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# Characterization of the heat shock factor *RcHsfA6* in *Rosa chinensis* and function in the thermotolerance of *Arabidopsis*

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## Abstract

**Background** Environmental stresses, especially high temperatures, severely limit the growth and development of many horticultural plants. As a woody ornamental flower with rich flower colors and flower types, rose (*R. chinensis)* leaves wilt and shriveled petals at high temperatures, which severely affects its growth and ornamental value. The defense mechanism of rose plants against high-temperature stress has not been fully elucidated.

**Results** In the present study, the transcriptomes of rose petals at normal (25 °C) and high (35 °C) temperature were compared. A total of 2519 differentially expressed genes (DEGs) were identified, including 1491 upregulated DEGs and 1028 downregulated DEGs. The plant hormone signal transduction pathway, especially the abscisic acid (ABA) signaling pathway, was the most enriched signaling pathway for DEGs in rose at high temperature. Heat shock factors (Hsfs), especially class A Hsfs, have been confirmed to be involved in thermotolerance mechanisms. Among the DEGs, eight genes were annotated as Hsfs, including 5 upregulated Hsfs at high temperature. *RcHsfA6* is rapidly induced by high temperatures and is a candidate regulatory factor in the plant ABA signaling pathway. Therefore, we focused on *RcHsfA6*. *RcHsfA6* encodes a protein containing 308 amino acids and contains typical Hsf domains, such as the DNA-binding domain (DBD), the N-terminal oligomerization domain (OD), the nuclear localization signal (NLS) and AHA motifs at the C-terminal activator domain (CTAD). The heterologous overexpression of *RcHsfA6* in *Arabidopsis* increased the thermotolerance of *Arabidopsis* seeds. In addition, *RcHsfA6* overexpression increased the ABA content and the expression of ABA biosynthetic gene *AtABI5* and signal transduction gene *AtPYL12*, thereby inhibiting the germination of *Arabidopsis* seeds under exogenous ABA conditions.

**Conclusions** Taken together, our results suggest that *RcHsfA6* is involved in the high-temperature response of rose and its heterologous overexpression in *Arabidopsis* increased the thermotolerance of *Arabidopsis* at high temperatures via the ABA signaling pathway.

**Keywords** *R. chinensis*, Transcriptome, Heat shock transcription factor, *RcHsfA6*, ABA, Thermotolerance

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## Background

Rose (*R. chinensis*) is an important horticultural plant that often encounters high temperatures during flowering, which severely affects its ornamental value. When encountering high temperatures, plants being sessile cannot cope with adverse environments by moving [1]. Therefore, it is necessary for plants to initiate their own complex regulatory mechanisms to acquire heat acclimation [2]. Transcription factors (Hsf) play an important role in regulating the response of plants to high temperatures [3, 4].

Plants *Hsfs* are structurally and functionally conserved throughout the eukaryotic kingdom. Typically, plant Hsf proteins can be subdivided into three major subclasses (A, B, and C), which contain a DNA-binding domain (DBD), an N-terminal oligomerization domain (OD), a nuclear localization signal (NLS), a nuclear export signal (NES), and a C-terminal activator domain (CTAD) [5]. In most plants, there is an activating factor AHA motif (Aromatic, hydrophobic and acidic amino acid residues) in the CTAD domain of class A *Hsfs*, which is the core of the activating domain [6]. The number of class A *Hsf* genes is greatest, and these genes play important roles in the response to heat stress and are mainly responsible for the transcriptional regulation of heat stress proteins, which has been reported in *Arabidopsis*, tomato and maize [6–9].

As positive regulators of environmental response genes, class A Hsfs increase the thermotolerance levels of plants by regulating the structural expression of heat shock proteins (HSPs) and other proteins [10–12]. When encountering high temperatures, plants rapidly induce the transcription and translation of *Hsf* genes and can further bind to the HSE (5'-nGAAnnTTCn-3' or 5'-nTTCnnGAAn-3') elements of the target *Hsp* genes to initiate a complex heat stress response signaling pathways [13, 14]. Class A *Hsfs* can be divided into A1 ~ A9, and their function in the plant stress response has been widely studied [15]. *HsfA1* ~ *HsfA3* have important functions in high temperature response, but their specific functions have some differences. *AtHsfA1* is a key regulator of the integration of light and temperature signals and that the regulatory mechanism of *HsfA1-PIF4* module-mediated thermomorphogenesis allows *Arabidopsis* to adapt better to high temperatures [16]. *AtHsfA2* is the only HSF protein that mediates heat stress memory (HS memory), which specifically performs important functions in HS memory and is strongly induced by *AtHsfA1* after HS [4]. *AtHsfA3* can be specifically activated by DREB2C to induce the expression of *Hsp* genes, further regulating the heat tolerance of *Arabidopsis*. In addition, *AtHsfA3* overexpression increases galactinol levels and oxidative stress tolerance [17, 18]. *HsfA4* has been found to play an important role in plant cadmium tolerance. For example, the overexpression of *TaHsfA4a* can improve tolerance to cadmium (Cd), whereas the knockout of *OsHsfA4a* in rice can lead to Cd hypersensitivity [19]. *HsfA5* and *HsfA7* are positive regulators of plant salt tolerance by regulating the expression of downstream genes to improve salt tolerance [20, 21]. *MdHsfA8a* promotes flavonoid accumulation by enhancing the transcription of the downstream flavonoid synthesis genes *MdMYB12*, *MdANS* and *MdFLS* and stimulating ROS clearance to increase drought tolerance [22]. Class A Hsfs not only plays an important defense role to abiotic stresses, but also acts important functions such as plant nitrogen metabolism, leaf development, pollen, flower bud, seed development [23]. *HsfA9* was found to be a negative regulator of seed dormancy depth during seed development [24]. Unlike other Class A *Hsf* genes, few studies have investigated the function of *HsfA6*, which has been found to play an important role in the ABA signaling pathway [25, 26]. *TaHsfA6e* responds to high temperatures by regulating the expression of its target gene *TaHsp70*, and *HsfA6b* is activated by *bZIP60* and participates in maize chlorophyll degradation [27, 28]. In barley, *HvHsfA6a* upregulates the expression of *Hsps*, promotes metabolite accumulation involved in stress amelioration, and then enhances antioxidative potential at high temperatures [29]. Currently, there are few studies on the function of the *Hsf* gene in rose. Our previous study revealed that *RcHsf17* could be rapidly induced by temperatures above 30 °C, especially at 42 °C. The heterologous overexpression of *RcHsf17* increased increased CAT and POD activity, thereby improving the heat resistance of *Arabidopsis*. Moreover, the heterologous expression of *RcHsf17* affects seed germination by attenuating the expression of endogenous JA synthesis genes *AtJAT3* and *AtJAZ5* in *Arabidopsis* [30]. Many studies have shown that ABA and MeJA play a crosstalk role in regulating plant development and stress response. ABA and MeJA synergistically regulate stomatal opening and closing of *Arabidopsis* guard cells by cytosolic alkalization and cytosolic Ca $2^{+}$ oscillation [31]. In *Nicotiana tobacum*, MeJA co-operated with ABA in phytochrome-mediated salt stress tolerance [32]. Morever, ABA and MeJA induce reactive oxygen species (ROS) production and removal mediated by NAD(P)H oxidase, forming a complex feedback regulation network to regulate the defense response of plants [33]. It remains unclear whether ABA is involved in hormone-mediated stress response in rose.

