1a* gene was directly regulated by the FvWRKY75 TF. The overexpression of the *FvWRKY75* gene in *Arabidopsis* also improved heat tolerance. Overall, we have provided evidence that *FvHsfB1a* may play an important role in improving strawberry thermotolerance.

2. Results

2.1. Isolation and Characterization of *FvHsfB1a*

The *FvHsfB1a* gene was isolated from ‘Ruegen’ leaves and the CDS sequence of *FvHsfB1a* was 873 bp, encoding a protein containing 290 amino acids (Figure 1A). The predicted molecular weight and isoelectric point of *FvHsfB1a* protein were predicted to be 32.01 kDa and 5.96 M. The structure of *FvHsfB1a* contained a typical HSF domain for DNA binding at the N-terminus (Figure 1A). Phylogenetic analysis showed that the sequence of *FvHsfB1a* was the closest homolog to RcHSF24 (class B1) (Figure 1B).

The subcellular localization of *FvHsfB1a* protein was detected via green fluorescent protein (GFP) and red fluorescent protein (RFP) as a nuclear location marker. As shown in Figure 1C, the recombinant pCambia1302-*FvHsfB1a*-GFP was localized only in the nucleus.

2.2. Expression Profiles of *FvHsfB1a* in Diploid Strawberry

The tissue-specific expression patterns of *FvHsfB1a* were measured in the diploid strawberry ‘Ruegen’. The *FvHsfB1a* gene was differentially expressed in different tissues, with the lowest expression in leaves and the highest expression in the stem (Figure 2A). Then, we investigated the expression profiles of the *FvHsfB1a* gene by using mature strawberry leaves under abiotic stress conditions. Under heat stress treatment, *FvHsfB1a* gene expression was significantly induced and reached a peak with a 43.7-fold increase after 24 h of treatment (Figure 2B).

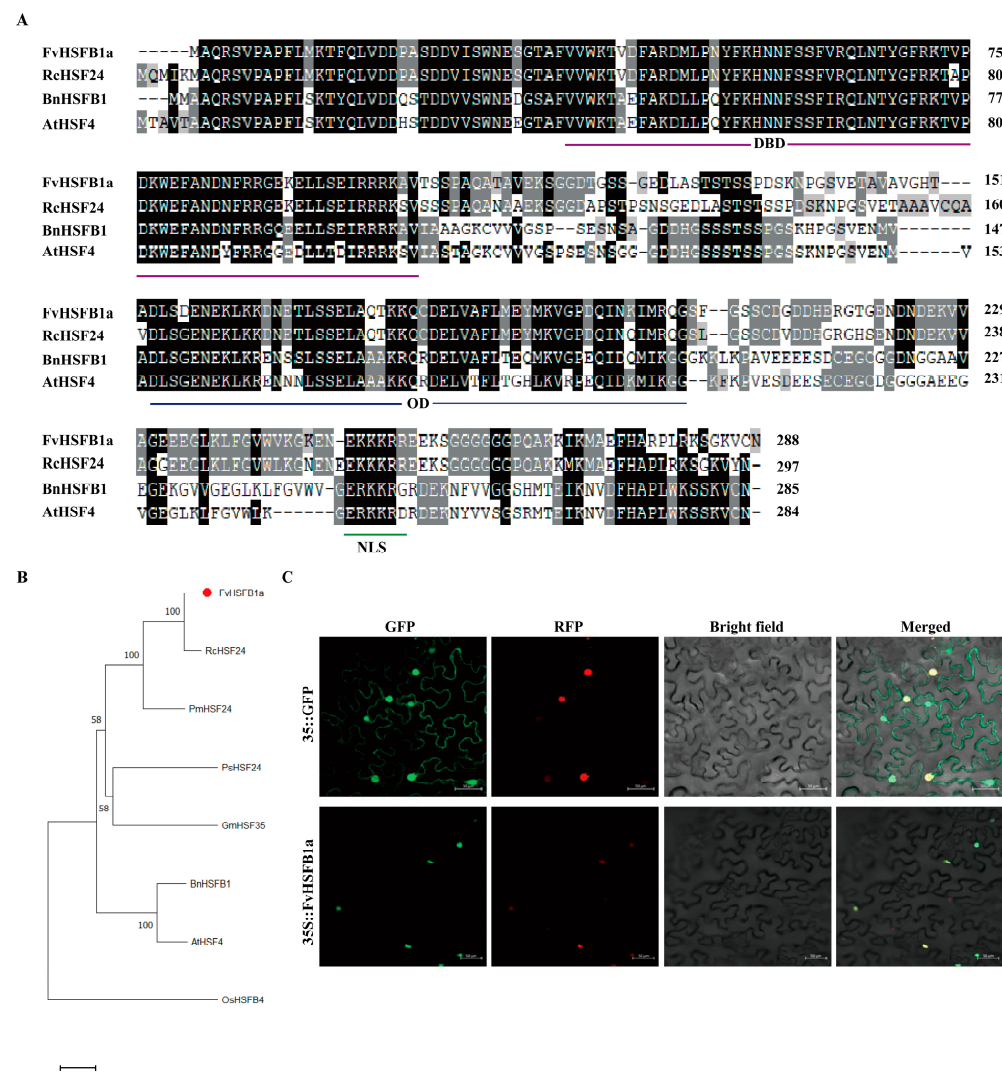


Figure 1. Analysis of FvHsfb1a transcription factor. (A) Amino acid sequence alignment of FvHsfb1a; DBD: DNA-binding domain; OD: oligomerization domain; NLS: nuclear localization signal of *Fragaria vesca*, *Rosa chinensis*, *Brassica napus*, and *Arabidopsis thaliana*. (B) Phylogenetic tree analysis of FvHsfb1a. (C) Subcellular localization of FvHsfb1a in tobacco leaves and RFP as a nuclear location marker.

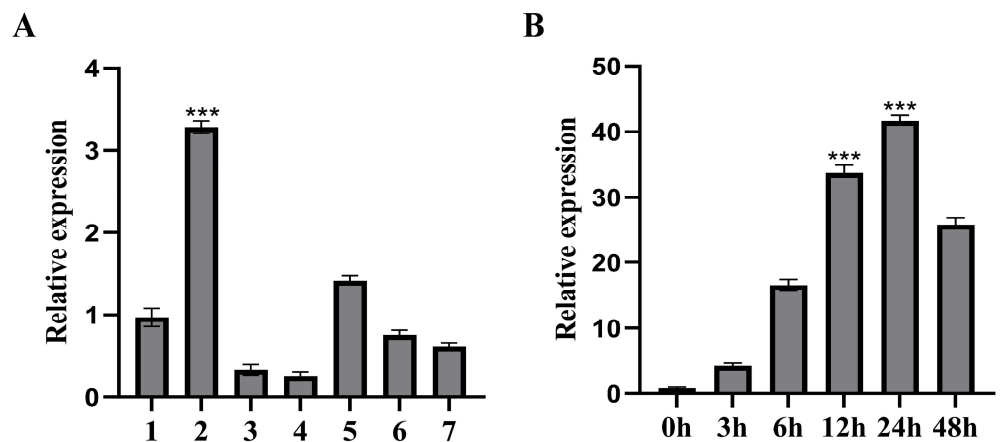


Figure 2. The expression profile of *FvHsfb1a* in diploid strawberry. (A) The tissue-specific expression of the *FvHsfb1a* gene in diploid strawberry. 1: Root; 2: stem; 3: young leaves; 4: mature leaves; 5: flowers; 6: young fruits; 7: mature fruits. (B) Relative expression levels of the *FvHsfb1a* gene under heat stress (42 °C). Three independent biological replicates were used for the experiment. *** $p < 0.001$.

