

Review

Molecular Hubs of Plant Heat Stress Memory: Structure, Function, and Regulatory Mechanisms of HSFs

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Highlights

What are the main findings?

- HSFs act as central regulators of plant heat stress memory across species.
- HSFs control heat stress memory through transcriptional regulation, post-translational modifications, epigenetic remodeling, hormone signaling, and protein interactions.

What are the implications of the main findings?

- This study helps explain how plants cope with recurring heat stress and provides a basis for improving crop thermotolerance.

Abstract

Global warming is associated with an increased frequency and intensity of heat waves, which severely threaten crop production and sustainable agriculture. As sessile organisms, plants evolved complex heat stress memory mechanisms to cope with recurring heat waves. Heat shock transcription factors (HSFs) are at the core of plant heat stress responses and memory. They regulate basal thermotolerance, acquired thermotolerance, and the maintenance of acquired thermotolerance. These processes involve multiple mechanisms, including temperature perception, activation of heat shock protein expression, and integration of hormonal and epigenetic signals. Here, we review the pivotal role HSFs play in the formation of heat stress memory, their structural characteristics, functional differentiation, and signal perception and transcriptional regulatory mechanisms. We further discuss the functional conservation and the diversity of HSFs across multiple species—for instance, *HSFA2* acts as a conserved regulator of heat stress memory in *Arabidopsis*, tomato, wheat, and barley—and outline future research directions, including the functional characterization of heat shock transcription factor (HSF) subfamilies, investigation of their roles under stress combination, and strategies to balance stress tolerance with growth and development. We hope that our review will provide a theoretical foundation for the genetic improvement of crop thermotolerance as well as contribute to efforts directed at ensuring food security in the face of climate change.

Academic Editor: Alessandra Francini

Received: 29 May 2026

Revised: 26 June 2026

Accepted: 3 July 2026

Published: 5 July 2026

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Keywords: heat stress response; heat shock transcription factors; heat stress memory; thermotolerance

1. Introduction

Latest observational data indicate that the 2024 annual mean surface air temperature over global land regions was 2.1 °C above the 1951–1980 climatological baseline, marking a record-high anomaly. The decade spanning 2015 to 2024 ranks as the warmest ten-year period ever documented [1,2]. As a consequence of global warming, the frequency and intensity of heat stress (HS), or heat wave, events are steadily increasing, and HS has become one of the major abiotic stressors limiting crop growth, reducing yields, and threatening sustainable agriculture development [3,4].

Plants are sessile organisms and cannot escape high temperatures. Instead, they rely on molecular and physiological acclimation and adaptation mechanisms for survival. Understanding how plants perceive stress signals and adapt to adverse environments is therefore critical for global agriculture [5]. In natural environments, plants frequently encounter recurrent HS events during their life cycle. To cope with repeated HS exposure, plants have evolved complex stress memory mechanisms. These involve specific molecular responses that enable a more effective response to subsequent HS events [6]. Heat stress memory (HSM) is a multi-layered cellular phenomenon involving epigenetic modifications, protein quality control, metabolic reprogramming, and broader physiological adjustments [6,7].

Upon exposure to excessively high temperatures, plants activate the heat shock response (HSR), an evolutionarily conserved protective mechanism [8,9]. Heat-stressed plants display leaf wilting and curling, retarded growth, aberrant flowering, and compromised reproductive development. At the physiological level, heat stress provokes membrane lipid peroxidation, suppresses photosynthetic efficiency, and triggers a burst of reactive oxygen species (ROS). In response, plants activate antioxidant enzymes (e.g., SOD and CAT) and accumulate proline and soluble sugars to sustain osmotic balance [7]. Heat shock transcription factors (HSFs) are the core transcriptional regulators of the HSR, orchestrating multiple protective processes that maintain proteostasis and cellular integrity by inducing the expression of heat shock proteins (HSPs), antioxidants, and other key factors [8–10]. Plant HSFs bind to the heat shock element (HSE, 5'-nGAAnnTTCn-3') in the promoters of target genes under heat stress, thereby activating HSR-related genes that are central to the acquisition of abiotic stress tolerance [11]. In addition, HSFs integrate multiple signaling pathways and interact with epigenetic regulatory mechanisms to contribute to the establishment of HSM, which is essential for the development and maintenance of acquired thermotolerance in plants [12]. This review examines the central role of HSFs in plant heat stress responses and memory formation, focusing on their structural features, functional differentiation, mechanisms of signal perception, and transcriptional regulatory networks. It provides a comprehensive framework for understanding the molecular mechanisms underlying plant adaptation to recurrent HS, and offers valuable insights into crop thermotolerance breeding and stress-resilience improvement strategies.

2. Formation of Plant Heat Stress Memory (HSM)

2.1. Damage to Plants Caused by HS

In nature, plants are exposed to dynamically fluctuating temperatures. Mild to moderately elevated temperatures can induce thermomorphogenesis, characterized by hypocotyl elongation, altered flowering time, and other morphological changes [13]. In

contrast, abrupt or extreme elevated temperatures that cause HS can severely impair plant physiological functions, leading to leaf senescence and abscission, growth inhibition, and reduced seed vigor [5]. These detrimental effects occur because HS disrupts membrane fluidity and protein conformation, impairs photosynthesis and respiration, triggers excessive accumulation of reactive oxygen species (ROS) and disturbs cellular and metabolic homeostasis. Collectively, these changes result in cellular damage and may ultimately lead to cell death [14–16].

2.2. Basal Thermotolerance, Acquired Thermotolerance, and Maintenance of Acquired Thermotolerance

Plant thermotolerance can be mediated by basal thermotolerance (BT), acquired thermotolerance (AT), and/or maintenance of acquired thermotolerance (MT) [17]. BT refers to the inherent heat tolerance of plants without prior priming; in this case, plants grown under control temperatures are directly exposed to high-temperature stress [18,19]. AT denotes the adaptive capacity acquired after exposure to a sublethal HS (priming), which enables plants to survive a subsequent severe or lethal HS that would otherwise be fatal in the absence of priming [18,20]. MT represents the prolonged retention of AT via plant memory, allowing plants to better withstand recurring HS events over an extended period of time [17,21]. Different plant species require distinct temperature conditions to induce AT and MT [20]. For example, in 4-day-old *Arabidopsis* seedlings, AT can be induced following priming at 37 °C for 1 h and recovery at 23 °C for 1.5 h [17] (Figure 1A). Inducing MT involves an additional priming by a heat treatment (44 °C for 45 min) compared with AT, followed by exposure to a lethal temperature (44 °C) for 80–180 min during the test phase [17] (Figure 1A). By contrast, BT treatment is performed on 4-day-old seedlings without any prior priming; seedlings are directly subjected to 44 °C high temperature for 10–60 min to evaluate the basal thermotolerance of plants [17] (Figure 1A). In tomato seedlings (approximately 20 days old), Sun et al. [22,23] established optimized protocols for assessing AT (priming at 35 °C for 14 h, recovery at 25 °C for 90 min, followed by testing at 45 °C for 24 h) and MT (with an additional priming at 45 °C for 4 h and recovery at 25 °C for 24 h) (Figure 1B). Overall, both *Arabidopsis* and tomato seedlings exhibit significantly enhanced thermotolerance under AT and MT treatments compared to BT conditions [17,22,23].

2.3. Generation of HSM

In natural environments, HS typically occurs repeatedly over extended periods, driving plants to evolve strategies to memorize prior stress exposure(s). After experiencing a mild, sublethal priming stress, plants retain specific beneficial modifications induced by the initial stimulus. This enables them to “remember” previous stress cues and enhances their tolerance to subsequent stress events [12,24] (Figure 2A). The initial sublethal HS induces the expression of genes associated with HSP synthesis, redox regulation, phytohormone regulation, calcium signaling, and autophagy. These responses trigger stress perception and signal transduction, thereby enabling a more rapid and robust activation of the defense mechanisms upon subsequent exposure to related conditions [6,25–27].