Abscisic acid (ABA) is an important plant hormone, and its signaling pathways are involved in the regulation of biotic and abiotic stresses in plants [34, 35]. Changes in endogenous ABA levels in plants are affected by many complex regulatory mechanisms. Current studies have shown that the ABA signaling pathway is involved in the development of embryos and seeds; seed dormancy;

germination; plant growth and development; and the plant response to various stresses [36–38]. *ABI4* (abscisic acid insensitive 4) and *ABI5* are two important transcription factors involved in ABA signal transduction, and *ABI4* and *ABI5* mutants are insensitive to ABA during seed germination [39]. *PYL5* encodes the receptor protein of ABA, and its overexpression can increase the sensitivity of *Arabidopsis* to ABA, which manifests as the inhibition of seed germination and root development [40]. ABI1 can interact with SnRK2s and dephosphorylate them, inactivate SnRK2 kinases, and then negatively regulate the seed germination process inhibited by ABA signaling [41]. In *Arabidopsis*, *HOS15* is a negative regulator of drought stress, the synthesis of ABA is significantly increased in the *hos15* mutant, and its drought resistance is increased [42, 43]. The ABA signaling receptor gene *RCAR5* is involved in regulating the cold resistance of *Arabidopsis* [44]. The germination of *RCAR5*-overexpressing *Arabidopsis* seeds is significantly reduced under low-temperature conditions, whereas the expression levels of the ABA response genes *ABI3*, *ABI4* and *ABI5* are significantly increased [45]. *CYP707A*, which dominates the catabolic and inactivation of ABA, is inhibited by high temperatures in sweet cherry fruits, resulting in a decrease in ABA levels, which in turn leads to slower process of ripening [34].

In this study, we performed comparative transcriptome analysis in rose at high temperatures to identify the key *RcHsf* genes, in which *RcHsfA6* might be a key potential effector at high temperatures. We characterized *RcHsfA6* and its function in response to high temperatures. The role of *RcHsfA6* in thermotolerance was examined via a heterologous expression system in *Arabidopsis*. These analyses provide new insights into the functional divergence of *RcHsfs* and provide a theoretical basis for the genetic engineering and breeding of rose.

## Materials and methods

### Plant materials

Roses (*R. chinensis* 'Semperflorens' cv. 'Slater's Crimson China') grown in greenhouse and resource nurseries at the Institute of Forestry and Pomology, Beijing Academy of Agriculture and Forestry Sciences, Beijing, China, were used for high-temperature treatment and the sampling of different tissues. The division and phenotypic description of flowers in the five stages of rose have been presented in our previous studies [30, 46]. The petals, young leaves, old leaves, stylus, anthers, stems, and roots of the roses were sampled and stored at -80°C after freezing in liquid nitrogen.

*Arabidopsis thaliana* ecotype Columbia grown on 1/2 MS basal media was used as the wild-type and heterologous transformation material. The seeds were stratified in the dark at 4 °C for 2 d to induce germination and then transferred to an artificial climate box at 23 °C. The light intensity of the artificial climate box was set at 4,800 lx, and the light cycle was 8 h dark/16 hours light. The relative humidity was approximately 60%.

### High temperature treatments of roses

Three-year-old potted rose seedlings grown in a greenhouse were transferred to an artificial climate box for high-temperature treatment. The parameters of the artificial climate box settings were the same as above. The high-temperature treatments were set at 31 °C, 33 °C, 35 °C, 37 °C, and 42 °C for 15 min, 30 min and 1 h, respectively. 25 °C was the control temperature. When the temperature was higher than 35 °C, roses could not complete the flowering process normally, the buds would wilt and the petals would curl. Therefore, we presumed that 35 °C was the key testing temperature to study the high temperature response of rose. Base on this, the petals stored at 35 °C were used as the material for transcriptome sequencing.

### Sample preparation and RNA-seq analysis

Rose petal samples at the full bloom stage were collected after treating with 25 °C (CK) and 35 °C (HT) for 1 h for RNA-seq analysis. The samples from at least three flowers were pooled, frozen in liquid nitrogen and stored at -80 °C until RNA extraction. Total RNA was isolated via a VeZol-Pure Total RNA Isolation Kit (Vazyme Biotech, Nanjing China). Transcriptome library construction and sequencing were performed as described in our previous study [47]. Six transcriptome profiles have been deposited in the China National GenBank DataBase (CNP0006864).

Differentially expressed genes (DEGs) were identified via the DESeq2 R package (version 1.20.0) on the basis of the threshold standard ($P$ value < 0.01 and |log2-fold change| >1.5). The expression of DEGs was analyzed via the fragments per kilobase of transcript sequence per million base pairs sequenced (FPKM) method.

### RT–PCR assay

Total RNA extraction and first-strand cDNA extraction were performed with a VeZol-Pure Total RNA Isolation Kit and a HiScript II 1st Strand cDNA Synthesis Kit (Vazyme Biotech, Nanjing China) according to the manufacturer's instructions. A Bio-Rad CFX96 PCR system (Bio-Rad, California, CA, United States) was used for qPCR amplification. SYBR™ Green I (Thermo Scientific, Waltham, MA, United States) was used in a 10 μL reaction mixture during qPCR amplification, which included 5 μL of SYBR™ Green I, 1 μL of forward primer (10 μM), 1 μL of reverse primer (10 μM), 1 μL of cDNA and 2 μL of $ddH_2O$. The sequences of the primers are listed in Table S1. The relative expression ratio of genes was calculated

via the $2^{-\Delta\Delta Ct}$ method [48]. All reactions were repeated three times.

#### Structure and phylogenetic analysis

The full-length cDNA sequence of *RcHsfA6* was cloned and sequenced (GenBank accession: PV089626, unreleased) from petal cDNA according to the methods of Kang et al. [30]. The sequences of the primers are listed in Table S1. GOR IV (http://npsa-pbil.ibcp.fr/cgi-bin/npsa_automat.pl?page=/NPSA/npsa_sopma.html) was used to predict the secondary structure of *RcHsfA6*.

To establish the molecular evolutionary relationship of *RcHsfA6*, a phylogenetic tree of *RcHsfA6* was constructed via the neighbor-joining method via orthologs from *Arabidopsis (Arabidopsis thaliana)* and tomato (*S. lycopersicum*) and then designed via ITOL (https://itol.embl.de/) [49, 50].

#### Subcellular localization and transactivation activity analysis

The full-length cDNA of *RcHsfA6* was amplified as described above and then integrated into the 5′ end of the super1300-GFP vector with a CaMV 35 S promoter and a C-terminal GFP Label protein to construct the *RcHsfA6* recombinant vector (*35 S*::RcHsfA6) via SpeI and KpnI. The vetor information was described in our previous study [30]. The sequences of the primers used for vector construction are listed in Table S1. Three-week-old *Arabidopsis* rosettes were used to extract protoplasts. The *Arabidopsis* protoplast transformation method was used for subcellular localization analysis, and the method was described in our previous studies [30, 51].

The yeast one-hybrid (Y1H) method was used for transcriptional activity analysis of RcHsfA6. *RcHsfA6* was integrated into the pGBKT7 vector via SalI and NdeI, and the pGBKT7-RcHsfA6 fusion vector was subsequently transformed into Y187 yeast cells (WEIDI, YC1020). The sequences of the primers used for vector construction are listed in Table S1. After being cultured on SD/-Trp media for 72 h, four well-growing monoclones were transferred to SD/-Trp media supplemented with X-α-gal for 48 ~ 72 h to observe whether the yeast cells turned blue. pGBKT7-p53 (fused with the p53 gene) was used as a positive control, and the pGBKT7 empty vector was used as a negative control [30].

#### Generation and high-temperature treatment of transgenic *Arabidopsis*

To explore the function of *RcHsfA6*, the GFP-tagged recombinant plasmid *35 S*::RcHsfA6 was heterologously transformed into *Arabidopsis* via the classical floral dip method [52]. Bolting and flowering *Arabidopsis* plants were used as heterologously transformed materials. The lateral tillers or branches of the seedlings were removed in time. *RcHsfA6*-overexpressing transgenic homozygous *Arabidopsis* plants were selected with 50 mg/L hygromycin. The identified homozygous *Arabidopsis RcHsfA6*-overexpressing transgenic lines were subjected to high-temperature treatment.

After vernalization, wild-type and transgenic seeds were germinated at 42 °C for 2 h and then transferred to 23 °C conditions for recovery. After 14 d, the phenotype and germination rates were analyzed. Moreover, wild-type and transgenic seedlings were cut, immediately frozen in liquid nitrogen and stored at -80 °C for RT−PCR and ABA content determination.

#### ABA treatment

The seeds of the wild-type and transgenic lines were cultured on 1/2 MS basal media plates supplemented with 0.1 μM ABA. The phenotype was photographed, and the germination rate was determined after 14 days. Moreover, the seedlings were sampled to determine the gene expression profiles.