2.3. *FvHsfB1a* Improves Thermotolerance in *Arabidopsis*

To examine the function of *FvHsfB1a* under heat stress, we generated *FvHsfB1a* transgenic *Arabidopsis* plants, and two *FvHsfB1a*-OE T3 homozygous lines were selected by using PCR and qRT-PCR for future tests (Figure S1). As shown in Figure 3, no significant differences in seed germination and seedling growth were observed between the WT and OE lines under normal conditions. After treatment with heat stress for 7 d, the WT seeds exhibited the lowest germination rates, especially under the 42 °C treatment (Figure 3A,B). In contrast, the *FvHsfB1a*-OE seeds had higher germination rates in 37 °C and 42 °C treatments for 7 d compared to WT (Figure 3A,B). Meanwhile, the performance of *FvHsfB1a*-OE and WT *Arabidopsis* seedlings was evaluated after 45 °C treatment for 3 h. Compared with WT, there were better performances, higher survival rates, and longer root lengths in *FvHsfB1a*-OE seedlings observed (Figure 3C,D). Additionally, the heat tolerance of WT and *FvHsfB1a*-OE mature plants was also detected. Compared with WT, *FvHsfB1a*-OE lines showed a lower degree of leaf wilting, higher chlorophyll and proline contents, lower MDA and O₂[−] contents, and better superoxide (SOD) and peroxidase (POD) activities after heat stress treatment (Figure 4A–G). These results suggest that the *FvHsfB1a* gene could improve thermotolerance in *Arabidopsis*.

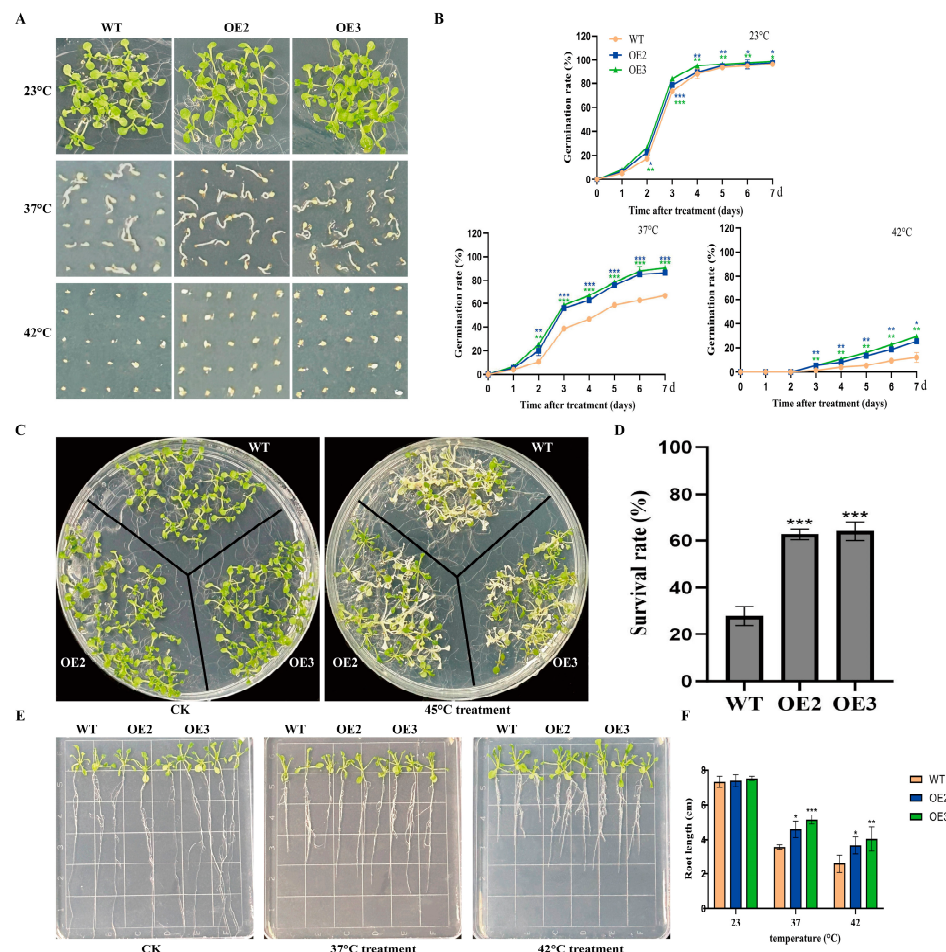


Figure 3. Heat tolerance analysis of WT and *FvHsfB1a*-OE lines. (A,B) Germination phenotypes and germination rates of *FvHsfB1a*-OEs and WT seeds under heat stress conditions. (C,D) Phenotypes and survival rates of *FvHsfB1a*-OEs and WT seedlings under heat stress. A sample of 7 d old seedlings grown on 1/2 MS medium was treated at 45 °C for 4 h, and then underwent recovery for 7 d. (E,F) The phenotype and root length of WT and *FvHsfB1a*-OE seedlings before and after 37 °C and 42 °C treatments, respectively. Three independent biological replicates were used for the experiment. *** $p < 0.001$, ** $p < 0.01$, * $p < 0.05$.