The mechanisms underlying plant stress memory are complex, and current studies span multiple cellular levels, including transcriptional and post-transcriptional regulation, translational regulation, and post-translational modifications [7] (Figure 2B). Transcriptional regulation and post-transcriptional regulation primarily involve activation of transcription factors, differential gene expression, and alternative splicing [28]. Translation-level stress responses include ribosomal pausing, selective mRNA degradation, and regulation of stress-specific protein (e.g., HSPs, CBFs, CSDs, RBD1) synthesis by translation initiation factors [29]. Post-translational regulation encompasses protein

phosphorylation, ubiquitination, SUMOylation-mediated degradation, oligomerization, and protein–protein interactions [9]. Epigenetic modifications represent another mechanism by which plants rapidly respond to abiotic stress, including histone modifications, chromatin remodeling, DNA methylation, transposon activation, and regulation by non-coding RNAs, all of which play important roles in plant stress memory [30–33].

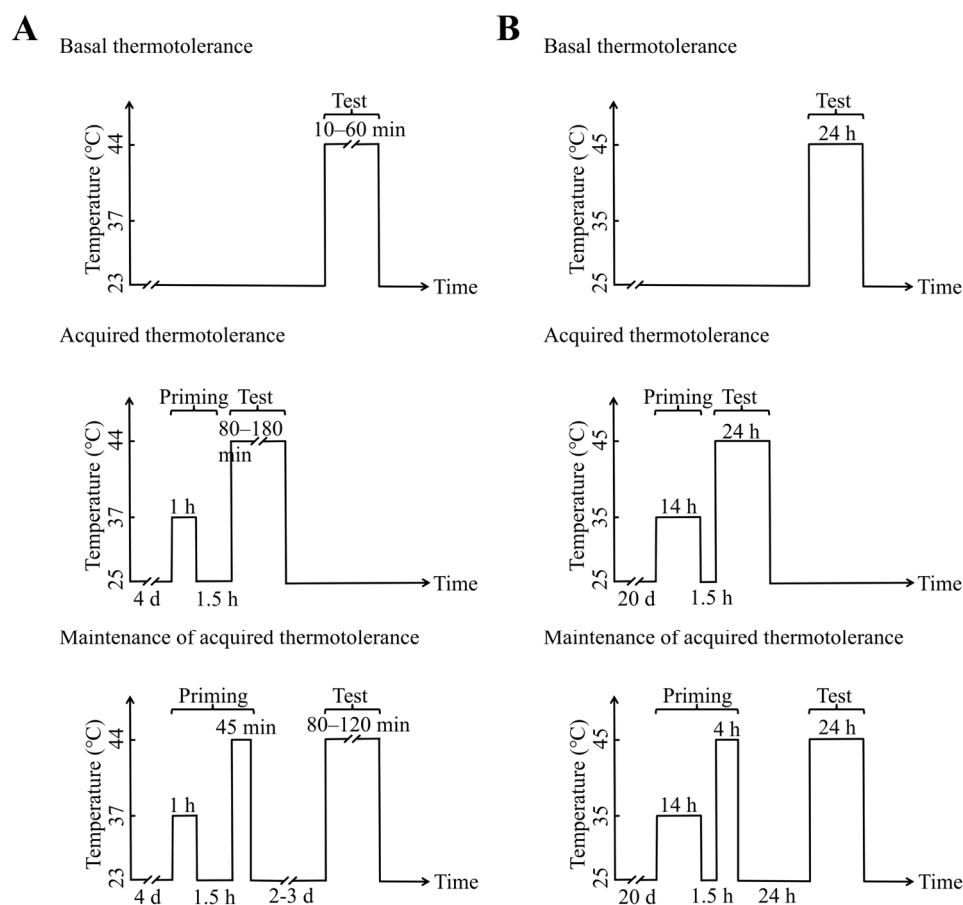


Figure 1. Experimental procedures for assessing thermotolerance in *Arabidopsis* and tomato. **(A)** *Arabidopsis* (4-day-old seedlings). Basal thermotolerance (BT): seedlings without priming are directly exposed to lethal high temperature for testing. Acquired thermotolerance (AT): seedlings are primed once with sublethal heat, recovered, and then tested at lethal temperature. Maintenance of acquired thermotolerance (MT): seedlings are primed twice, with recovery between the two priming events, and then tested at lethal temperature. **(B)** Tomato (approximately 20-day-old seedlings). Basal thermotolerance (BT): seedlings without priming are directly exposed to lethal high temperature for testing. Acquired thermotolerance (AT): seedlings are primed once with sublethal heat, recovered, and then tested at lethal temperature. Maintenance of acquired thermotolerance (MT): seedlings are primed twice, with recovery between the two priming events, and then tested at lethal temperature. Arrows indicate the sequential order of priming, recovery, and testing phases (based on [17,22,23]).