#### Statistical analysis

Mean values between different wild-type and transgenic lines were defined as significant when the value was lower than or equal to 0.05 probability. The statistical analysis of all the data were performed via SPSS Statistics 26 (IBM Corp. Armonk, NY, USA) via Duncan's test, and differences at $p < 0.05$ were labeled for the statistical tests [30]. Each experiment was repeated three times.

## Results

#### Transcriptome sequencing, assembly and DEG analysis

A total of 147,453,694 reads were produced from three cDNA libraries of *R. chinensis* under the 25 °C control (CK), and 141,334,292 reads were produced from three cDNA libraries of *R. chinensis* under the 35 °C high-temperature (HT) treatment (Table 1). The six sets of reads were subsequently aligned to the reference genome, and the mapping ratios ranged from 88.88 to 90.62% (Table S2). The average GC content in CK and HT was 46.54%. The Q20 and Q30 base percentages were greater than 98.00% and 93.81%, respectively (Table 1). A total of 2519 DEGs were identified (DESeq2 padj < = 0.01; |log2FoldChange| >=1.5), including 1491 upregulated DEGs and 1028 downregulated DEGs in HT (Table S3).

According to the GO enrichment analysis, 2519 DEGs were categorized into 667 functional groups, of which 1491 upregulated DEGs were categorized into 562 functional groups and 1028 downregulated DEGs were categorized into 483 functional groups (Fig. 1A). Among the DEGs, the number of genes clustered into molecular function categories was the greatest, and the number of genes clustered into cell component modules was the smallest (Fig. 1A). In the GO enrichment functional

**Table 1** Summary of the results of transcriptome sequencing of *R. chinensis* in the CK and HT treatments

| Samples | Raw reads | Clean reads | Mapped reads | Mapped ratio (%) | GC_pct | Q20 | Q30 |
|---|---|---|---|---|---|---|---|
| CK1 | 49,112,442 | 47,835,272 | 43,134,869 | 90.17% | 47.16 | 97.16 | 92.76 |
| CK2 | 49,078,720 | 48,051,502 | 42,976,760 | 89.44% | 46.34 | 97.38 | 93.07 |
| CK3 | 49,262,532 | 48,148,212 | 42,797,489 | 88.89% | 46.33 | 97.16 | 92.59 |
| Total | **147,453,694** | **144,034,986** | **128,909,118** | | | | |
| HT1 | 48,543,532 | 47,600,406 | 42,307,919 | 88.88% | 45.53 | 97.17 | 92.56 |
| HT2 | 46,417,320 | 45,342,000 | 40,901,507 | 90.21% | 46.69 | 97.3 | 92.93 |
| HT3 | 46,373,440 | 44,786,420 | 40,587,361 | 90.62% | 47.16 | 97.31 | 92.9 |
| Total | **141,334,292** | **137,728,826** | **123,796,787** | | | | |

groups, the number of upregulated genes was greater than the number of downregulated genes, except for genes related to DNA-binding transcription factor activity and transcription regulation activity. In the biological process category, only the upregulated genes were enriched in the protein folding category (Fig. 1A).

According to the KEGG pathway analysis of the DEGs, the significantly enriched pathways differed between the 1491 upregulated DEGs and the 1028 downregulated DEGs. Among the upregulated DEGs, significantly enriched pathways were putatively identified as 'protein processing in endoplasmic reticulum' and 'plant hormone signal transduction' (Fig. 1B). However, 'plant hormone signal transduction', 'biosynthesis of various plant secondary metabolites', and 'MAPK signaling pathway' were significantly enriched in the KEGG pathway among the downregulated DEGs (Fig. 1C). Plant hormones are signaling compounds that regulate key aspects of plant growth, development, and environmental stress responses. The enrichment results of the 'plant hormone signal transduction' pathway among the DEGs revealed the important function of hormones in response to high temperature in rose. ABA- and auxin-related genes were significantly enriched in the 'plant hormone signal transduction' pathway, including 11 upregulated genes and 7 downregulated genes in HTs, among which the difference in the transcription of abscisic acid insensitive *ABI5* (LOC112170122) and abscisic acid receptor *PYL12* (LOC112202757) was the most significant between CK and HT (Table 2). Therefore, we speculated that the ABA signal transduction pathway may play an important role in the response of rose petals to high temperature.

### Identification of transcription factors involved in the heat stress response of petals

To explore the possible transcriptional regulatory mechanism under high temperatures, we predicted the transcription factors (TFs) in upregulated and downregulated DEGs, respectively. Among the upregulated DEGs, 318 genes were predicted as transcription factors. while 210 genes were predicted as transcription factors within downregulated DEGs (Fig. 2).

As an important transcriptional regulator in heat stress response, heat shock factor (HSF) would quickly deploy the adaptive mechanism to cope with high temperature stress. To study the transcriptional regulatory mechanism under high temperatures in rose, Hsf genes were focused. Among the DEGs, eight TFs were annotated as Hsf genes, including 5 Hsfs among the upregulated TFs (Fig. 2A) and 3 Hsfs among the downregulated TFs (Fig. 2B). The expression levels of 8 Hsf genes under high-temperature conditions were detected via RT–PCR, and the results were consistent with those for the transcripts (Fig. 2C, D). By annotating 8 Hsf genes, we found that the *RcHsfA6* (LOC112172194) gene was significantly highly expressed at high temperature. In this study, we found that the plant hormone signal transduction pathway was the most enriched signaling pathway for DEGs in rose under high temperature. Therefore, we speculated that *RcHsfA6* may be involved in the high-temperature response mediated by plant hormones.

### Isolation and characterization of *RcHsfA6* in *R. chinensis*

The full-length cDNA sequence of *RcHsfA6* was obtained from the petals of *R. chinensis*. RcHsfA6 is 308 amino acids in size and consists of three main structures: α-helixes (45.13%), β-sheets (4.22%), and random coils (50.65%) (Fig. 3A). Phylogenetic analysis revealed that RcHsfA6 is closely related to AtHsfA6 and SlHsfA6 (Fig. 3B). RcHsfA6 contains a DNA-binding domain, an HR-A/B region with 21 amino acid residues inserted, an NLS, and an AHA activation domain in the CTAD region (Fig. 3C). Multiple sequence alignments with *Arabidopsis* and tomato HsfA proteins revealed that RcHsfA6 had 55.9% and 61.2% sequence similarity to AtHsfA6 and SlHsfA6, respectively (Fig. 3C). RcHsfA6 is highly conserved in the DBD, OD and NLS structures with AtHsfA6 and SlHsfA6 but differs greatly in the AHA domain, which was different from another subfamily A2 Hsf gene *RcHsf17* in rose [30]. RcHsf17 has the AHA domain and transcriptional self-activation function. Base on the structural feature of RcHsfA6, we speculated that *RcHsfA6* may not have a transcriptional self-activation function.