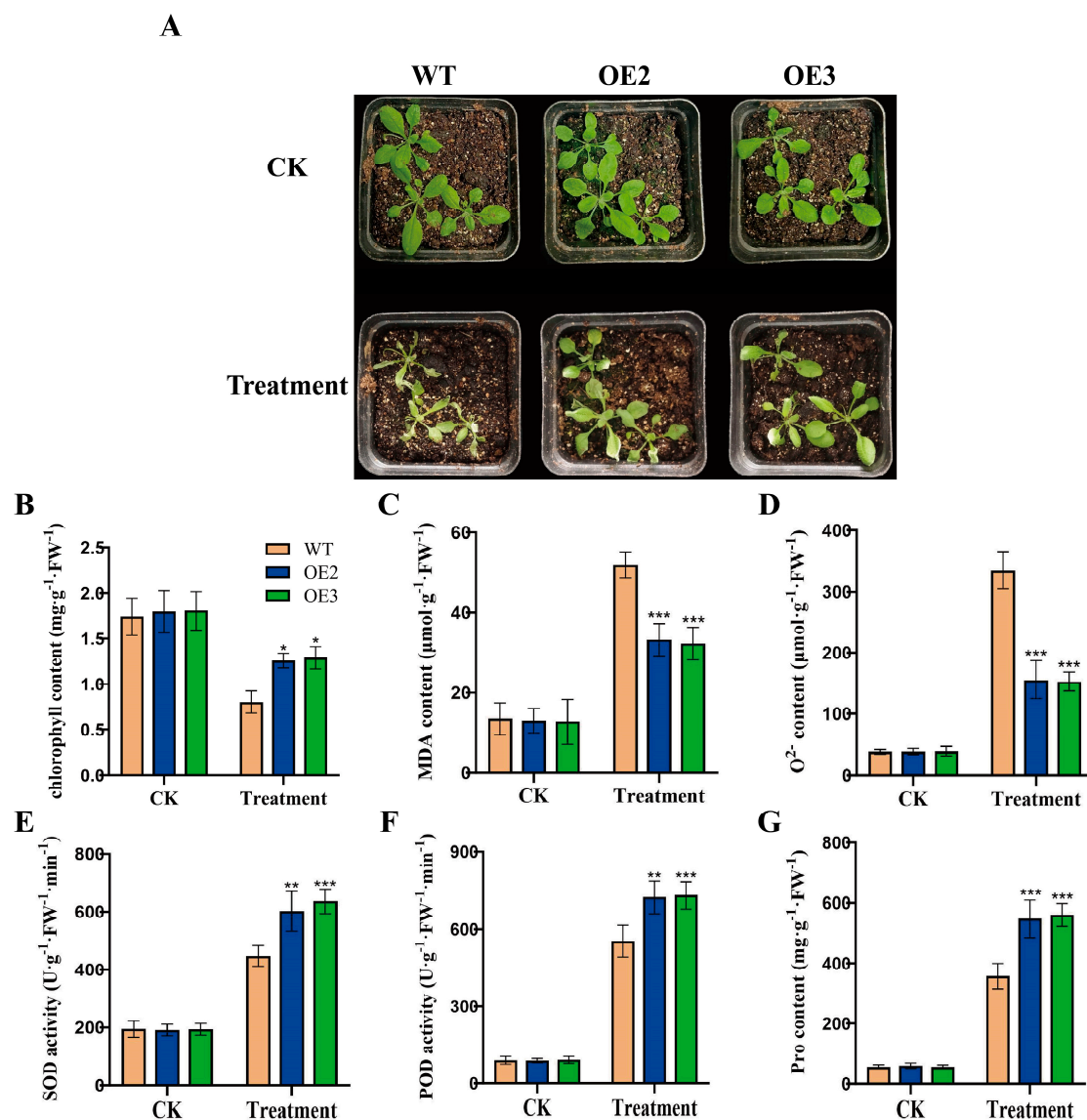


Figure 4. Thermotolerance analysis of WT and *FvHsfB1a*-OE lines. (A) Phenotypes of *FvHsfB1a*-OEs and WT plants before and after heat stress treatment for 7 d. (B–G) The chlorophyll, MDA, $O_2^{\cdot-}$, SOD activity, POD activity, and proline content of WT and *FvHsfB1a*-OE plants under heat stress, respectively. Three independent biological replicates were used for the experiment. *** $p < 0.001$, ** $p < 0.01$, * $p < 0.05$.

2.4. Overexpression of *FvHsfB1a* Regulates the Expression of Stress-Related Genes

To further investigate the regulation of *FvHsfB1a* in response to heat stress, the expression profiles of several heat-responsive and stress-related genes were detected by using qRT-PCR in the WT and *FvHsfB1a*-OE lines. After treatment with heat stress, the expression levels of heat-responsive genes (*AtHSP70b* and *AtHSP101*) and stress-related genes (*AtP5CS1*, *AtSOD*, and *AtPOD*) were significantly upregulated in *FvHsfB1a*-OE lines as compared with the WT (Figure 5).

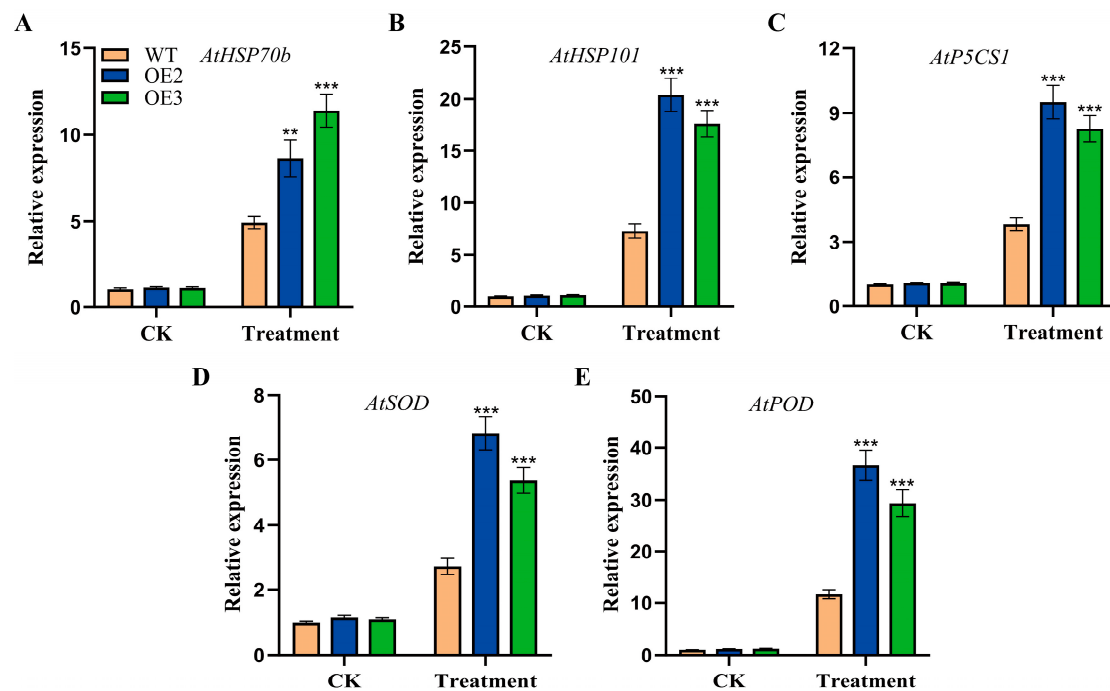


Figure 5. The expression of stress-related genes in WT and *FvHsfB1a*-OE lines under cold stress. The gene expression levels of 2-week-old *Arabidopsis* seedlings treated at 42 °C for 2 d were detected by using qRT-PCR. (A–E) The transcript level of *AtHSP70b*, *AtHSP101*, *AtP5CS1*, *AtSOD* and *AtPOD* gene in mature plants under heat stress, respectively. Three independent biological replicates were used for the experiment. *** $p < 0.001$, ** $p < 0.01$.

2.5. *FvWRKY75* Can Active the Promoter of *FvHsfB1a* Gene

Bioinformatics analysis showed that the promoter of the *FvHsfB1a* gene contains several WRKY binding elements (Figure 6A). Based on previous findings, *FvWRKY70* and *FvWRKY75* were selected for a yeast one-hybrid assay to determine their relationship with *FvHsfB1a*. As shown in Figure 6B,C, the *FvWRKY75* directly bound to the *FvHsfB1a* promoter and influenced its expression in the yeast one-hybrid and LUC assays.

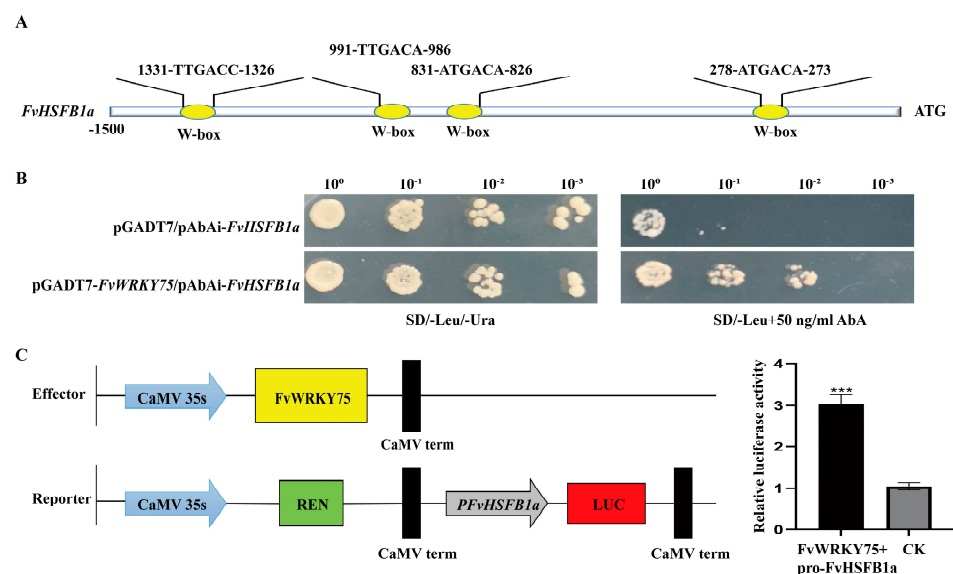


Figure 6. *FvWRKY75* activated *FvHsfB1a* expression. (A) Bioinformatics analysis showed that the promoter of *FvHsfB1a* contained WRKY binding elements. (B) Analysis of the activation of *FvHsfB1a* transcription by *FvWRKY75* using a yeast one-hybrid assay. (C) The luciferase activity assay showed that *FvWRKY75* binds to the *FvHsfB1a* promoter. *** $p < 0.001$.