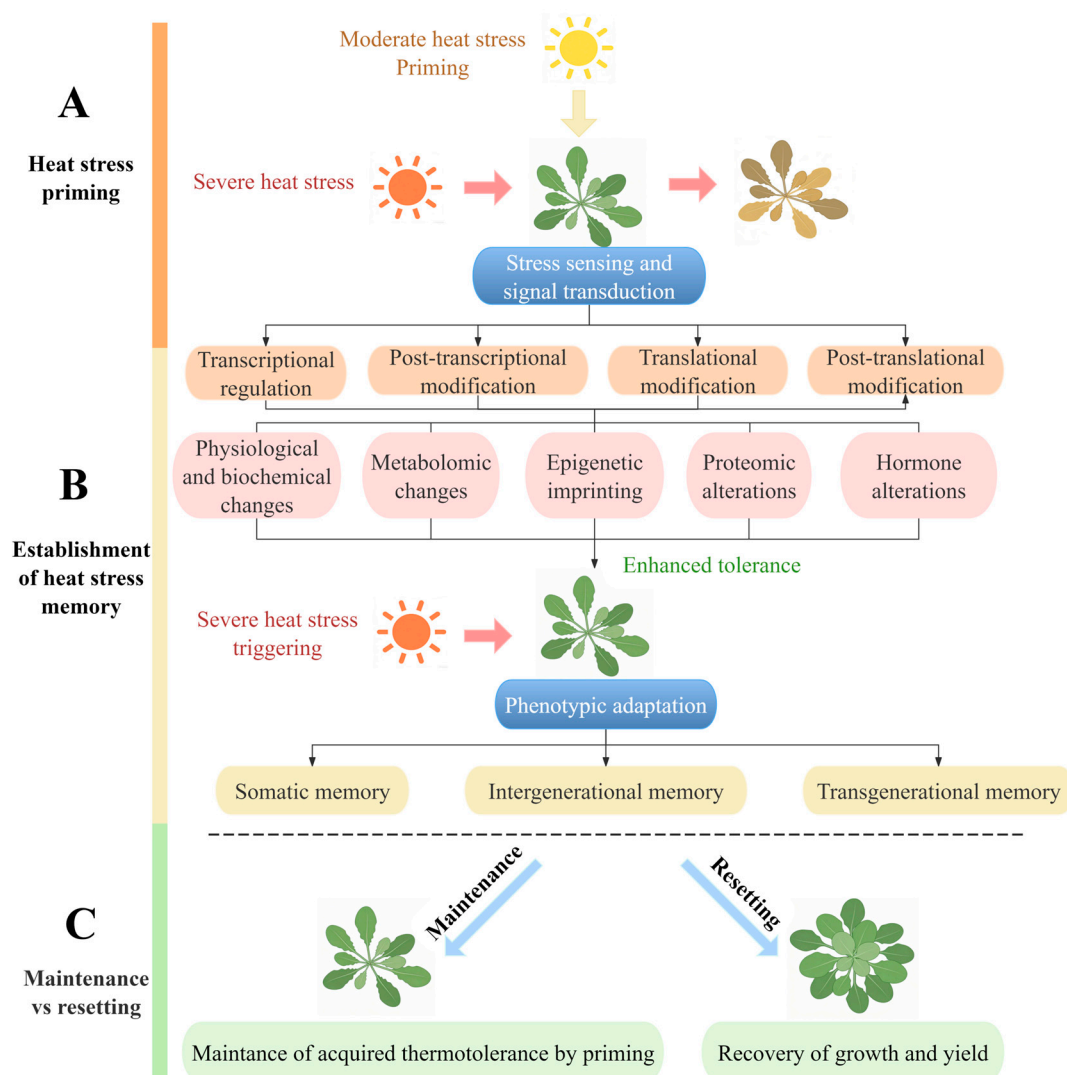


Figure 2. Schematic overview of the pathways and mechanisms for the acquisition of heat stress memory in *Arabidopsis*. **(A)** Heat stress priming: moderate heat stress (priming) triggers stress sensing and signal transduction, followed by transcriptional regulation, post-transcriptional modification, translational regulation, post-translational modification, physiological and biochemical changes, metabolomic changes, epigenetic imprinting, proteomic alterations, and hormone alterations. **(B)** Establishment of heat stress memory: upon severe heat stress triggering, the primed plants exhibit enhanced tolerance, phenotypic adaptation, and three types of memory—somatic memory, intergenerational memory, and transgenerational memory. **(C)** Maintenance vs. resetting: heat stress memory can be maintained through the maintenance of acquired thermotolerance by priming, or reset to allow recovery of growth and yield.

The duration of memory varies. Most memory-related characteristics in plants are transient and revert to pre-stress levels once heat stress subsides [6,34]. When memory persists only within a single generation, it is referred to as somatic memory (Figure 2B). For instance, HSM typically lasts up to 7 days in *Arabidopsis* seedlings [17,35]. In addition to somatic memory, heritable intergenerational memory and transgenerational memory have also been described (Figure 2B). During the maintenance of acquired thermotolerance, epigenetic modifications at loci of heat shock memory-related genes are altered, leading to sustained gene expression [6]. To a large extent, epigenetic HSM can be inherited by offspring, including intergenerational memory (affecting the F1 generation) and transgenerational memory (affecting F2 and F3 generations) [36,37].

Under native physiological homeostasis, activation of the HSR network redirects energy and resources toward stress tolerance processes, potentially resulting in impaired growth and development [38]. Although distinct from the constitutive stress response, stress memory is an induced state established after priming, and its persistence and intensity can strongly influence the plant's physiological state and energy allocation. During the recovery period following stress, most transcripts, proteins, metabolites, and physiological parameters return to the pre-stress state (Figure 2C). The duration and recovery dynamics of stress memory vary with developmental stage and species. In *Arabidopsis*, seedlings can reset *HSFA2*-associated H3K4me3 marks within 3 days, and the expression of memory-related genes such as *APX2* largely returns to baseline levels by 28 h of recovery [39]. In mature plants, however, the same memory genes (e.g., *APX2* and *HSP22*) require over 3 days for complete resetting of both epigenetic marks (e.g., H3K4me3) and expression levels, a process that depends on *CSN5A*-mediated clearance of histone modifications [40]. Heat stress memory in *Arabidopsis* typically persists for approximately 7 days [7]. By contrast, in wheat, heat stress memory acquired at the heading stage can persist through the grain-filling stage [41]. This resetting of defense mechanisms enables plants to prioritize growth and reproduction, while also serving as a key factor in ensuring plant survival and sustained growth under the challenging conditions imposed by climate change [7,42].

Among the multiple regulatory components involved in heat stress memory, HSFs emerge as central integrators linking acute stress perception to long-term adaptive responses.

3. The Plant HSF Family and Its Functions

HSFs act as central hubs in the trade-off between plant stress adaptation and growth development [32,43]. During the resetting of defense mechanisms, stress-induced molecules such as HSPs are degraded, stress signaling is terminated via HSF inactivation, and cellular metabolism and redox balance are re-established—collectively, these processes represent a critical step toward the restoration of homeostasis [44]. Multiple mechanisms can suppress HSF activity, including attenuation of upstream transcription factors, targeted degradation of transcripts, and alterations in epigenetic marks [44].

3.1. Introduction to the HSF Family

HSF genes have been identified in all plant species studied, and their genome-wide expression profiles under various abiotic stresses have been extensively characterized, revealing complex regulatory networks [9,45]. The HSF family of plants is large and highly diverse, functioning as key components of signal transduction pathways that mediate the expression of numerous stress-responsive genes [9]. Plant HSF proteins typically exhibit a highly conserved modular structure, including a DNA-binding domain (DBD), an oligomerization domain (OD, also known as the HR-A/B region), and a nuclear localization signal (NLS); some members also contain a nuclear export signal (NES) [9]. The DBD specifically recognizes and binds to the HSE in target gene promoters, thereby activating the transcription of stress-induced genes [46]. Based on amino acid sequence characteristics of the OD, plant HSFs are classified into three major classes: HSFA, HSFB, and HSFC. These classes are further subdivided into nine (A1–A9), five (B1–B5), and two (C1–C2) subclasses, respectively [9,47]. HSFB members possess a compact HR-A/B region, whereas HSFA and HSFC members contain longer HR-A/B regions with insertions of 21 (HSFAs) and 7 (HSFCs) amino acid residues between the HR-A and HR-B segments [47]. The C-terminal activation domain of HSFA contains short peptide motifs (AHA motifs), which are essential for their transcriptional activation activity but are absent in HSFB or HSFC proteins [45,48]. Notably, most HSFB family members (with the exception of HSFB5)

harbor a conserved C-terminal tetrapeptide LFGV, which functions as a repression domain [28]. To date, no canonical activator or repressor motifs have been identified in HSF members [43].

3.2. Functions of HSFs in Plant HSR

In various plant species, HSFs have been identified as key regulators of the HS response. They participate in temperature sensing, signal integration, and multi-layered regulatory mechanisms, functioning as central ‘controllers’ of the plant HSR [43,49]. HSFs also act as molecular bridges linking initial stress response with long-term stress memory. By coordinating immediate transcriptional reprogramming with sustained regulatory changes, they influence plant adaptation to HS and the establishment of memory, thereby enabling plants to better cope with long-term and recurring HS events [7].

3.2.1. HSFs Participate in HS Signal Perception and Transduction

RNA molecules adopt secondary and tertiary structures that are highly temperature-sensitive, enabling them to function as thermosensors [50]. Similar hairpin-like structures have been identified in the transcripts of *HSFA2*, *WRKY22*, and *PIF7*. Upon HS induction in plants, these structures melt, thereby allowing protein translation. This finding suggests that temperature-sensing mRNA secondary structures may be widespread in plants [51]. Temperature-dependent alternative splicing of HSF transcripts produces distinct isoforms that regulate thermal responses. This is exemplified by *RtHSFA2*, *RtHSFA9*, and *TaHSFA6e*. These examples establish alternative splicing as an additional layer of temperature sensing beyond protein-level control [52,53]. Hahn et al. [54] proposed a classic model for proteostasis-based heat sensing mechanisms: Under optimal temperatures, HSFs are bound and inhibited by the molecular chaperones HSP70 and HSP90. Upon acute HS, large amounts of misfolded proteins (displaying higher affinity for HSPs) accumulate, sequestering these chaperones and releasing HSFs, which subsequently translocate to the nucleus and activate transcription.