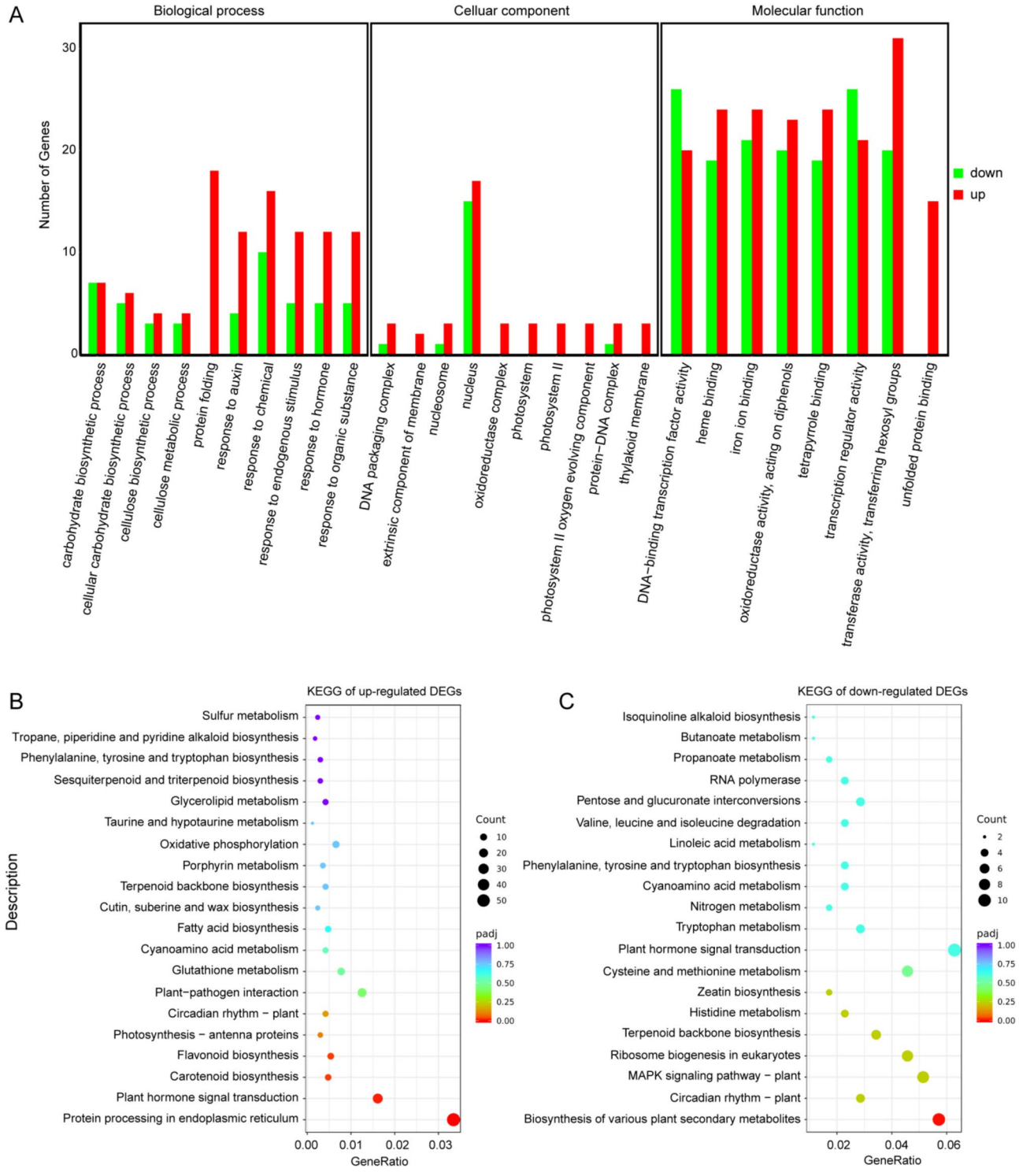


**Fig. 1** GO and KEGG enrichment analyses of DEGs in the CK and HT treatments. (**A**) GO enrichment analysis of DEGs in the CK and treatment groups. (**B**) KEGG enrichment analysis of upregulated DEGs in HT. (**C**) KEGG enrichment analysis of downregulated DEGs in HT

### Expression profiles of *RcHsfA6*, localization, and transcriptional activity

To explore the spatiotemporal expression patterns of *RcHsfA6*, the expression of *RcHsfA6* at different developmental stages and in different tissues of roses was detected via RT−PCR. The results indicated that *RcHsfA6* expression increased at the late blooming stage (S5) of flower development (Fig. 4A). However, *RcHsfA6* had the

**Table 2** Summary and transcripts of DEGs enriched in the plant hormone signal transduction pathway

| Differently expression genes | Gene name | Gene description | Transcripts of CK | Transcripts of HT | Fold change |
|---|---|---|---|---|---|
| Upregulated DEGs | ***LOC112170122*** | **Abscisic acid insensitive 5 (ABI5)** | **3.584609533** | **61.50842543** | **17.15903082** |
| | *LOC112196014* | Auxin-induced protein 22D | 3.652210697 | 46.74944422 | 12.80031414 |
| | *LOC112186843* | Auxin-induced protein 15 A-like | 2.300072432 | 20.54741292 | 8.933376461 |
| | *LOC112190257* | Auxin-responsive protein IAA29-like | 0.791386341 | 6.92818622 | 8.754493043 |
| | *LOC112188016* | Auxin-responsive protein IAA17-like | 1.672180887 | 10.56599219 | 6.318689724 |
| | *LOC112180833* | Indole-3-acetic acid-induced protein ARG7-like | 5.028679225 | 29.79743574 | 5.925499402 |
| | *LOC112200817* | Auxin-responsive protein IAA14-like | 172.6511978 | 851.3692223 | 4.931151553 |
| | *LOC112173536* | Auxin-responsive protein IAA11 | 4.468081282 | 19.01044476 | 4.254722232 |
| | *LOC112189436* | Serine/threonine-protein kinase BSK7 | 2.481615424 | 9.746686137 | 3.927557043 |
| | *LOC112189737* | Protein TIFY 10a | 11.34964016 | 40.78514488 | 3.593518762 |
| | *LOC112188720* | Protein TIFY3-like | 10.5869116 | 30.71147486 | 2.900890838 |
| Downregulated DEGs | ***LOC112202757*** | **Abscisic acid receptor PYL12-like** | **26.72614235** | **1.728406884** | **15.46287659** |
| | *LOC112186569* | BTB/POZ domain and ankyrin repeat-containing protein NPR1 | 8.64937282 | 1.076326785 | 8.862120451 |
| | *LOC112188712* | Transcription factor TGA4 | 1.951627562 | 0.221672378 | 8.804108019 |
| | *LOC112180920* | Auxin-responsive protein SAUR36-like | 25.65832839 | 3.602946778 | 7.121484153 |
| | *LOC112170562* | Probable indole-3-acetic acid-amido synthetase GH3.1 | 233.3676639 | 66.38401246 | 3.515419681 |
| | *LOC112179087* | Serine/threonine-protein kinase SAPK3-like | 35.30351731 | 12.26066057 | 2.879413968 |
| | *LOC112169890* | Transport inhibitor response protein | 26.71871325 | 9.341982568 | 2.86006884 |

highest expression in old leaves and the lowest expression in petals (Fig. 4B).

Subcellular localization of RcHsfA6 was analyzed in *Arabidopsis* protoplasts. The GFP-tagged recombinant plasmid *35 S*::RcHsfA6 was conducted by seamless cloning (Fig. 4C), and then was transformed into *Arabidopsis* protoplasts to observe the GFP fluorescence signal. The results revealed that the green fluorescence signal of the *35 S*::RcHsfA6 fusion protein was emitted only in the nucleus, indicating that RcHsfA6 was localized in the nucleus (Fig. 4D).

Yeast one-hybrid was used to analyze the transcriptional activity of RcHsfA6. The recombinant plasmid pGBKT7-RcHsfA6 was transformed into Y1H yeast cells, which were spread on SD/-Trp media for 48 ~ 72 h, after which four well-grown monoclonals were streaked on SD/-Trp media supplemented with X-α-gal. After 24 ~ 48 h, the yeast cells were observed to turn blue and photographed. As shown in Fig. 4E, yeast cells transformed with pGBKT7-p53 were blue, but those transformed with pGBKT7-RcHsfA6 and pGBKT7 were not blue. On the basis of these results, RcHsfA6 had no self-activation activity.

### Expression profile of *RcHsfA6* under high temperatures

To study the function of *RcHsfA6* in the high-temperature response, three-year-old potted rose seedlings in bloom stage were treated with 31 °C, 33 °C, 35 °C, 37 °C, and 42 °C. The expression profiles of *RcHsfA6* in petals of rose were detected. The results revealed that *RcHsfA6* rapidly increased at 31 °C for 30 min (Fig. 5A). When the treatment temperature was greater than 33 °C, the expression of *RcHsfA6* significantly increased at 15 min. When the plants were subjected to high temperatures for 1 h, the expression of *RcHsfA6* began to decrease (Fig. 5B-E). To further explore the expression profiles of *RcHsfA6* after 1 h of high-temperature treatment, we increased the treatment time at 42 °C to 48 h. The results revealed that the expression of *RcHsfA6* gradually decreased at 42 °C and that almost no expression was detected after 3 h. The above results indicated that *RcHsfA6* was rapidly induced by high temperatures and that its expression decreased rapidly with increasing high-temperature treatment time.