2.6. *FvWRKY75* Enhances Thermotolerance in *Arabidopsis*

Previously, we cloned the *FvWRKY75* gene and generated two *FvWRKY75*-OE transgenic *Arabidopsis* plants [34]. In this study, we examined the role of the *FvWRKY75* gene in the response to high-temperature stress. During heat treatment, the *FvWRKY75*-OE transgenic plants exhibited greater insensitivity to heat stress with a better phenotype compared to the WT (Figure 7A). Compared with the WT, lower MDA and O_2^- contents, higher chlorophyll and proline contents, and better peroxidase activity were observed in the *FvWRKY75*-OE plants (Figure 7B–G), which suggested that *FvWRKY75* is also a positive regulator of heat tolerance.

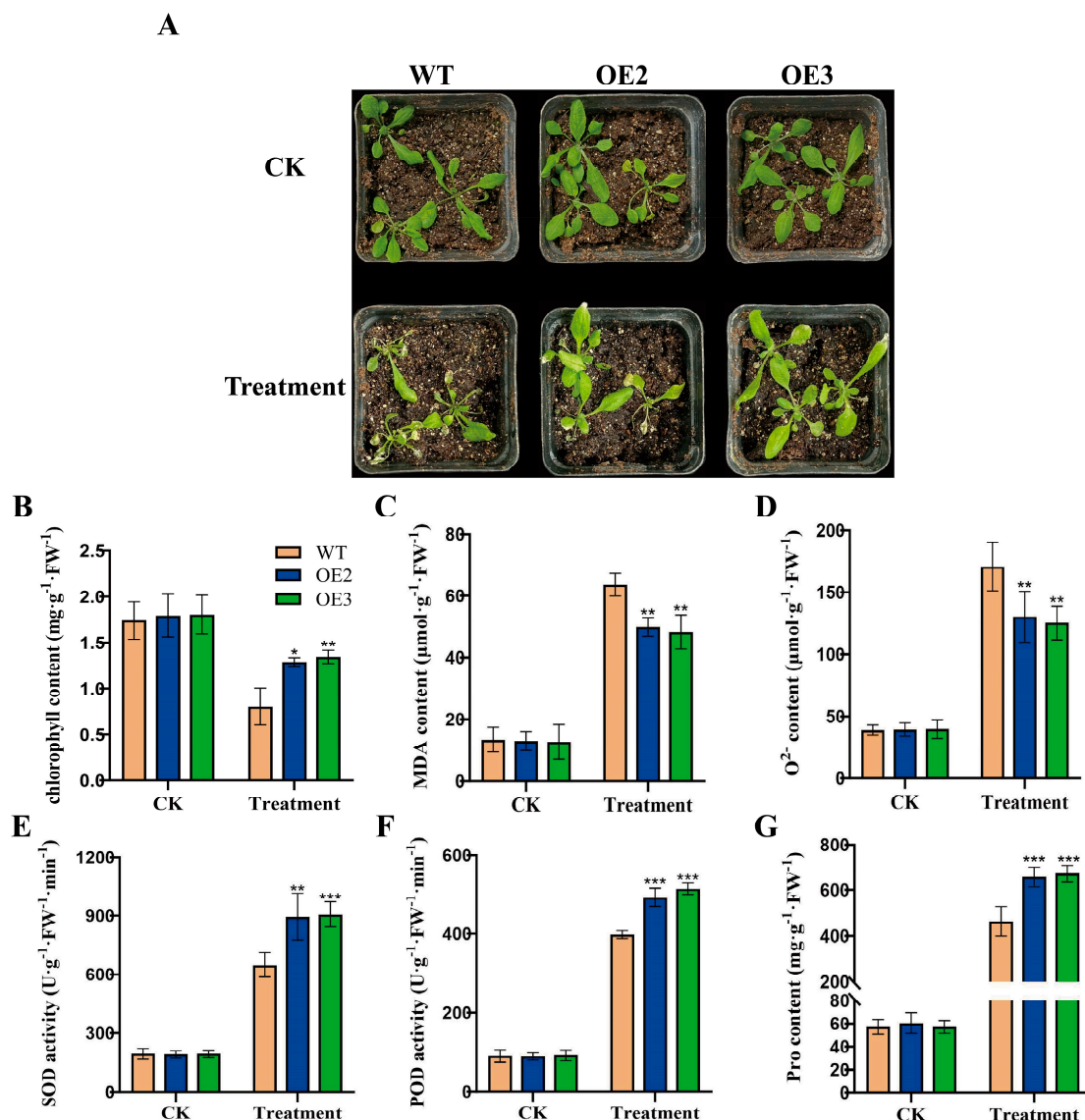


Figure 7. Heat tolerance analyses of WT and *FvWRKY75*-OE lines. (A) Phenotypes of *FvWRKY75*-OEs and WT plants with or without heat treatment for 7 d. (B–G) The chlorophyll, MDA, O_2^- , SOD activity, POD activity, and proline content of WT and *FvWRKY75*-OE plants under heat stress, respectively. Three independent biological replicates were used for the experiment. *** $p < 0.001$, ** $p < 0.01$, * $p < 0.05$.

3. Discussion

Heat stress severely affects the growth, development, production, and yields of most agricultural crops [35–38]. The Hsf family has been well-identified in many plant species

and functions as a key regulatory component involved in various stress responses [10,12,39]. In strawberries, 17 *Hsfs* were identified based on the wild diploid woodland strawberry (*Fragaria vesca*) [33]. Here, we cloned an Hsf TF gene, *FvHsfB1a*, from ‘Ruegen’ leaves, and explored its characterization. The amino acid sequence of FvHsfB1a showed all the characteristics of Class B HSFs, and the sequence of FvHsfB1a showed high homology with that of RcHsf24, which belongs to Class B1 Hsfs (Figure 1A,B). The FvHsfB1a protein was localized only in the nucleus (Figure 1C,D). Some Hsf B TF genes, including *OsHSF2b*, *CarHSFB2*, and *ZmHsf08*, were upregulated under abiotic stresses and proved to play a role in heat stress response [23,24,26]. In the current study, we found that the *FvHsfB1a* gene was induced by heat stress (Figure 2), which suggested that the *FvHsfB1a* gene may be involved in the heat stress response in strawberry.