In *Arabidopsis*, upstream sensing-type HSFs (e.g., *HSFA1*) can act as primary heat sensors. Through prion-like domains (PrDs), HSFs undergo protein phase transition, directly sensing temperature changes and forming transcriptionally active condensates (speckles). These speckles bind to target genes, and promote chromatin looping, thereby enhancing gene expression [55]. HSFs can also function as ROS sensors, linking redox status to HS response [56–58]. For example, *HSFA4a* and *HSFA8* are proposed to act as redox-sensitive transcription factors under heat stress. They perceive oxidative changes in the cytoplasm via redox-sensitive cysteine residues, which triggers their translocation to the nucleus [57,58]. The activity of *PpHSFA2a* is regulated by the upstream Ca^{2+} -ROS signaling pathway: *PpCDPK29* phosphorylates serine residues within the nuclear localization signal (NLS) domain of *PpHSFA2a*, promoting its nuclear translocation and enhancing its binding affinity to downstream target genes encoding heat shock proteins, antioxidant enzymes, and osmolyte-related factors [59]. Additionally, He et al. [60] showed that nitric oxide (NO) serves as a signaling molecule in plant heat sensing. NO originates in the shoot apical meristem and triggers plant HSR by activating *HSFA2* expression via the GT-1 transcription factor, thereby initiating the HSR. Furthermore, the histone variant H2A.Z within nucleosomes plays a critical role in sensing ambient temperature. Binding of *Arabidopsis* *HSFA1a* to specific cis-elements under high temperature is required for Z-nucleosome depletion, resulting in the rapid eviction of H2A.Z from target genes [61]. Under non-inductive conditions, H2A.Z-containing nucleosomes help maintain transcriptional repression by limiting promoter and gene accessibility. Heat-induced histone dissociation and reduced H2A.Z occupancy increase chromatin accessibility, thereby facilitating the recruitment of additional transcription factors or cofactors during HS [62]. Taken together,

HSFs are widely involved in the perception and transduction of the HS signal through multiple pathways, including ROS and NO signaling, phase separation, and chromatin remodeling.

3.2.2. HSFs Participate in HSR Activation and Regulation

Plants employ a series of interconnected yet independent mechanisms to sense environmental temperature fluctuations and activate signaling cascades that regulate HSF-dependent gene networks [63]. At the transcriptional level, HSFs integrate multiple hormone signals, including abscisic acid (ABA), indole-3-acetic acid (IAA), gibberellin (GA), ethylene, and salicylic acid (SA), among others. They also cooperate with other transcription factors and regulatory proteins (e.g., DREB2A, DREB2B, bZIP, complex protein MBF1C, kinase CBK3) to activate HS response genes [64,65].

The HSFA1 subfamily function as the master regulator of the HSR, playing a pivotal role in activating the transcription of HS-induced genes. HSFA1 members trigger transcriptional cascades by inducing the expression of numerous downstream regulators, including other HSFs [66]. Upon HS-induced activation of HSFA1, additional HSFs are transcriptionally upregulated. Activated HSF subunits (either as homo-oligomers or in combination with transcription factors from other families) form hetero-oligomeric complexes. These complexes are phosphorylated and subsequently translocated to the nucleus, where they bind to HSP-containing promoters of HSP genes, thereby upregulating HSP expression and initiating the HSR [15,67].

In tomato, *HSFA1a* has been identified as the primary regulator of the HSR. *HSFA1a*, *HSFA2*, and *HSFB1* form a functionally interacting HSF triad that coordinates transcription during the HSR [9,68]. *Arabidopsis* HSFA1s (*HSFA1a/b/d/e*) act as master regulators of heat responses, with *hsfa1a/b/d* triple mutants showing loss of heat shock gene expression and thermotolerance, and quadruple mutants exhibiting severe growth defects, underscoring their functional redundancy and essential roles in growth. HSFA1s form oligomers and interact with HSP90, which negatively regulates their nuclear import [69]. *HSFA2* acts as the principal co-activator of *HSFA1a*. Whereas *HSFA1* is rapidly induced upon HS and is responsible for the initial activation of HSR genes, *HSFA2* accumulates during prolonged heat acclimation and extends AT, thereby promoting the establishment of plant HSM [70–72]. Downstream of HSFA1s, DREB2A and DREB2C function as key regulators of drought and heat responses. These factors directly activate *HSFA3* expression, thereby triggering multiple abiotic stress responses in plants. Consequently, *HSFA3* also plays an important role in heat acclimation [64,73]. *HSFA4* acts as a positive regulator of HSR gene expression, whereas HSFA5 functions as a specific inhibitor of HSFA4 activity. These two proteins can form a complex that modulates ROS homeostasis [74,75]. HSFA6b, as a downstream regulator of ABA-mediated stress signaling, is essential for thermotolerance [76]. In tomato, HSFA7 positively regulates thermotolerance by activating HSPs and antioxidant enzymes (e.g., ascorbate peroxidase), thereby reducing ROS accumulation [77].

In contrast, HSFB4a is a negative regulator of HS tolerance. In the heat-tolerant tomato cultivar ‘CLN’, HSFB4a transcripts are suppressed through Sly-miR4200-mediated degradation, relieving the inhibition on HSPs [77]. The function of plant HSFB1 exhibits marked functional divergence across species [47]. For instance, *Arabidopsis* HSFB1 acts as a negative regulator, repressing the expression of heat-inducible HSFs (e.g., *HSFA2*, *HSFA7a*, *HSFB1*, *HSFB2b*) and HSPs (e.g., *HSP101*, *HSP70*, *HSP90*) [78]. Controversially, tomato HSFB1 displays dual functionality, acting as both repressor and co-activator to modulate the transition from mild to severe HSR. Its co-activator function with HSFA1 depends on the recruitment of the histone acetyltransferase HAC1 [79,80]. To date, no activator or repressor motifs have been reported in HSFC members. Only a few studies

suggest that some HSFCs may function as transactivators, similar to HSFA, and participate in abiotic stress responses in various plant species [81,82]. Overall, the functional diversification of the HSF family reflects their evolutionary adaptation to HS in plants.

Beyond their established function in activating the heat shock response, specific HSF members also play pivotal roles in the establishment, maintenance, and resetting of heat stress memory, as detailed in the following sections.

4. Functions of HSFs During Plant HSM Formation

A variety of factors involved in the regulation of HSM have been identified, including HSFs such as *HSFA1*, *HSFA2*, *HSFA3*, and *HSFA7* [31,83,84]. In addition, several HSPs, including HSP18.2, HSP21, HSP22.0, HSP17.6c, HSP101, HSP90.0, and HSA32, function as molecular chaperones that contribute to the regulation of HSM [85–88]. As described below, HSFs play a central regulatory role in the establishment and maintenance of HSM by sustaining HSP expression and coordinating transcriptional and epigenetic regulatory mechanisms [32].

4.1. Roles of *HSFA1* in HSM

In *Arabidopsis*, *HSFA1* primarily contributes to the establishment of HSM by regulating *HSFA2* expression and/or forming regulatory complexes [89] (Table 1). *HSFA1a* modulates chromatin accessibility through three-dimensional reorganization of promoter–enhancer interactions, thereby playing a crucial role in the dynamic transcriptional regulation responses during HS [83]. Members of the *Arabidopsis* *HSFA1* subfamily (e.g., *AtHSFA1a/b/d/e*) function as master regulators of the HSR, and their deficiency significantly reduces both BT and AT [90] (Table 1). Recent evidence indicates that *HSFA1b* is also involved in regulating heritable transgenerational memory. Long-term HS suppresses *HSFA1b* expression, leading to the downregulation of *HSP70-3* and the promotion of E3 ligase SGIP1-mediated degradation of the heat-induced protein SUPPRESSOR OF GENE SILENCING 3 (*SGS3*), which in turn reduces tasiRNA accumulation [91] (Table 1). De-repression of the tasiRNA target *HEAT-INDUCED TAS1 TARGET 5* (*HTT5*) promotes early flowering but compromises immunity; notably, this altered physiological state can be transmitted across generations [91] (Table 1). In maize, ectopic overexpression of *HSFA1*-class genes *ZmHSF06* and *ZmHSF12* enhances both BT and AT when expressed in transgenic *Arabidopsis* plants [92,93] (Table 1).