### Generation of *RcHsfA6*-overexpressing Transgenic homozygous *Arabidopsis*

To investigate the multiple functions of *RcHsfA6* in response to high temperatures, the GFP-tagged recombinant plasmid *35 S*::RcHsfA6 was transformed into *Arabidopsis*. Three *RcHsfA6*-overexpressing transgenic homozygous lines were obtained after screening with 50 mg/L hygromycin (Fig. 6A). By extracting the protoplasts of three transgenic homozygous lines and the wild type, we determined that the GFP was expressed in the nucleus of the *35 S*::RcHsfA6 transgenic line (Fig. 6B). The expression of GFP protein indicated that *RcHsfA6* was successfully expressed in *Arabidopsis*. PCR and qRT−PCR were used to further verify the expression of *RcHsfA6* in three *35 S*::RcHsfA6 transgenic lines (Fig. 6C, D). On the basis of the above

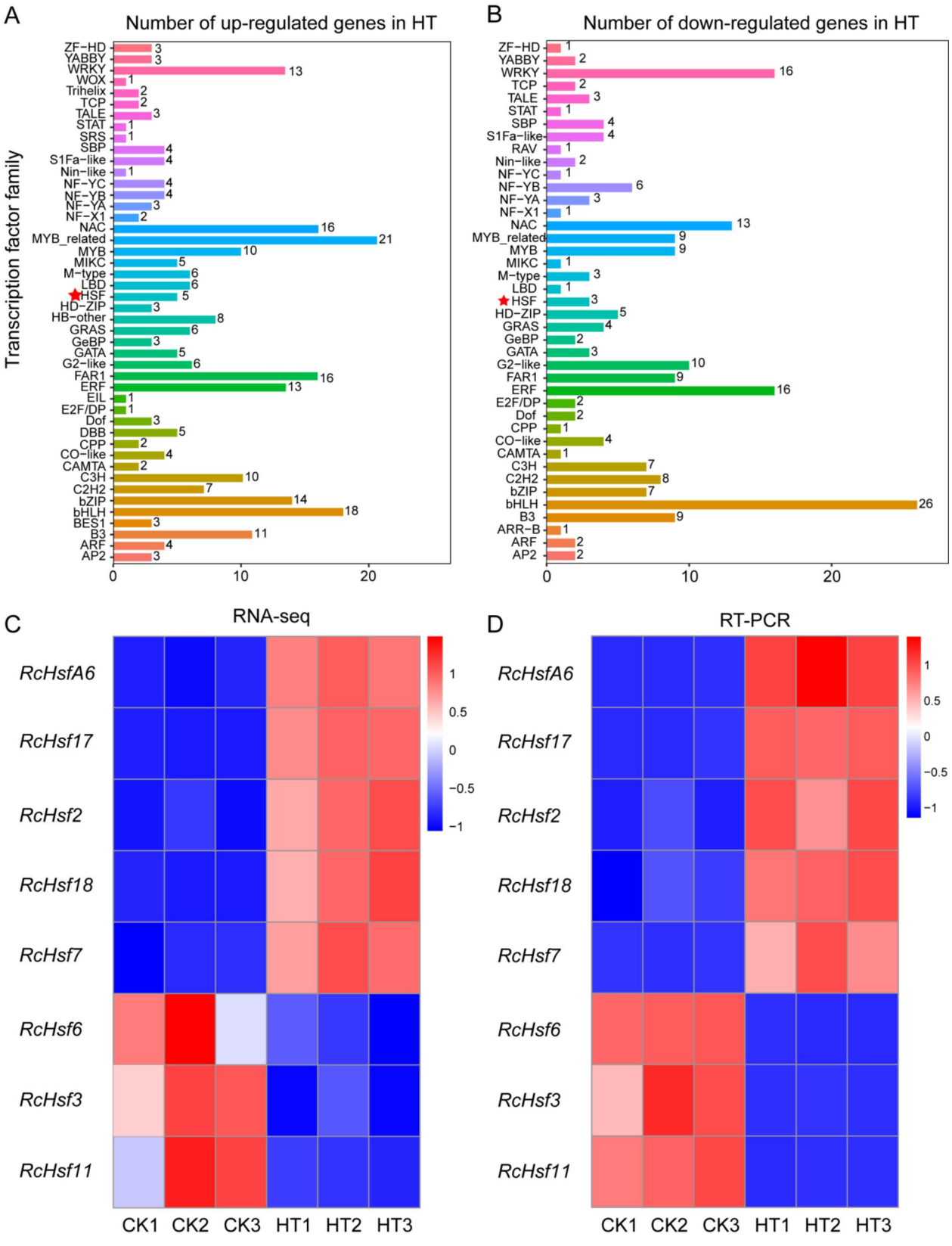


**Fig. 2** Transcription factor annotation and expression of DEGs. (**A**) Transcription factor annotation of upregulated genes in HT. (**B**) Transcription factor annotation of downregulated genes in HT. (**C**) RNA-seq heatmap of 8 Hsfs among the DEGs. (**D**) RT–PCR analysis of the expression of 8 Hsfs in CK and HT