Previous studies reported that *HsfBs* genes may play diverse roles in abiotic stress responses. For example, the overexpression of *VdHSFB1* and *VvHSFB1* in grapes improved heat tolerance, and the overexpression of *CarHSFB2* and *GmHsfB2b* improved resistance to drought and salt stress in transgenic plants [22,23,25]. However, *OsHsfB2b* and *ZmHsf08* (a member of *HSFB1*) negatively regulated drought and salt tolerance in rice and maize [24,26]. To better understand the function of the *FvHsfB1a* gene in strawberry, two *FvHsfB1a*-OE *Arabidopsis* lines were generated and used for future tests (Figure S1). After heat stress treatment, *FvHsfB1a*-OE lines showed increased insensitivity to high temperature, longer root length, higher germination rates, and higher survival rates than the WT (Figure 3E,F), which suggested that *FvHsfB1a* may regulate the growth of roots in response to heat stress. Under stress conditions, chlorophyll degradation and membrane lipid peroxidation occur in plants, resulting in a decrease in chlorophyll content and an increase in ROS ($O_2^{\cdot-}$ and H_2O_2) and MDA content. Therefore, chlorophyll, MDA, $O_2^{\cdot-}$, and H_2O_2 contents are commonly used to assess the severity of stress damage in plants [40–42]. In addition, antioxidant enzymes, such as POD, CAT, and APX, play important roles in scavenging oxidative damage caused by ROS accumulation [43,44]. In this study, it was noticed that *FvHsfB1a*-OE lines had a lower MDA content and higher chlorophyll and proline contents than WT after stress treatment; meanwhile, a lower $O_2^{\cdot-}$ content and higher SOD and POD activities were presented for the former rather than the latter (Figure 4), which suggested that *FvHsfB1a* could attenuate heat stress damage to plants by modulating ROS scavenging and increasing antioxidant enzyme activity.

Heat shock proteins (HSPs) were known as the target genes of HSFs and play important roles in heat stress regulation [45,46]. *HSP70* and *HSP101* are induced by heat stress and confer improved heat tolerance in *Arabidopsis* [47,48]. In the current study, *AtHSP70b* and *AtHSP101* were significantly induced in *FvHsfB1a*-OE lines compared to in WT plants under heat stress (Figure 5A,B). This suggests that *FvHsfB1a* could influence the expression of these *HSP* genes to improve thermotolerance in transgenic plants. The *P5CS* gene participated in the synthesis of proline, which contributes to the positive regulation of plant dehydration and osmotic stress [49]. The upregulation of the *AtP5CS1* gene in the *FvHsfB1a*-OE lines under heat treatment may contribute to the accumulation of proline, which can reduce the injury caused by stress (Figure 5C). Many studies reported the connection between antioxidant enzyme genes and Hsfs in scavenging ROS [50,51]. In the current study, the stress-related genes (*AtSOD* and *AtPOD*) showed higher expression levels in transgenic plants under heat stress conditions (Figure 5D,E). All of the results suggest that the overexpression of the *FvHsfB1a* gene improves thermotolerance by upregulating the expression of heat-responsive genes (*AtHSP70b* and *AtHSP101*) and stress-related genes (*AtP5CS1*, *AtSOD*, and *AtPOD*).

In this study, the Y1 H and LUC assays showed that the FvWRKY75 transcription factor directly binds to the promoter region of the *FvHsfB1a* gene (Figure 6). It has been

reported that the WRKY TFs play important roles in abiotic stress responses, especially WRKY75 [52–54]. For example, the overexpression of *PtWRKY75* in poplar and *AhWRKY75* in peanuts improves heat, drought, and salt tolerance; the overexpression of *MdWRKY75* in apple regulates the expression of *Hsf* genes to enhance thermotolerance [55–57]. In a previous study, we cloned the *FvWRKY75* gene and found that the *FvWRKY75* gene plays an active role in response to salt stress [34]. Herein, we explored the role of the *FvWRKY75* gene in the response to high-temperature stress. It was noticed that the *FvWRKY75*-OE transgenic *Arabidopsis* plants exhibited a better phenotype compared to the WT under heat treatment, and lower MDA and O_2^- contents, higher chlorophyll and proline contents, and better SOD and POD activities were observed in the former than in the latter (Figure 7). This suggests that *FvWRKY75* is also a positive regulator of thermotolerance in strawberry.

In conclusion, we suggest a model of the WRKY-HSF transcriptional regulatory cascade for regulating heat stress in strawberry. In response to heat stress, *FvHsfB1a* acts as a positive regulator to improve thermotolerance by modulating ROS scavenging, enhancing antioxidant enzyme activity, and being directly regulated by the *FvWRKY75* TF. These results provide physiological and molecular evidence highlighting the significance of *FvHsfB1a* in plant responses to heat stress.

4. Materials and Methods

4.1. Plant Materials, Growth Conditions, and Stress Treatment

Diploid strawberry cultivar ‘Ruegen’ (*Fragia vesca*), Nc89 tobacco (*Nicotiana tabacum*), and *Arabidopsis thaliana* (Columbia) plants were grown in a growth chamber at 23 °C, a 12 h light (4000 lux)/12 h dark photoperiod, and 60% relative humidity in Anhui Agricultural University (Hefei, China). The leaf, stem, root, flower, green fruit, and mature fruit of ‘Ruegen’ plants were harvested and used to detect the tissue specificity of the *FvHsfB1a* gene. The two-month-old seedlings were subjected to 42 °C treatment in an artificial climate incubator, and mature leaves were collected at a series of time points (0 h, 3 h, 6 h, 12 h, 24 h, and 48 h).

4.2. Structure and Sequence Analysis of *FvHsfB1a*

The CDS sequence of *FvHsfB1a* (XM_004288037.1) was collected from NCBI (www.ncbi.nlm.nih.gov, accessed on 10 April 2022) and cloned from ‘Ruegen’ cDNA using the specific primers (Supplemental Table S1). DANMAN (version 6.0) software was used to analyze the homologs of *FvHsfB1a* and other species (accessed on 20 May 2022). MEGA (version 7.0) software was used to construct the phylogenetic tree by the maximum-likelihood (ML) method (accessed on 20 May 2022).

4.3. Subcellular Localization of *FvHsfB1a* Protein

The CDS region without the stop codon of *FvHsfB1a* was cloned and used to construct the fusion vector 35S::FvHsfB1a-eGFPm and then introduced into the *A. tumefaciens* GV3101 strain to infiltrate the tobacco leaves. Leica fluorescence confocal microscopy (Leica TCS SP8) was used to observe the GFP signal. The subcellular localization assays were used according to the methods of a previous study [58]. The primers used are listed in Table S1.

4.4. Genetic Transformation of *Arabidopsis*

The CDS sequence of *FvHsfB1a* was cloned and inserted into the pRI101-AN vector to construct the pRI101-FvHsfB1a fusion vector. The fusion vector was transferred into the *A. tumefaciens* GV3101 strain and then transferred into the *Arabidopsis* Columbia-0 (Col-0, abbreviated WT) plants at flower budding by the floral dip method [59]. T3 homozygous

transgenic lines were measured by PCR and qRT-PCR with the specific primers, and used for further tests. All of the primers used are listed in Table S1.

4.5. RNA Extraction and RT-qPCR

The CTAB methods were used to isolate the total RNA of strawberry and *Arabidopsis* plants. Each cDNA sample was reverse-transcribed and then used for quantitative real-time PCR (qRT-PCR) according to the established protocols. Three technical replicates of each sample were analyzed for qRT-PCR. The primers used are listed in Table S1.

4.6. Heat Stress Assay of Transgenic *Arabidopsis* Plants

To test the function of *FvHsfB1a* and *FvWRKY75* genes, WT, *FvHsfB1a*-OE, and *FvWRKY75*-OE lines were treated with heat stress. At first, seeds of WT and *FvHsfB1a*-OE lines were treated in a chamber at 37 °C and 42 °C in darkness for 7 days for heat stress. After heat treatment, all *Arabidopsis* seeds were transferred into a growth chamber for 4 days under normal growing conditions (23/18 °C day/night), and then the germination rates were measured. Meanwhile, seeds of *FvHsfB1a*-OE lines and WT were sterilized and seeded on an MS medium plate. A sample of 7-day-old *Arabidopsis* seedlings was subjected to heat stress at 45 °C for 3 h, and then underwent recovery for 5 days under normal conditions to record phenotypes and count survival rates. Furthermore, 3-week-old WT, *FvHsfB1a*-OE, and *FvWRKY75*-OE lines were subjected to heat stress at 42/37 °C (day/night) for 7 days, and samples were collected to measure the physiological parameters. A sample of 3-week-old plants was subjected to heat stress at 42 °C for 3 h, and the leaves were harvested to analyze the expression profiles of related genes.