Table 1. Regulatory mechanisms of heat shock transcription factor A1 (*HSFA1*) during the formation of plant heat stress memory.

Plant Species	Function	Stress Condition	References
<i>Arabidopsis thaliana</i> L.	<i>HSFA1</i> may participate in memory by forming heteromeric complexes or regulating downstream factors such as <i>HSFA2</i> .	Priming: 37 °C for 1 h; Recovery: 22 °C for 3 d to 7 d; A second heat stress: 37 °C for 1 h. The persistence of memory was tested by varying the duration of the recovery period.	[89]
<i>Arabidopsis thaliana</i> L.	The <i>hsfA1a/b/d/e</i> quadruple mutant shows defective HSP expression and significantly reduced BT, AT, and TMHT.	BT: 43 °C for 26 min; SAT: 37 °C for 1 h, 22 °C for 2 h, 44 °C for 140 min; LAT: 37 °C for 1 h, 22 °C for 2 d, 44 °C for 50 min; TMHT: 35 °C for 7 d.	[90]
<i>Arabidopsis thaliana</i> L.	The <i>HSFA1B</i> - <i>HSP70</i> module affects transgenerational temperature memory	The 10-day-old seedlings were treated at 30 °C for 14 consecutive days and	[91]

	by regulating the stability of the heat-in-then moved to 22 °C for seed harvest-induced key protein SUPPRESSOR OF GENE SILENCING 3 (SGS3). Unexposed progeny exhibits early flowering and reduced disease resistance.	ing. The progeny was grown at 22 °C for phenotype assessment.	
<i>Zea mays</i> L.	Overexpression of <i>ZmHSF12</i> in <i>Arabidopsis</i> enhanced both BT and AT. Overexpressing lines showed increased chlorophyll content and survival rate compared to wild-type seedlings under heat stress.	BT: 45 °C for 50 min, 22 °C for 8 d; AT: 37 °C for 1 h, 22 °C for 2 d, 46 °C for 1 h, 22 °C for 8 d.	[92]
<i>Zea mays</i> L.	Overexpression of <i>ZmHSF06</i> in <i>Arabidopsis</i> enhanced BT and AT, which also conferred enhanced drought tolerance and growth advantages.	BT: 45 °C for 1 h, 22 °C for 7 d; AT: 37 °C for 2 h, 22 °C for 2 d, 46 °C for 2 h, 22 °C for 7 d.	[93]

Note: BT, basal thermotolerance; AT, acquired thermotolerance; SAT, short-term acquired thermotolerance; LAT, long-term acquired thermotolerance; TMHT, thermotolerance to moderately high temperature.

4.2. Roles of HSFA2 in HSM

HSFA2, which is strongly induced by HS, serves as a key regulator of HSM at both physiological and transcriptional levels and plays a central role in memory establishment, maintenance, and resetting. In *Arabidopsis*, *hsfa2* knockout mutants exhibit impaired AT, reduced expression of memory-associated genes (e.g., *HSPs* and *HSA32*), and suppressed seed formation, seedling growth, root length, and reproductive development compared with Col-0, whereas both BT and growth under non-stress conditions remain largely normal [66]. The expression of *HSFA2* is directly regulated by *HSFA1*. While *HSFA2* is not essential for the early activation of AT, it positively regulates the expression of heat-induced genes following initial *HSFA1* activation, such as maintaining *HSP* gene expression to extend AT in *Arabidopsis* [12,66,70] (Table 2). In maize, Class A2 family members *ZmHSF05* and *ZmHSF04* also contribute to thermotolerance [94,95]. Overexpression of *ZmHSF05* enhances both BT and AT in *Arabidopsis*, whereas *ZmHSF04* overexpression positively regulates short-term acquired thermotolerance and improves plant tolerance to salt stress and ABA [94,95] (Table 2). In tomato, *SlHSFA2* acts as a co-activator of *HSFA1a* and is involved in AT regulation [71] (Table 2). Similarly, wheat *TaHSFA2-10* and tall fescue *FaHSFA2c* have been shown to positively enhance BT and MT [96,97] (Table 2).

Domain analysis indicates that *HSFA2* lacks a temperature-dependent regulatory domain (TDR), whereas the oligomerization domain (OD) regulates HSF complex composition and function. This domain promotes the formation of heteromeric complexes between *HSFA2* and other HSFs, such as *HSFA3* and *HSFA7B*, supporting long-term transcriptional activation [98] (Table 2). The mediator kinase module subunits, CYCLIN-DEPENDENT KINASE 8 (CDK8) and MEDIATOR 12 (MED12), are recruited by *HSFA2* to HSM genes under repeated HS. This recruitment immediately affects RNA polymerase II activity, promoting transcriptional hyper-induction [99]. Sustained expression of memory genes also relies on histone 3 lysine 4 (H3K4) hyper-methylation and the balance between activating H3K4me3 and repressive H3K27me3 [100,101]. Chromatin remodeling is an ATP-dependent process mediated by enzymes that alter histone–DNA connections. These remodelers utilize the energy derived from ATP hydrolysis to modulate histone–DNA interactions and regulate DNA accessibility through nucleosome assembly, sliding, eviction, and histone variant incorporation. Importantly, chromatin remodeling complexes are emerging as key factors in establishing and maintaining a permissive chromatin state

at memory-associated loci. During early HS, HSFA2 transiently binds to the promoters of HSM genes, thereby promoting H3K4me3 accumulation and increasing chromatin accessibility, enabling faster and stronger transcriptional responses upon subsequent heat stress encounters [12,89,102,103] (Figure 3A). HSFA2 can also directly activate the histone demethylase REF6, removing the repressive H3K27me3 mark from small HSPs (HSP22, HSP21, and HSP17.6c) associated with heat memory, facilitating transcriptional activation of memory-related genes [100] (Figure 3A). HSFA2 and REF6 form a positive, heritable feedback loop, coordinating with tasiRNA to induce and transmit transgenerational heat memory in *Arabidopsis*, ensuring reproductive success under repeated high-temperature conditions [37] (Table 2) (Figure 3B).