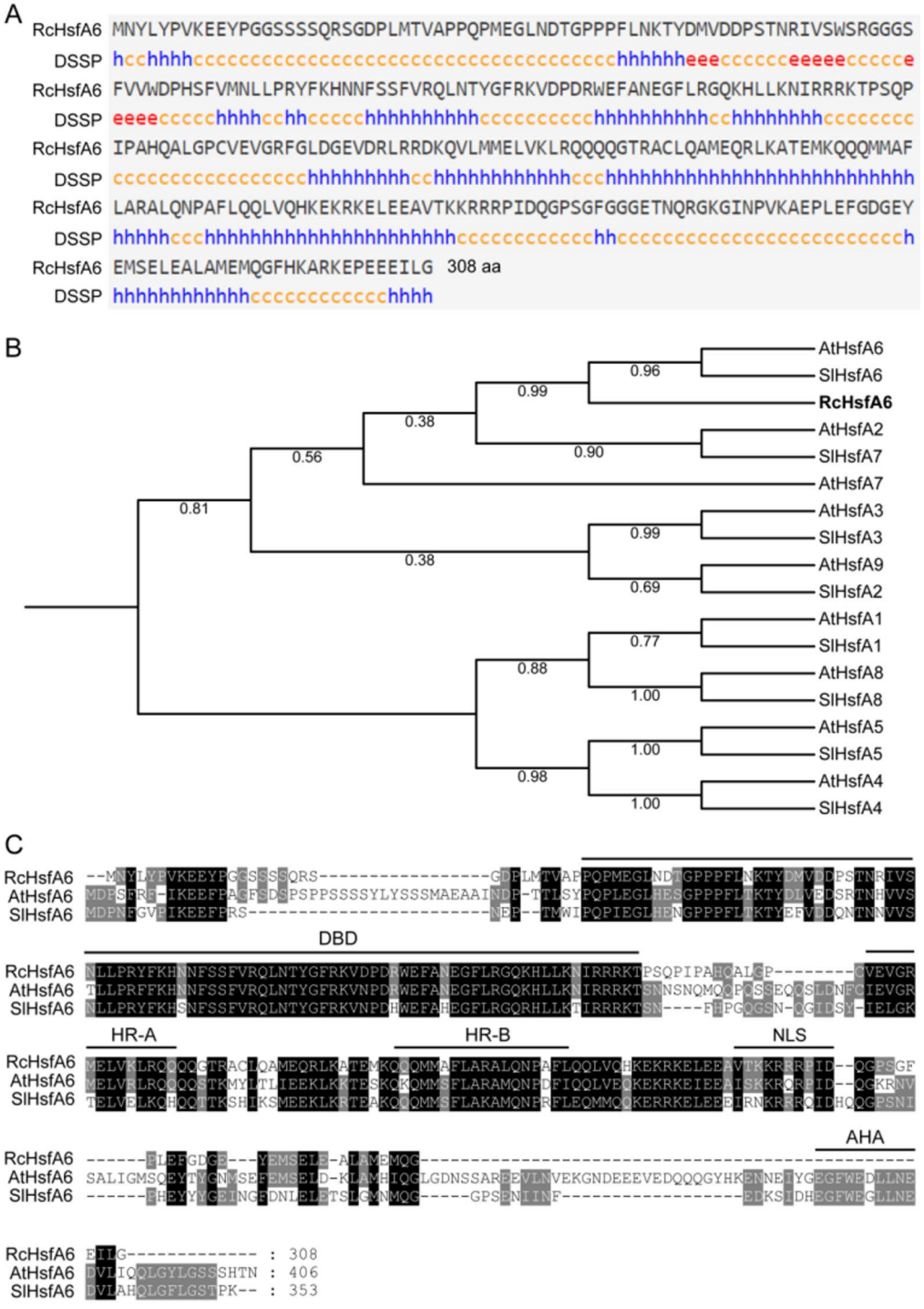


**Fig. 3** Structures and phylogenetic analysis of the RcHsfA6 protein. (**A**) Prediction of the secondary structure of RcHsfA6 by GOR IV. h: α-helixes; e: β-strands (extended strands); c: random coils. DSSP: Dictionary of Protein Secondary Structure. (**B**) Phylogenetic tree of RcHsfA6 (PV089626, unreleased), AtHsfAs (AtHsfA6, AT5G54070; AtHsfA2, AT2G26150; AtHsfA7, AT3G51910; AtHsfA3, AT5G16820; AtHsfA9, AT4G18870; AtHsfA1, AT4G17750; AtHsfA8, AT1G67970; AtHsfA5, AT4G13980; AtHsfA4, AT4G19630), and SlHsfAs (SlHsfA6, Solyd09g069710; SlHsfA7, Solyd08g060630; SlHsfA3; SlHsfA2, Solyd07g059160; SlHsfA1, Solyd08g068180; SlHsfA8, Solyd09g062650; SlHsfA5, Solyd12g072820; SlHsfA4, Solyd07g069210). (**C**) Multiple sequence alignments of RcHsfA6, AtHsfA6, and SlHsfA6

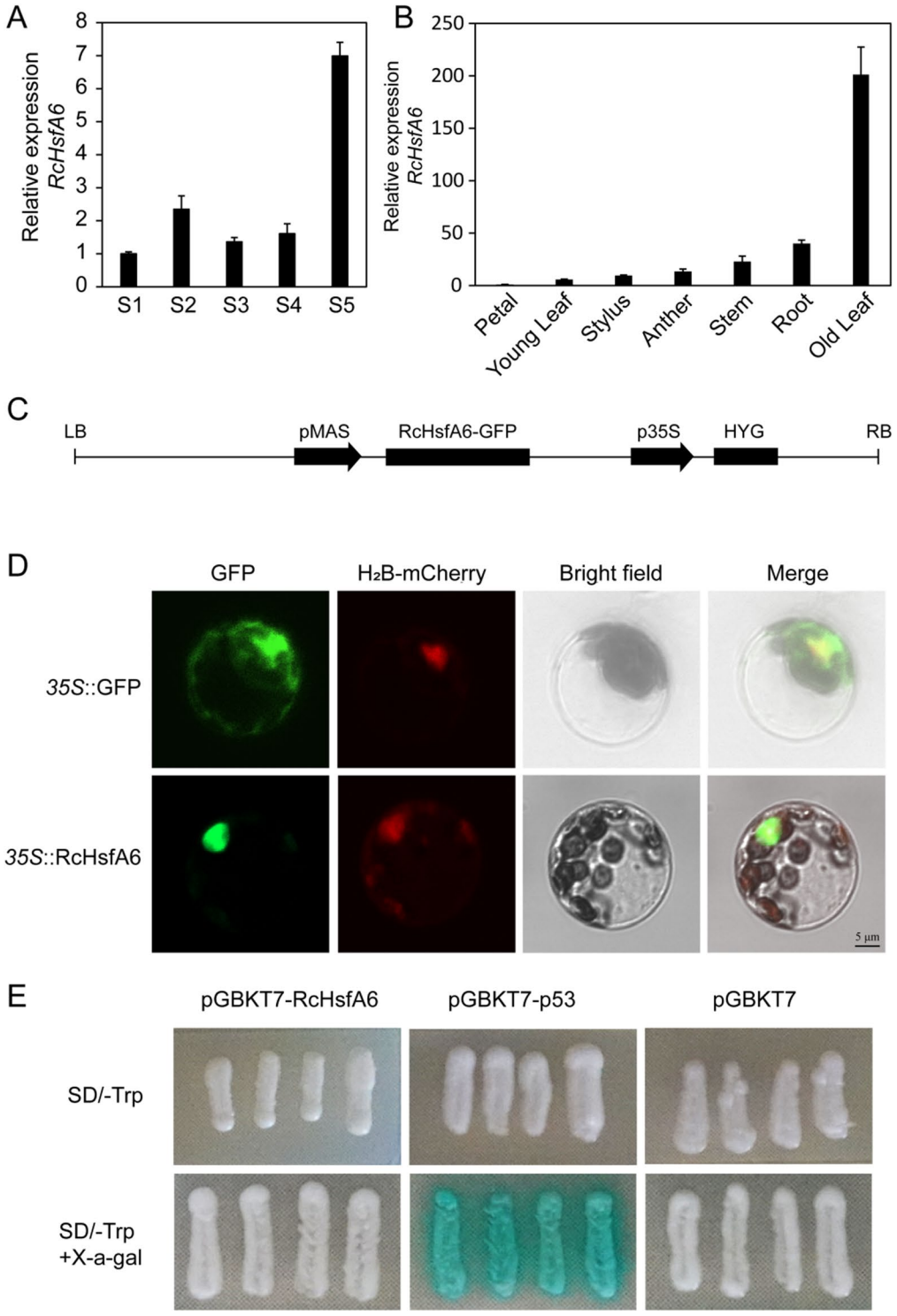


**Fig. 4** Expression profiles and localization and transcriptional activity of RcHsfA6. (**A**) Expression of *RcHsfA6* in the 5 stages of flower development. (**B**) Expression of *RcHsfA6* in different tissues. The analyzed tissues included the root, stem, old leaf, young leaf, petal, anther, and stylus. (**C**) The pictorial diagram of the GFP-tagged recombinant plasmid 35 S::RcHsfA6. pMAS: MAS promoter; GFP: GFP protein; p35S: CaMV 35 S promoter; HYG: Hygromycin. (**D**) Subcellular localization of RcHsfA6. GFP: *35 S*::RcHsfA6 fusion protein with GFP; $H_2B$-mCherry: $H_2B$ fusion protein with RFP protein (nucleus marker). Scale bar: 5 μm. (**E**) Transcriptional activity assay of RcHsfA6. SD/-Trp: SD basal media without tryptophan, SD/-Trp + X-a-gal: SD basal media without tryptophan and containing X-a-gal. pGBKT7-p53 and pGBKT7 were used as positive and negative controls, respectively