4.7. Yeast One-Hybrid Assay

The CDS region of *FvWRKY75* (XM_004310052.2) was cloned and inserted into the plasmid vector pGADT7 with the GAL4 activation domain. PlantCARE online software (<http://bioinformatics.psb.ugent.be/webtools/plantcare/html/>, accessed on 20 May 2022) was used to identify the cis-acting elements in the promoter region. The promoter region (1500 bp) of *FvHsfB1a* was cloned and inserted into the pAbAi vector. Yeast one-hybrid assays were conducted according to the previously reported methods [58]. The primers used for the yeast one-hybrid assays are listed in Table S1.

4.8. Dual Luciferase Activity Assay

The CDS region of *FvWRKY75* was cloned and inserted into the pGreenII-62SK vector, named pGreenII-62SK-*FvWRKY75*, and defined as an effector. The promoter region (1500 bp) of *FvHsfB1a* was inserted into the pGreenII-0800-LUC vector and named pGreenII-0800-*FvHsfB1a*, which acts as a reporter. Dual luciferase activity assays were conducted according to the previously reported methods [58]. All of the primers used are listed in Table S1.

4.9. Data Analysis

The data are the average of three repeated experiments and are expressed as the mean $\pm$ SD. The statistical analysis was determined by using SPSS system (version 20) software. Duncan's multiple range test was used to identify significant differences among treatment means (* $p < 0.05$, ** $p < 0.01$, and *** $p < 0.001$).

Supplementary Materials: The following supporting information can be downloaded at <https://www.mdpi.com/article/10.3390/plants14152392/s1>, Figure S1: The identification of *FvHsfB1a* transgenic *Arabidopsis thaliana* lines. A: PCR assay of *FvHsfB1a* transgenic lines. B: Relative expression

of *FvHsfB1a* gene in different transgenic lines. Note: 1: Wild-type *Arabidopsis thaliana*; 2: negative; 3: line 1; 4: line 2; 5: line 3; 6: positive; Table S1: The primers used in this study.

Author Contributions: Conceptualization, H.X. (Hao Xue) and Q.C.; validation and formal analysis, Q.C., T.M., S.L. and H.X. (Hanxiu Xie); data curation, Q.C. and T.M.; writing—original draft preparation, T.M. and Q.C.; writing—review and editing, H.X. (Hao Xue) and K.Y.; funding acquisition, H.X. (Hao Xue). All authors have read and agreed to the published version of the manuscript.

Funding: This work was supported by the Natural Science Foundation of Anhui Province (No. 2108085QC120).

Data Availability Statement: Data are contained within the article and can be made available on request.

Conflicts of Interest: The authors have no conflict of interest to declare.

References

- Choudhury, F.K.; Rivero, R.M.; Blumwald, E.; Mittler, R. Reactive oxygen species, abiotic stress and stress combination. *Plant J.* **2017**, *90*, 856–867. [\[CrossRef\]](#)
- Wahid, A.; Gelani, S.; Ashraf, M.; Foolad, M.R. Heat tolerance in plants: An overview. *Environ. Exp.* **2007**, *61*, 199–223. [\[CrossRef\]](#)
- Hasanuzzaman, M.; Nahar, K.; Alam, M.M.; Roychowdhury, R.; Fujita, M. Physiological, biochemical, and molecular mechanisms of heat stress tolerance in plants. *Int. J. Mol. Sci.* **2013**, *14*, 9643–9684. [\[CrossRef\]](#)
- Gechev, T.; Petrov, V. Reactive oxygen species and abiotic stress in plants. *Int. J. Mol. Sci.* **2020**, *21*, 7433. [\[CrossRef\]](#) [\[PubMed\]](#)
- Zandalinas, S.I.; Frittschi, F.B.; Mittler, R. Global warming, climate change, and environmental pollution: Recipe for a multifactorial stress combination disaster. *Trends Plant Sci.* **2021**, *26*, 588–599. [\[CrossRef\]](#) [\[PubMed\]](#)
- Zhu, J.K. Abiotic stress signaling and responses in plants. *Cell* **2016**, *167*, 313–324. [\[CrossRef\]](#) [\[PubMed\]](#)
- Erpen, L.; Devi, H.S.; Grosser, J.W.; Dutt, M. Potential use of the DREB/ERF, MYB, NAC and WRKY transcription factors to improve abiotic and biotic stress in transgenic plants. *Plant Cell Tissue Organ Cult.* **2017**, *132*, 1–25. [\[CrossRef\]](#)
- Wang, W.; Vinocur, B.; Shoseyov, O.; Altman, A. Role of plant heat-shock proteins and molecular chaperones in the abiotic stress response. *Trends Plant Sci.* **2004**, *9*, 244–252. [\[CrossRef\]](#)
- Xue, G.P.; Sadat, S.; Drenth, J.; McIntyre, C.L. 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. *J. Exp. Bot.* **2014**, *65*, 539–557. [\[CrossRef\]](#)
- Guo, M.; Liu, J.H.; Ma, X.; Luo, D.X.; Gong, Z.H.; Lu, M.H. The plant heat stress transcription factors (HSFs): Structure, regulation, and function in response to abiotic stresses. *Front. Plant Sci.* **2016**, *7*, 114. [\[CrossRef\]](#)
- Doring, P.; Treuter, E.; Kistner, C.; Lyck, R.; Chen, A.; Nover, L. The role of AHA motifs in the activator function of tomato heat stress transcription factors HsfA1 and HsfA2. *Plant Cell* **2000**, *12*, 265–278. [\[CrossRef\]](#)
- Jacob, P.; Hirt, H.; Bendahmane, A. The heat-shock protein/chaperone network and multiple stress resistance. *Plant Biotechnol. J.* **2017**, *15*, 405–414. [\[CrossRef\]](#)
- Liu, H.C.; Charn, Y.Y. Common and distinct functions of arabidopsis class A1 and A2 heat shock factors in diverse abiotic stress responses and development. *Plant Physiol.* **2013**, *163*, 276–290. [\[CrossRef\]](#)
- Chen, S.; Yu, M.; Li, H.; Wang, Y.; Lu, Z.; Zhang, Y.; Liu, M.; Qiao, G.; Wu, L.; Han, X.; et al. SaHsfA4c from *Sedum alfredii* hance enhances cadmium tolerance by regulating ROS-scavenger activities and heat shock proteins expression. *Front. Plant Sci.* **2020**, *11*, 142. [\[CrossRef\]](#)
- Gai, W.X.; Ma, X.; Li, Y.; Xiao, J.J.; Khan, A.; Li, Q.H.; Gong, Z.H. CaHsfA1d improves plant thermotolerance via regulating the expression of stress- and antioxidant-related genes. *Int. J. Mol. Sci.* **2020**, *21*, 8374. [\[CrossRef\]](#) [\[PubMed\]](#)
- Wang, R.; Mao, C.J.; Jiang, C.H.; Zhang, L.; Peng, S.Y.; Zhang, Y. One heat shock transcription factor confers high thermal tolerance in clematis plants. *Int. J. Mol. Sci.* **2021**, *22*, 2900. [\[CrossRef\]](#)
- Bharti, K.; Koskull–Döring, P.V.; Bharti, S.; Kumar, P.; Tintschl–Körbitzer, A.; Treuter, E.; Nover, L. Tomato heat stress transcription factor HsfB1 represents a novel type of general transcription coactivator with a histone-like motif interacting with the plant CREB binding protein ortholog HAC1. *Plant Cell* **2004**, *16*, 1521–1535. [\[CrossRef\]](#) [\[PubMed\]](#)
- Giorno, F.; Guerriero, G.; Baric, S.; Mariani, C. Heat shock transcriptional factors in *Malus domestica*: Identification, classification and expression analysis. *BMC Genom.* **2012**, *13*, 639. [\[CrossRef\]](#) [\[PubMed\]](#)
- Hu, Y.; Han, Y.; Zhang, K.; Zhao, F.L.; Li, Y.J.; Zheng, Y.; Wang, Y.J.; Wen, Y.Q. Identification and expression analysis of heat shock transcription factors in the wild Chinese grapevine (*Vitis pseudoreticulata*). *Plant Physiol. Biochem.* **2016**, *99*, 1–10. [\[CrossRef\]](#)
- Ikeda, M.; Mitsuda, N.; Ohme-Takagi, M. Arabidopsis HsfB1 and HsfB2b act as repressors of the expression of heat-inducible Hsfs but positively regulate the acquired thermotolerance. *Plant Physiol.* **2011**, *157*, 1243–1254. [\[CrossRef\]](#)