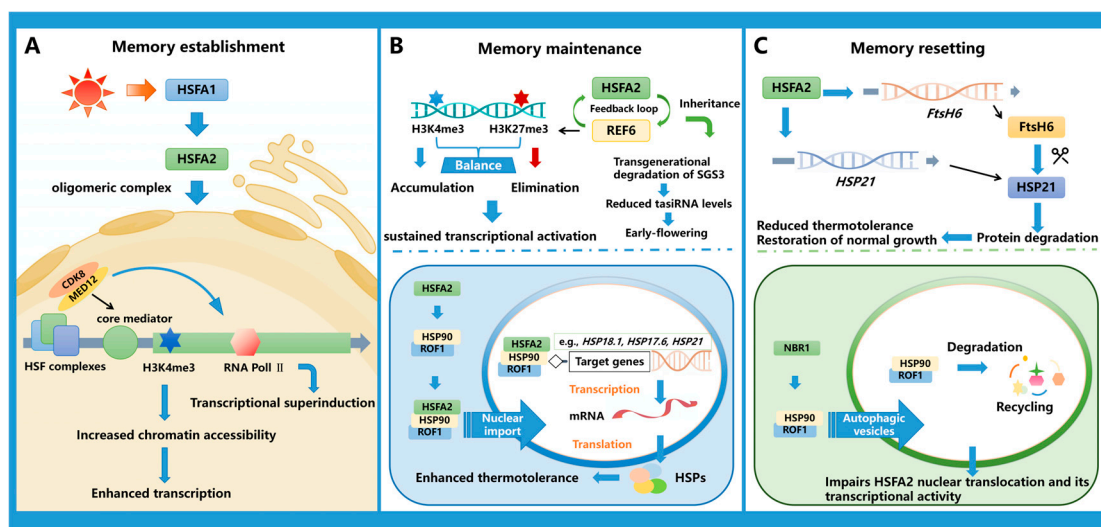


Figure 3. Schematic diagram of HSFA2 in regulating the establishment, maintenance, and resetting of heat stress memory in plants. (A) Memory establishment: Initial HS activates HSFA1 and its downstream target HSFA2. HSFA2 promotes memory gene transcription by recruiting the mediator kinase module (CDK8/MED12) to enhance RNA polymerase II activity, and facilitating H3K4me3 accumulation while activating REF6 to remove H3K27me3, thereby establishing an active chromatin state for rapid re-induction. (B) Memory maintenance: During recovery and repeated HS, HSFA2 forms a nuclear complex with HSP90.1-ROF1 to sustain sHSP (e.g., HSP21) expression for memory prolongation, while also activating FtsH6 to degrade HSP21 for fine-tuning memory intensity. Additionally, HSFA2 and REF6 form a heritable positive feedback loop and modulate tasiRNA pathways to contribute to transgenerational memory inheritance. (C) Memory resetting: The selective autophagy receptor NBR1 recognizes cytosolic HSP90.1-ROF1 complexes and mediates their autophagic degradation, thereby attenuating HSFA2-driven HSP expression. This process relieves growth suppression (restoration of normal growth) and reduces thermotolerance, achieving a balance between stress defense and growth recovery.

After HS exposure, HSFA2 interacts with HSP90.1, facilitating translocation of the ROF1-HSP90.1 complex to the nucleus [104]. The ROF1-HSP90.1-HSFA2 complex maintains continuous synthesis of small HSP (sHSP) during the recovery period [104] (Figure 3B). In responses to repeated HS, the selective autophagy receptor NBR1 (NEIGHBOR OF BRCA1) binds to cytosolic HSP90-ROF1 complexes and traffics them to autophagic vesicles for degradation, attenuating HSP expression regulated by the HSFA2. In this sense, loss-of-function mutations of NBR1 display an enhanced HS memory phenotype [105] (Table 2) (Figure 3C). Sustained high levels of the plastid-localized HSP21 are crucial for maintaining the primed state and the duration of heat memory, and HSFA2 can both induce and maintain HSP21 expression [106]. HSFA2 also regulates stability by activating the plastid metalloprotease FtsH6, which degrades HSP21 in vivo, thereby limiting HSM

capacity [107] (Table 2) (Figure 3B). Collectively, these complex feedback and regulatory loops illustrate how HSFA2 coordinates HSM activation and resetting, balancing the prolongation of heat memory with its timely termination.

Table 2. Regulatory mechanisms of heat shock transcription factor A2 (HSFA2) during the formation of plant heat stress memory.

Plant Species	Function	Stress Condition	References
<i>Arabidopsis thaliana</i> L.	HSFA2 primarily regulates the duration, not the initial induction, of AT.	37 °C for 1 h; Recovery at 24 °C for different durations (2 h~3 d); 44 °C for 45 min.	[70]
<i>Arabidopsis thaliana</i> L.	The functions of HSFA1 and HSFA2 are different and complementary. HSFA1a/b/d are crucial for response to sustained moderate heat stress above 27 °C, whereas HSFA2 plays a dominant role in SAT and LAT.	SAT: 37 °C for 1 h, 22 °C for 2 h, 44 °C for 155 min; LAT: 37 °C for 1 h, 22 °C for 2 d, 44 °C for 50 min; TMHT: 35 °C for 5 d.	[66]
<i>Arabidopsis thaliana</i> L.	HSFA2 transiently binds to and regulates a set of memory genes, inducing persistent chromatin state changes that enable faster and stronger expression upon secondary stress.	Priming HS: 37 °C for 1 h, 23 °C for 90 min, 44 °C for 45 min; Recovery phase: 23 °C for 2 d~3 d; Triggering HS: 44 °C for 80~120 min.	[12]
<i>Zea mays</i> L.	Overexpression of <i>ZmHSF05</i> in <i>Arabidopsis</i> seedlings improves both BT and AT.	BT: 45 °C for 50 min, 22 °C for 8 d; AT: 37 °C for 1 h, 22 °C for 2 d, 46 °C for 1 h, 22 °C for 8 d.	[94]
<i>Zea mays</i> L.	Overexpression of <i>ZmHSF04</i> in <i>Arabidopsis</i> enhances heat tolerance and positively regulates SAT. <i>ZmHSF04</i> also significantly improves tolerance to salt and abscisic acid (ABA) in transgenic <i>Arabidopsis</i> .	BT: 44 °C for 2 h; SAT: 37 °C for 1 h, 22 °C for 2 h, 44 °C for 220 min; LAT: 37 °C for 1 h, 22 °C for 2 d, 44 °C for 220 min.	[95]
<i>Solanum lycopersicum</i> L.	Tomato HSFA2 acts as a major co-activator of the master regulator HSFA1a, which was involved in acquired but not basal thermotolerance or responses to acute heat shock.	BT: 39/42/25 °C for 90 min, 25 °C for recovery; AT: 37.5 °C for 90 min, 25 °C for 3 to 24 h, 47.5 °C for 90 min, 25 °C for 5 d.	[71]
<i>Triticum aestivum</i> L.	Wheat <i>TaHSFA2-10</i> regulates the expression of <i>HSA32</i> and <i>HSP101</i> , improving both BT and AT in transgenic <i>Arabidopsis</i> seedlings.	BT: 45 °C for 50 min, 22 °C for 8 d; AT: 37 °C for 1 h, 22 °C for 2 d, 46 °C for 1 h, 22 °C for 8 d.	[96]
<i>Festuca arundinacea</i> Schreb.	Ectopic overexpression of <i>FaHSFA2c</i> improved BT and AT in wild-type <i>Arabidopsis</i> , and restored the heat-sensitive defect in <i>hsfa2</i> mutants, where the function of <i>FaHSFA2c</i> is specific in monocots.	BT: 45 °C for 4 h, 25 °C for 7 d; AT: 37 °C for 2 h, 25 °C for 2 d, 45/40 °C (day/night) for 3.5 d, 25 °C for 7 d.	[97]
<i>Arabidopsis thaliana</i> L.	The absence of the temperature-dependent regulatory domain (TDR) region on HSFA2, along with oligomerization domain (OD)-dependent complex formation and stable protein activity, constitutes a critical mechanism for its role in mediating heat stress memory.	Priming HS: 37 °C for 1 h, 23 °C for 1.5 h, 44 °C for 45 min; Recovery phase: 23 °C for 3 d; Triggering HS: 44 °C for 80~90 min.	[98]