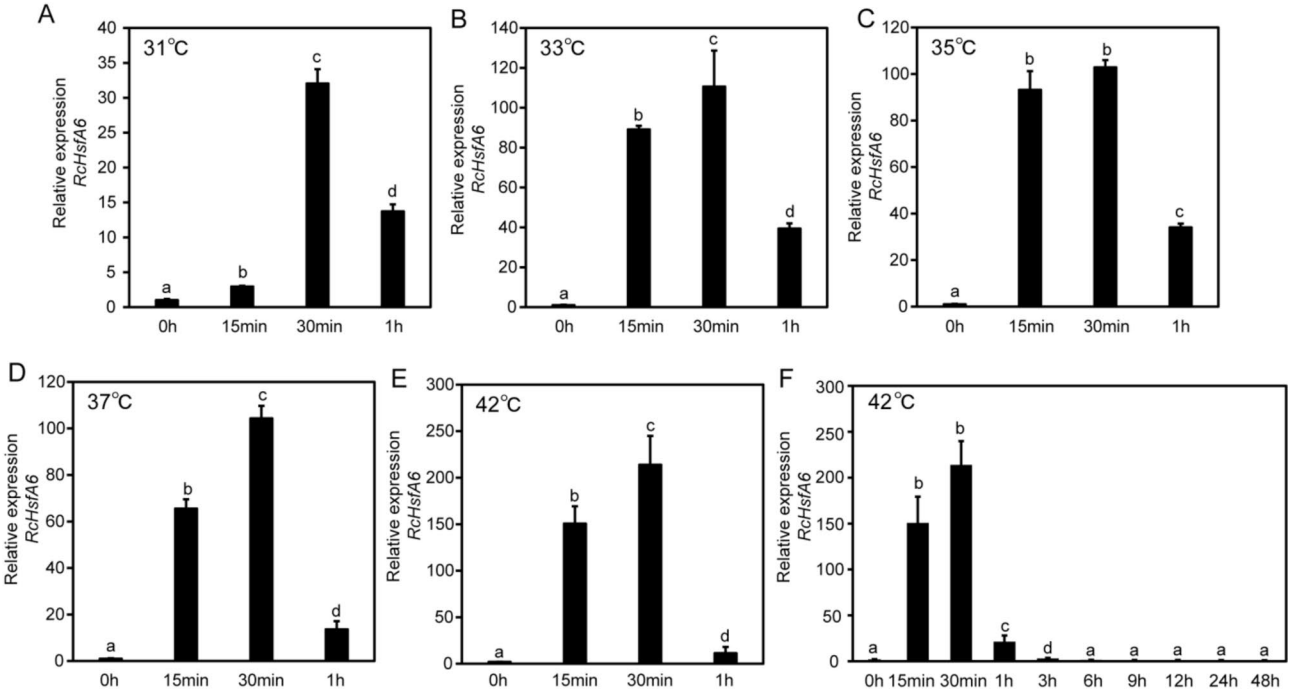


**Fig. 5** Expression level of *RcHsfA6* in petals of rose under high temperatures. (**A**-**E**) Expression levels of *RcHsfA6* in petals of rose at 31 °C, 33 °C, 35 °C, 37 °C, and 42 °C for 15 min, 30 min, and 1 h. (**F**) Expression level of *RcHsfA6* in petals of rose at 42 °C for 15 min, 30 min, 1 h, 3 h, 6 h, 9 h, 12 h, 24 h, and 48 h. *R. chinensis* actin (GenBank accession: KC514920) was used as the internal control. The letters indicate significant differences. $p < 0.05$

results, we confirmed that *RcHsfA6*-overexpressing transgenic homozygous lines were generated.

### *RcHsfA6* overexpression promoted thermotolerance in transgenic *Arabidopsis* seedlings

To validate the role of *RcHsfA6* under high temperatures, *RcHsfA6* transgenic lines and wild-type seeds were subjected to 42 °C (HT) for 2 h. After 20 d, the phenotypic data and related gene expression were measured. As shown in Fig. 7A, most wild-type seeds could not germinate normally at HT, and the germination rate was only approximately 25%, while the germination rate of the *RcHsfA6* transgenic lines reached more than 70%, which was significantly greater than that of the wild type (Fig. 7B). The gene expression of the *Hsp* genes *AtHsp18* (Fig. 7C), *AtHsp25* (Fig. 7D), *AtHsp26* (Fig. 7E), *AtHsp70* (Fig. 7F), and *AtHsp90* (Fig. 7G) at high temperatures was assayed for thermotolerance analysis. The results revealed that *AtHsp18*, *AtHsp25*, *AtHsp26*, *AtHsp70*, and *AtHsp90* in *the* wild type were significantly induced by high temperatures. In the *RcHsfA6* transgenic lines, *AtHsp18*, *AtHsp25*, *AtHsp26*, and *AtHsp70* were significantly induced at high temperatures, whereas there was no significant difference in the expression of *AtHsp90* between CK and HT (Fig. 7G). These results indicated that *RcHsfA6* overexpression enhanced the thermotolerance of *Arabidopsis*.

### *RcHsfA6* overexpression improved the ABA content in transgenic *Arabidopsis* under high temperatures

To explore whether ABA is involved in *the* response of RcHsfA6 to high temperatures, the ABA content in the wild-type and *RcHsfA6* transgenic lines was detected after high-temperature treatment. The results revealed that the ABA content of the transgenic lines was significantly greater than that of the wild type, indicating that the overexpression of *RcHsfA6* promoted the synthesis of endogenous ABA in *Arabidopsis* (Fig. 8A). ABA can promote thermotolerance and increase ABA signaling, which is beneficial for heat acclimation in *Arabidopsis* [53]. Therefore, the overexpression of *RcHsfA6* increased the ABA content, thereby improving the thermotolerance of *Arabidopsis*. At high temperatures, the ABA content of the wild type increased, whereas the ABA content of the transgenic lines decreased (Fig. 8A). The expression of *RcHsfA6* in the transgenic lines decreased at high temperatures, which was consistent with the ABA content (Fig. 8B). Therefore, we hypothesized that *RcHsfA6* overexpression reduced the increase in ABA content caused by high temperatures and had a positive regulatory effect on the ABA content at high temperatures.

Transcriptome data analysis revealed that the transcripts of the ABA signal pathway genes *ABI5* and *PYL12* among the DEGs were the most significant at high temperatures in rose, and their homologous genes in *Arabidopsis*, including *AtABI5* and *AtPYL12*, were identified