21. Tan, B.; Yan, L.; Li, H.N.; Lian, X.D.; Cheng, J.; Wang, W. Genome-wide identification of HSF family in peach and functional analysis of PpHSF5 involvement in root and aerial organ development. *PeerJ* **2021**, *9*, e10961. [\[CrossRef\]](#)
22. Chen, H.Y.; Liu, X.N.; Li, S.C.; Yuan, L.; Mu, H.Y.; Wang, Y.; Li, Y.; Duan, W.; Fan, P.G.; Liang, Z.C.; et al. The class B heat shock factor HsFB1 regulates heat tolerance in grapevine. *Hortic. Res.* **2023**, *10*, uhad001. [\[CrossRef\]](#)
23. Ma, H.; Wang, C.T.; Yang, B.; Cheng, H.Y.; Wang, Z.; Mijiti, A.; Ren, C.; Qu, G.H.; Zhang, H.; Ma, L. *CarHSFB2*, a class B heat shock transcription factor, is involved in different developmental processes and various stress responses in chickpea (*Cicer arietinum* L.). *Plant Mol. Biol. Rep.* **2016**, *34*, 1–14. [\[CrossRef\]](#)
24. Xiang, J.H.; Ran, J.; Zou, J.; Zhou, X.Y.; Liu, A.L.; Zhang, X.W.; Peng, Y.; Tang, N.; Luo, G.Y.; Chen, X.B. Heat shock factor OsHsfB2b negatively regulates drought and salt tolerance in rice. *Plant Cell Rep.* **2013**, *32*, 1795–1806. [\[CrossRef\]](#)
25. Bian, X.H.; Li, W.; Niu, C.F.; Wei, W.; Hu, Y.; Han, J.Q.; Lu, X.; Tao, J.J.; Jin, M.; Qin, H.; et al. A class B heat shock factor selected for during soybean domestication contributes to salt tolerance by promoting flavonoid biosynthesis. *New Phytol.* **2020**, *225*, 268–283. [\[CrossRef\]](#)
26. Wang, J.; Chen, L.; Long, Y.; Si, W.; Cheng, B.; Jiang, H. A novel heat shock transcription factor (ZmHsf08) negatively regulates salt and drought stress responses in maize. *Int. J. Mol. Sci.* **2021**, *22*, 11922. [\[CrossRef\]](#)
27. Schmidt, R.; Schippers, J.H.; Welker, A.; Mieulet, D.; Guiderdoni, E.; Mueller-Roeber, B. Transcription factor OsHsfC1b regulates salt tolerance and development in *Oryza sativa* ssp. *japonica*. *AoB Plants* **2012**, *1*, pls011. [\[CrossRef\]](#)
28. Zhuang, L.L.; Cao, W.; Wang, J.; Yu, J.J.; Yang, Z.M.; Huang, B.R. Characterization and functional analysis of *FaHsfC1b* from *festuca arundinacea* conferring heat tolerance in Arabidopsis. *Int. J. Mol. Sci.* **2018**, *19*, e2702. [\[CrossRef\]](#)
29. Jiao, S.Z.; Guo, C.; Yao, W.K.; Zhang, N.B.; Zhang, J.Y.; Xu, W.R. An Amur grape VaHsfC1 is involved in multiple abiotic stresses. *Sci. Horti.* **2022**, *295*, 110785. [\[CrossRef\]](#)
30. Hu, X.J.; Chen, D.D.; Lynne, M.C.; Fernanda, D.M.; Zhang, Z.B.; Drenth, J.; Kalaipandian, S.; Chang, H.P.; Xue, G.P. Heat shock factor C2a serves as a proactive mechanism for heat protection in developing grains in wheat via an ABA-mediated regulatory pathway. *Plant Cell Environ.* **2017**, *41*, 79–98. [\[CrossRef\]](#) [\[PubMed\]](#)
31. Long, S.P.; Ort, D.R. More than taking the heat: Crops and global change. *Curr. Opin. Plant Biol.* **2010**, *13*, 241–248. [\[CrossRef\]](#)
32. Teixeira, E.I.; Fischer, G.; Van Velthuizen, H.; Walter, C.; Ewert, F. Global hot-spots of heat stress on agricultural crops due to climate change. *Agric. For. Meteorol.* **2013**, *170*, 206–215. [\[CrossRef\]](#)
33. Hu, Y.; Han, Y.; Wei, W.; Li, Y.J.; Zhang, K.; Gao, Y.R.; Zhao, F.L.; Feng, J.Y. Identification, isolation, and expression analysis of heat shock transcription factors in the diploid woodland strawberry *Fragaria vesca*. *Front. Plant Sci.* **2015**, *6*, 736. [\[CrossRef\]](#) [\[PubMed\]](#)
34. Li, S.; Jiang, Y.; Xie, H.X.; Wang, K.W.; Yang, K.B.; Cao, Q.; Xue, H. FvWRKY75 positively regulates FvCRK5 to enhance salt stress tolerance. *Plants* **2025**, *14*, 1804. [\[CrossRef\]](#) [\[PubMed\]](#)
35. Nasser, S.; Kemal, K.; Schenk, P.M. Global plant stress signaling: Reactive oxygen species at the cross-road. *Front. Plant Sci.* **2016**, *7*, 187. [\[CrossRef\]](#)
36. Ohama, N.; Sato, H.; Shinozaki, K.; Yamaguchi-Shinozaki, K. Transcriptional regulatory network of plant heat stress response. *Trends Plant Sci.* **2017**, *22*, 53–65. [\[CrossRef\]](#)
37. Raza, A.; Razzaq, A.; Mehmood, S.S.; Zou, X.; Zhang, X.; Lv, Y.; Xu, J. Impact of climate change on crops adaptation and strategies to tackle its outcome: A review. *Plants* **2019**, *8*, 34. [\[CrossRef\]](#)
38. Hu, Y.M.; Zhao, T.L.; Guo, Y.F.; Wang, M.; Brachhold, K.; Chu, C.C.; Hanson, A.; Kumar, S.; Lin, R.C.; Long, W.J.; et al. 100 essential questions for the future of agriculture. *MODA* **2023**, *1*, 4–12. [\[CrossRef\]](#)
39. Liu, F.; Xi, M.W.; Liu, T.; Wu, X.Y.; Ju, L.Y.; Wang, D.J. The central role of transcription factors in bridging biotic and abiotic stress responses for plants' resilience. *New Crops* **2024**, *1*, 100005. [\[CrossRef\]](#)
40. Hu, L.; Li, H.; Pang, H.; Fu, J. Responses of antioxidant gene, protein and enzymes to salinity stress in two genotypes of Perennial ryegrass (*Lolium perenne*) differing in salt tolerance. *J. Plant Physiol.* **2012**, *169*, 146–156. [\[CrossRef\]](#)
41. Tomar, R.S.; Kataria, S.; Jajoo, A. Behind the scene: Critical role of reactive oxygen species and reactive nitrogen species in salt stress tolerance. *J. Agron. Crop Sci.* **2021**, *207*, 577–588. [\[CrossRef\]](#)
42. Sharma, P.; Jha, A.B.; Dubey, R.S.; Pessarakli, M. Reactive oxygen species, oxidative damage, and antioxidative defense mechanism in plants under stressful conditions. *J. Bot.* **2012**, *4*, 217037. [\[CrossRef\]](#)
43. Gill, S.S.; Tuteja, N. Reactive oxygen species and antioxidant machinery in abiotic stress tolerance in crop plants. *Plant Physiol. Biochem.* **2010**, *48*, 909–930. [\[CrossRef\]](#)
44. Sun, T.T.; Wang, C.; Liu, R.; Zhang, Y.; Wang, Y.C.; Wang, L.Q. ThHSFA1 confers salt stress tolerance through modulation of reactive oxygen species scavenging by directly regulating ThWRKY4. *Int. J. Mol. Sci.* **2021**, *22*, 5048. [\[CrossRef\]](#)
45. Hu, W.H.; Hu, G.C.; Han, B. Genome-wide survey and expression profiling of heat shock proteins and heat shock factors revealed overlapped and stress specific response under abiotic stresses in rice. *Plant Sci.* **2009**, *176*, 583–590. [\[CrossRef\]](#) [\[PubMed\]](#)
46. Wang, X.Y.; Huang, W.L.; Yang, Z.M.; Liu, J.; Huang, B.R. Transcriptional regulation of heat shock proteins and ascorbate peroxidase by CtHsfA2b from African bermudagrass conferring heat tolerance in Arabidopsis. *Sci. Rep.* **2016**, *6*, 28021. [\[CrossRef\]](#) [\[PubMed\]](#)