<i>Arabidopsis thaliana</i> L.	HSFA2 recruits the CYCLIN-DEPENDENT KINASE 8 (CDK8) and MED12 (MED12) to memory genes, promoting RNA polymerase II activity for transcriptional hyper-induction upon recurring heat stress.	Priming HS: 37 °C for 60 min, 22 °C for 90 min, 44 °C for 45 min; Recovery phase: 22 °C for 3 d; Triggering HS: 44 °C for 70~120 min.	[99]
<i>Arabidopsis thaliana</i> L.	HSFA2 directly activates the H3K27me3 demethylase <i>RELATIVE OF EARLY FLOWERING 6</i> (<i>REF6</i>) under heat stress. <i>REF6</i> and HSFA2 form a heritable feedback loop in <i>Arabidopsis</i> to coordinate transgenerational heat stress memory.	The 10-day-old seedlings grown at 22 °C were treated at 30 °C for 14 d and then moved to 22 °C for seed harvesting. Progeny were grown at 22 °C for assessment.	[37]
<i>Arabidopsis thaliana</i> L.	Next-to-BRCA1 (<i>NBR1</i>) inactivates the rotamase <i>FKBP1</i> (<i>ROF1</i>)-HSP90-HSFA2 protective mechanism by binding to cytoplasmic HSP90- <i>ROF1</i> and transporting it to autophagic vesicles for degradation, thereby impairing thermal memory capacity.	Priming HS: 37 °C for 90 min, 22 °C for 90 min, 44 °C for 45 min; Recovery phase: 22 °C for 3~4 d; Triggering HS: 44 °C for 90 min.	[105]
<i>Arabidopsis thaliana</i> L.	HSFA2 binds to and activates the promoter of the plastid metalloprotease <i>FILAMENTATION TEMPERATURE-SENSITIVE H6</i> (<i>FtsH6</i>). <i>FtsH6</i> resets heat stress memory during the recovery phase by degrading HSP21 back to basal levels.	Priming HS: 37 °C for 1 h, 22 °C for 90 min, 44 °C for 45 min; Recovery phase: 23 °C for 4 d; Triggering HS: 44 °C for 90 min.	[106]

Note: BT, basal thermotolerance; AT, acquired thermotolerance; SAT, short-term acquired thermotolerance; LAT, long-term acquired thermotolerance.

4.3. Roles of HSFA3 in HSM

HSFA3 is also necessary for HSM in plants. HSFA3 is essential for long-term heat stress memory through epigenetic regulation of chromatin states, but plays only a limited, synergistic role in heat acclimation/priming [39,108]. In *Arabidopsis*, HSFA2 and HSFA3 play central roles in long-term acquired thermotolerance, functioning within a shared heat-responsive pathway but with limited functional redundancy [109] (Table 3). ChIP-qPCR confirmed that HSFA3 directly binds to HSE motifs in the promoters of *HSP22* and *APX2* independently of HSFA2 [39]. Additionally, HSFA3 interacts with HSFA2 and forms heteromeric complexes with other HSFs in vivo, targeting low-expressed, chromatin-open memory genes [39]. These HSFA2/HSFA3 complexes recruit histone methyltransferases to sustain H3K4me3 enrichment, thereby maintaining long-term activation of these memory genes [31,39] (Table 3). The HSFA2/HSFA3 complex directly activates Type I memory genes (sustained induction type), whereas HSFA2 alone mediates the regulation of Type II memory genes (enhanced re-induction type) [39]. However, promoter-swap experiments demonstrated that HSFA3 could partially substitute for HSFA2 when expressed under the HSFA2 promoter, as evidenced by partial rescue of the survival defects of *hsfa2* mutants under heat stress, albeit not to wild-type levels [39]. In barley, *HvHSFA2* and *HvHSFA3* are both required for HSM, with *HvHSFA2* exerting a stronger effect on HSM than *HvHSFA3* [110]. These two genes have additive effects, as double mutants showed more severe defects than single mutants. However, their target genes and regulatory mechanisms have diverged, and unlike *Arabidopsis*, *HvHSFA2* and *HvHSFA3* do not enhance H3K4me3 modifications of memory genes but mediate transcriptional memory by amplifying gene expression in response to recurrent HS [110] (Table 3). In *Lilium longiflorum*, *LIHSFA3A* and *LIHSFA3B* are involved in proline-mediated stress

response regulation [111]. Overexpression of *LIHSFA3A* improves both BT and AT in *Arabidopsis*, whereas *LIHSFA3B* overexpression enhances only AT, reflecting functional divergence among HSFA3 subtypes within the same species [111]. Similarly, ectopic overexpression of tomato *SIHSFA3* in *Arabidopsis* enhances BT and AT and is associated with a late-flowering phenotype; *SIHSFA3* appears to influence flowering indirectly, either by activating the flowering repressor MAF5 or through the regulation of downstream stress-responsive genes [112] (Table 3).

Table 3. Regulatory mechanisms of heat shock transcription factor A3 (HSFA3) during the formation of plant heat stress memory.

Plant Species	Function	Stress Condition	References
<i>Arabidopsis thaliana</i> L.	HSFA2 and HSFA3 play a central role in plant LAT by co-regulating the expression of sHSPs. HSFA2 and HSFA3 share the same heat regulatory pathway and exhibit low functional redundancy.	BT: 45 °C for 6 h, 22 °C for 5 d; SAT: 38 °C for 90 min, 22 °C for 2 h, 45 °C for 150 min; LAT: 38 °C for 90 min, 22 °C for 2 d, 45 °C for 60 min.	[109]
<i>Arabidopsis thaliana</i> L.	HSFA2 and HSFA3 form heteromeric complexes that selectively recruit histone methyltransferases to memory genes, driving H3K4me3 enrichment, which is crucial for maintaining sustained gene induction after heat stress (Type I memory).	Priming HS: 37 °C for 1 h, 23 °C for 90 min, 44 °C for 45 min; Recovery phase: 23 °C for 3 d or 4 d; Triggering HS: 44 °C for 70~130 min.	[31,39]
<i>Hordeum vulgare</i> L.	<i>HvHSFA2</i> and <i>HvHSFA3</i> specifically control heat stress memory with additive effects, mediating transcriptional memory not via enhanced H3K4me3 at memory genes, but by amplifying the transcriptional response to recurrent heat stress.	Priming HS: 38 °C for 2 h, 23 °C for 1 h, 44 °C for 1 h; Recovery phase: 23 °C for 2~3 d; Triggering HS: 44 °C for 3 h.	[110]
<i>Lilium longiflorum</i> Thunb.	<i>LIHSFA3A</i> and <i>LIHSFA3B</i> are involved in proline-mediated stress response regulation. Overexpression of <i>LIHSFA3A</i> improves both BT and AT in <i>Arabidopsis</i> , while overexpression of <i>LIHSFA3B</i> only improves AT.	BT: 22 °C for 5 d, 45 °C for 50 min, 22 °C for 7 d; AT: 22 °C for 5 d, 37 °C for 60 min, 22 °C for 2 h, 45 °C for 90~220 min, 22 °C for 7 d.	[111]
<i>Solanum lycopersicum</i> L.	Ectopic overexpression of <i>SIHSFA3</i> enhances BT and AT in transgenic <i>Arabidopsis</i> and leads to a late-flowering phenotype.	BT: 22 °C for 8 d, 43 °C for 1 h, 22 °C for 6 d; AT: 22 °C for 5 d, 37 °C for 1 h, 22 °C for 3 h, 47 °C for 1 h, 22 °C for 6 d.	[112]

Note: BT, basal thermotolerance; AT, acquired thermotolerance; SAT, short-term acquired thermotolerance; LAT, long-term acquired thermotolerance.

4.4. Roles of the Other HSFs in HSM

HSFA6b acts as a downstream positive regulator of the ABA signaling pathway, enhancing both basal and acquired thermotolerance by activating heat shock protein genes (e.g., *HSP18.1*, *APX2*) and the DREB2A-HSFA3 pathway. *HSFA6* overexpression lines exhibited significantly improved heat stress survival even without ABA pretreatment, whereas dominant-negative mutants showed severe thermotolerance defects even with ABA treatment [76] (Table 4). *HSFA7b* enhances thermotolerance by binding to the *EIN3* promoter to modulate ethylene homeostasis and promote HSM establishment at the shoot apical meristem [84] (Table 4). In tomato, *SIHSFA7* functions as a novel regulatory switch

for HsfA1a in a temperature-dependent manner, transitioning from a co-repressor at mild temperatures to a co-activator under severe heat stress, thereby enhancing acquired thermotolerance [113] (Table 4). Unlike HSFAs, which function as evolutionarily conserved positive regulators of thermotolerance across different plant species, members of the HSFB subfamily display species-specific differences and dual regulatory functions. In *Arabidopsis*, *AtHSFB1* and *AtHSFB2b* repress the HSR under optimal temperatures, but act as positive regulators of AT [78] (Table 4). In tomato, *SlHSFB1* exhibits both repressive and co-activator activities; overexpression enhances thermotolerance but is associated with growth abnormalities [80] (Table 4). Similarly, overexpression of Class B2 member *VpHSF1* from Chinese wild grape reduces BT but significantly enhances AT [114] (Table 4). Functional divergence of HSFB thermotolerance regulators across species likely stems from promoter variations that alter their expression and target specificity. These examples highlight the complex and context-dependent regulatory roles of HSFB members in balancing plant growth and stress responses. Notably, while HSFB members contribute positively to heat stress memory and thermotolerance, they often simultaneously restrain normal growth and development, which limits their utility in molecular breeding.

Table 4. Regulatory mechanisms of the other heat shock transcription factors (HSFs) during the formation of plant heat stress memory.

Plant Species	Function	Stress Condition	References
<i>Arabidopsis thaliana</i> L.	HSFA6b acts as a positive regulator in abscisic acid (ABA)-mediated drought, salt, and heat tolerance. The <i>hsfa6b</i> mutant is severely impaired in both BT and AT.	BT: 22 °C for 9 d, 44 °C for 25 min, 22 °C for 10 d; AT: 22 °C for 9 d, 37 °C for 1 h, 22 °C for 2 h, 44 °C for 190 min.	[76]
<i>Arabidopsis thaliana</i> L.	The transcription factor HSFA7b controls heat stress memory in shoot apical meristems by regulating ethylene biosynthesis and signaling.	Priming HS: 37 °C for 1.5 h, 22 °C for 1.5 h, 44 °C for 45 min; Recovery phase: 22 °C for 3 d; Triggering HS: 44 °C for 1.5 h.	[84]
<i>Solanum lycopersicum</i> L.	HSFA7 acts in a co-repression mode under mild heat stress, which switches to a co-activator for HSFA1a in a temperature-dependent manner to enhance AT in tomato at elevated temperatures.	BT: 30 °C to 45 °C for 1 h, 25 °C for 1.5 h; AT: 37.5 °C for 30 min, 25 °C for 3 h, 45 °C for 1 h, 25 °C for 1 d.	[113]
<i>Arabidopsis thaliana</i> L.	HSFB1 and HSFB2b repress general heat shock response under non-heat stress conditions but positively regulate AT.	BT: 42 °C for 80 min, 23 °C for 15 d; AT: 32 °C for 30 min, 23 °C for 2 h, 43 °C for 2 h, 23 °C for 15 d.	[78]
<i>Solanum lycopersicum</i> L.	HSFB1 is a transcriptional repressor that can also function as a co-activator for HSFA1a, enhancing protective capacity and balancing growth and stress responses. Plants with elevated HSFB1 expression show altered development but BT and AT.	BT: 39/42/25 °C for 90 min, 25 °C for recovery; AT: 37.5 °C for 90 min, 25 °C for 3–24 h, 47.5 °C for 90 min, 25 °C for 5 d.	[80]
<i>Vitis pseudoreticulata</i> Wang.	Overexpression of <i>VpHSF1</i> from grapevine in tobacco reduces BT but improves AT.	BT: 48 °C for 2.5 h, 23 °C for 8 d; AT: 40 °C for 3 h, 50 °C for 2 h, 23 °C for 8 d.	[114]

Note: BT, basal thermotolerance; AT, acquired thermotolerance.

5. Summary and Outlook

Global warming is increasing the frequency, intensity, and duration of heat waves, which may occur against the background of other stressful conditions such as drought, salinity or nutrient deprivation. Compared to acute heat shock caused by short-term extreme high temperatures, the impact of long-term, recurrent heat stress events on crop productivity and ecosystem stability is more profound and complex. Therefore, understanding plant adaptation and survival mechanisms under repeated high-temperature stress holds greater practical significance. Plant HSFs serve as central hubs in the trade-off between plant growth, development, and stress response. They not only provide immediate protection by activating classical pathways, such as those involving HSPs, but also play key roles in integrating multiple signals and regulating the establishment and maintenance of acquired thermotolerance. By mediating HSM, HSFs enable plants exposed to sublethal high temperatures to develop stronger resistance to subsequent, more severe stress.

Here, we summarized the mechanisms by which HSF family members contribute to plant heat stress adaptation, including advances in understanding their role in heat stress perception, HSR gene regulation, and HSM. HSFA members hold central regulatory status in heat stress responses across different species. Significant progress has been made in identifying HSFA members, analyzing their functions, and elucidating their regulatory pathways. However, knowledge regarding the HSFB and HSFC subfamilies remains conspicuously scarce. To date, their physiological roles, downstream targets, and functional logic within the thermotolerance network are largely speculative, with only fragmentary experimental evidence available. Likewise, the mechanisms regulating HSF activity are not fully elucidated, and key upstream regulatory factors still require systematic identification and validation.

However, most existing studies on HSFs are limited to a few model plants (e.g., *Arabidopsis*) under single-stress conditions, making it difficult to simulate complex and variable field stress environments (e.g., high temperature combined with drought, or with high salinity, or fluctuating stress intensities). Consequently, the transition of these findings into practical field applications is often very limited. HSF-mediated stress responses can also impose temporary growth inhibition penalties, highlighting the need to explore molecular regulatory strategies to balance stress resistance with growth and development. Future research should address these gaps by conducting multi-dimensional, cross-species studies to provide precise molecular targets for crop improvement. In addition, future research should focus on tissue-specific HSM mechanisms. Ultimately, efforts will facilitate the breeding of stress-resilient, high-yielding crop varieties adapted to climate change, thereby contributing to global food security and sustainable agricultural development.

Beyond fundamental mechanistic insights, future research should increasingly focus on translational applications of HSF-based thermotolerance strategies. Genome editing technologies, particularly CRISPR/Cas systems, provide unprecedented opportunities to precisely modulate HSF activity, by altering either regulatory domains, promoter elements, or upstream signaling components to fine-tune stress responsiveness. However, constitutive overexpression of key HSFs often results in growth penalties due to sustained allocation of resources toward stress defense. Therefore, future breeding strategies should prioritize context-dependent and inducible modulation of HSFs rather than simple overexpression approaches. Fine-tuning HSF expression levels, temporal activation, or tissue-specific regulation may allow the optimization of the growth–stress tolerance balance, ultimately enabling the development of climate-resilient crops without compromising yield potential.

Author Contributions: The authors confirm their contributions to the paper as follows: study conception and design: R.Z. and Y.G.; draft manuscript preparation: Y.G. and Y.S.; table and figure preparation: R.Z., Y.G. and Y.S.; reviewing and editing with valuable comments: G.C.; J.L., F.J., Z.W., R.M.R. and R.M. All authors have read and agreed to the published version of the manuscript.

Funding: This study was funded by Fundamental Research Funds for the Central Universities (KJYQ2025027) and China Agriculture Research System (CARS-23).

Data Availability Statement: No new data were created or analyzed in this study. Data sharing is not applicable to this article.

Acknowledgments: We acknowledge the funding from Fundamental Research Funds for the Central Universities (KJYQ2025027), the earmarked fund for China Agriculture Research System (CARS-23), and the Bioinformatics Center of Nanjing Agricultural University.

Conflicts of Interest: The authors declare no conflicts of interest.

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