47. Wu, T.Y.; Juan, Y.T.; Hsu, Y.H.; Wu, S.H.; Liao, H.T.; Fung, R.W.; Charng, Y.Y. Interplay between heat shock proteins HSP101 and HSA32 prolongs heat acclimation memory posttranscriptionally in *Arabidopsis*. *Plant Physiol.* **2013**, *161*, 2075–2084. [[CrossRef](#)]
48. Guo, M.; Liu, J.H.; Ma, X.; Zhai, Y.F.; Gong, Z.H.; Lu, M.H. Genome-wide analysis of the *Hsp70* family genes in pepper (*Capsicum annuum* L.) and functional identification of *CaHsp70-2* involvement in heat stress. *Plant Sci.* **2016**, *252*, 246–256. [[CrossRef](#)]
49. Butt, H.I.; Yang, Z.E.; Gong, Q.; Chen, E.Y.; Wang, X.Q.; Zhao, G.; Ge, X.Y.; Zhang, X.Y.; Li, F.G. *GaMYB85*, an R2R3 MYB gene, in transgenic *Arabidopsis* plays an important role in drought tolerance. *BMC Plant Biol.* **2017**, *17*, 142. [[CrossRef](#)]
50. Li, F.; Zhang, H.; Zhao, H.; Gao, T.; Song, A.; Jiang, J.; Chen, F.; Chen, S. Chrysanthemum CmHSA4 gene positively regulates salt stress tolerance in transgenic chrysanthemum. *Plant Biotechnol. J.* **2018**, *16*, 1311–1321. [[CrossRef](#)]
51. Panchuk, I.I.; Volkov, R.A.; Schoffl, F. Heat stress- and heat shock transcription factor-dependent expression and activity of ascorbate peroxidase in *Arabidopsis*. *Plant Physiol.* **2002**, *129*, 838–853. [[CrossRef](#)] [[PubMed](#)]
52. Zhao, K.; Zhang, D.W.; Lv, K.W.; Zhang, X.M.; Cheng, Z.H.; Li, R.H.; Zhou, B.; Jiang, T.B. Functional characterization of poplar WRKY75 in salt and osmotic tolerance. *Plant Sci.* **2019**, *289*, 110259. [[CrossRef](#)]
53. Li, W.X.; Pang, S.Y.; Lu, Z.G.; Jin, B. Function and mechanism of WRKY transcription factors in abiotic stress responses of plants. *Plants* **2020**, *9*, 1515. [[CrossRef](#)]
54. Jiang, J.J.; Ma, S.H.; Ye, N.H.; Jiang, M.; Cao, J.S.; Zhang, J.H. WRKY transcription factors in plant responses to stresses. *J. Integr. Plant Biol.* **2017**, *59*, 86–101. [[CrossRef](#)]
55. Zhang, Y.; Zhou, Y.; Zhang, D.; Tang, X.; Li, Z.; Shen, C.; Han, X.; Deng, W.; Yin, W.; Xia, X. PtrWRKY75 overexpression reduces stomatal aperture and improves drought tolerance by salicylic acid-induced reactive oxygen species accumulation in poplar. *Environ. Exp. Bot.* **2020**, *176*, 104117. [[CrossRef](#)]
56. Zhu, H.; Jiang, Y.N.; Guo, Y.; Huang, J.B.; Zhou, M.H.; Tang, Y.Y.; Sui, J.M.; Wang, J.S.; Qiao, L.X. A novel salt inducible WRKY transcription factor gene, *AtWRKY75*, confers salt tolerance in transgenic peanut. *Plant Physiol. Bioch.* **2021**, *160*, 175–183. [[CrossRef](#)]
57. Zhang, Z.; Yang, C.; Xi, J.; Wang, Y.; Guo, J.; Liu, Q.; Liu, Y.; Ma, Y.; Zhang, J.; Ma, F.; et al. The MdHSC70-MdWRKY75 module mediates basal apple thermotolerance by regulating the expression of heat shock factor genes. *Plant Cell* **2024**, *36*, 3631–3653. [[CrossRef](#)] [[PubMed](#)]
58. Ma, Y.; Xue, H.; Zhang, F.; Jiang, Q.; Yang, S.; Yue, P.T.; Wang, F.; Zhang, Y.Y.; Li, L.G.; He, P.; et al. The miR156/SPL module regulates apple salt stress tolerance by activating *MdWRKY100* expression. *Plant Biotech. J.* **2020**, *19*, 311–323. [[CrossRef](#)] [[PubMed](#)]
59. Clough, S.J.; Bent, A.F. Floral dip: A simplified method for *Agrobacterium*-mediated transformation of *Arabidopsis thaliana*. *Plant J.* **1998**, *16*, 735–743. [[CrossRef](#)]

Disclaimer/Publisher’s Note: The statements, opinions and data contained in all publications are solely those of the individual author(s) and contributor(s) and not of MDPI and/or the editor(s). MDPI and/or the editor(s) disclaim responsibility for any injury to people or property resulting from any ideas, methods, instructions or products referred to in the content.