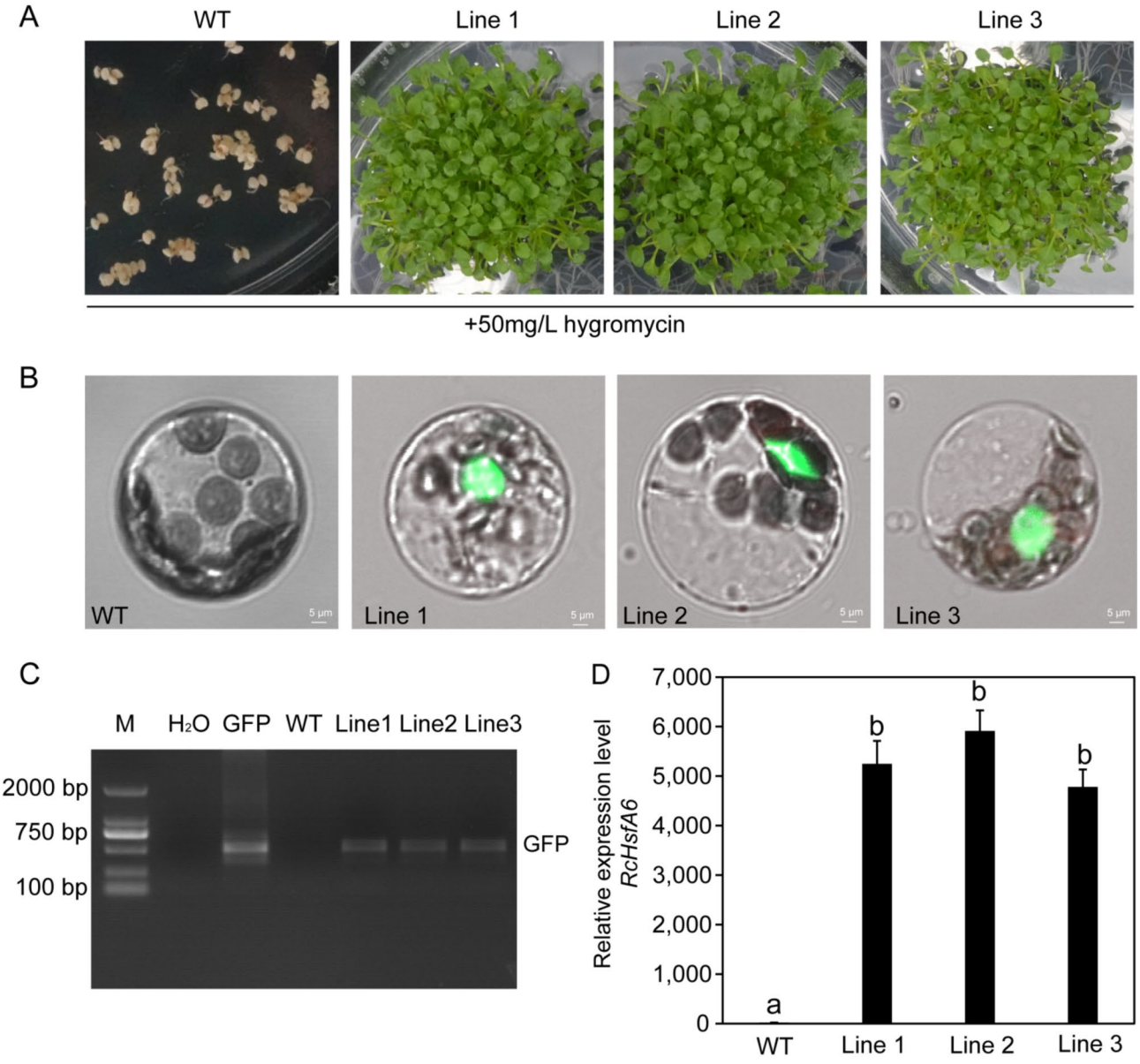


**Fig. 6** Physiological characterization and identification of *RcHsfA6* transgenic *Arabidopsis* seedlings. (**A**) Phenotype of *RcHsfA6* transgenic T3 generation *Arabidopsis* seedlings after screening with 50 mg/L hygromycin. WT: wild type, Line1/2/3: Overexpression *RcHsfA6* transgenic lines. (**B**) Expression at the subcellular level of RcHsfA6 protein fused with GFP protein in protoplasts of *RcHsfA6* transgenic lines. (**C**) PCR identification of the GFP gene. M: 2000 DNA marker; GFP: *35 S*::RcHsfA6 plasmid. (**D**) Expression of *RcHsfA6* in the wild-type and *RcHsfA6* transgenic lines under normal growth conditions. The letters indicate significant differences. $p < 0.05$

via *BLAST*. The expression of ABA biosynthetic gene *AtABI5* and *AtABA2*, signal transduction gene *AtPYL12* and catabolic gene *AtCYP707A* in *Arabidopsis* were detected, and the results revealed that the overexpression of *RcHsfA6* decreased the expression of the ABA catabolic gene *AtCYP707A* and increased the expression of *AtABI5* and *AtPYL12*, thereby increasing the ABA content of the transgenic lines (Fig. 8B-F). At high temperatures, the expression of *AtABI5* and *AtPYL12* and the ABA catabolic gene *AtCYP707A* in the transgenic lines was significantly greater than that in the wild type, whereas the expression of *AtABI5* and *AtABA2* did not differ (Fig. 8B-F). On the basis of the complex expression patterns of ABA biosynthetic genes *AtABI5*, *AtABA2*, and catabolic gene *AtCYP707A*, we speculated that high temperatures significantly reduced the ABA content in the *RcHsfA6*-overexpressing transgenic lines, which may be regulated by a complex network involving other genes.

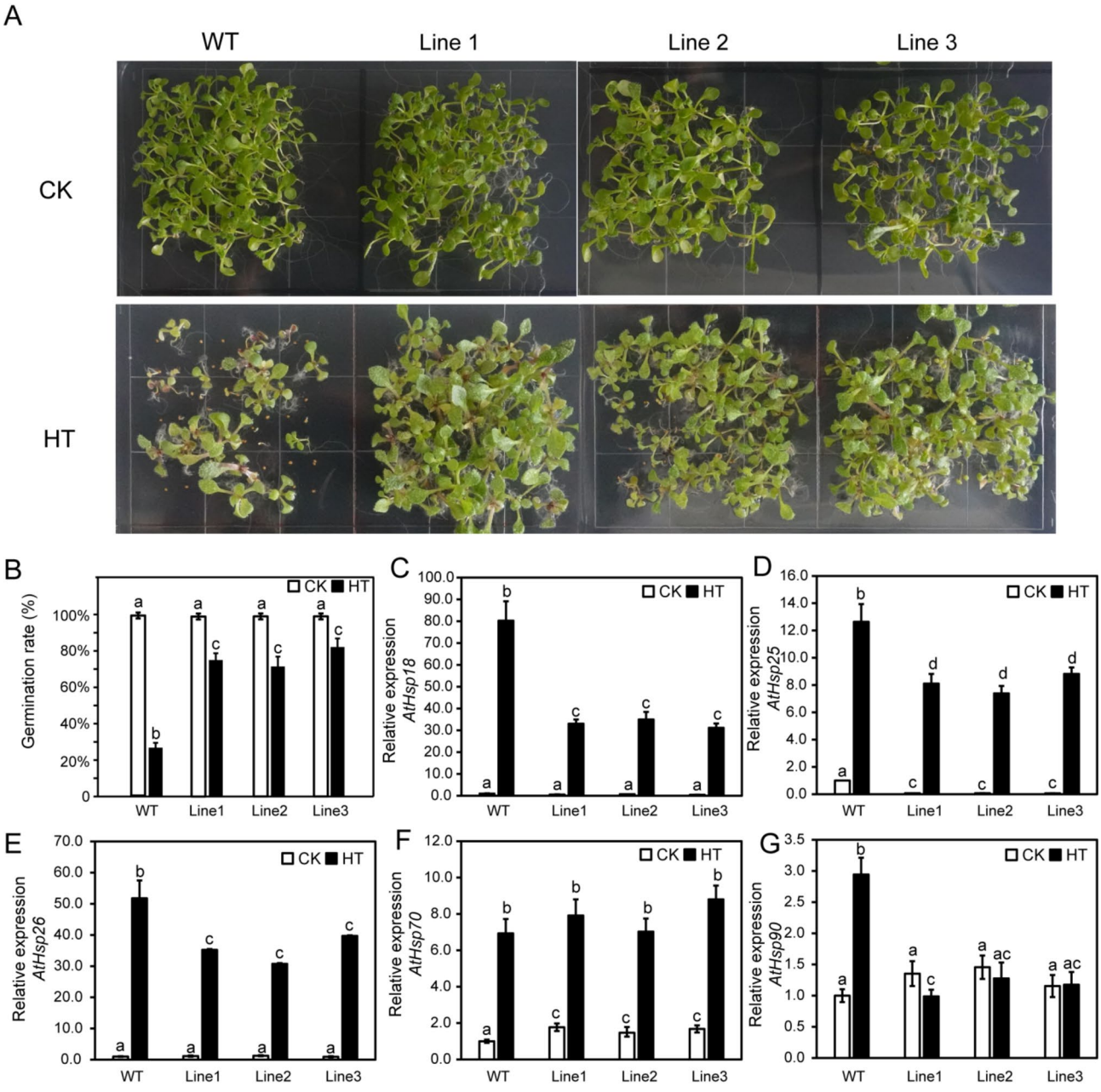


**Fig. 7** Thermotolerance analyses of *RcHsfA6*-overexpressing *Arabidopsis* seeds. (**A**) Phenotypes of wild-type and *RcHsfA6*-overexpressing *Arabidopsis* seedlings at control temperature (CK) and high temperature (HT). The high-temperature treatment was set to 42 °C for 2 h and then 22 °C for 14 d. (**B**) Germination rates of *RcHsfA6*-overexpressing *Arabidopsis* seeds and wild-type *Arabidopsis* seeds. (**C**-**G**) Expression of *AtHsp18*, *AtHsp25*, *AtHsp26*, *AtHsp70* and *AtHsp90* in wild-type and *RcHsfA6*-overexpressing *Arabidopsis* after high-temperature treatment. The letters indicate significant differences. $p < 0.05$

### Overexpression of *RcHsfA6* inhibited seed germination in *Arabidopsis* under ABA treatment

To verify the response of the *RcHsfA6*-overexpressing transgenic lines to ABA, wild-type and transgenic line seeds were treated with 0.1 μM ABA. We found that most of the transgenic *Arabidopsis* seeds did not germinate normally on 1/2 MS media supplemented with 0.1 μM ABA, and the growth of the transgenic *Arabidopsis* seedlings was significantly slower than that of the wild-type plants (Fig. 9A). Compared with that of the WT seeds, the germination rate of the transgenic *Arabidopsis* seeds was only 20%~40% (Fig. 9B). Under 0.1 μM ABA treatment, *RcHsfA6* overexpression significantly increased the expression of *AtABI1*, *AtABI5*, *AtPYL12*, and *AtCYP707A*. However, the expression of *AtABA2* did not differ between the wild-type and transgenic lines under 0.1 μM ABA treatment (Fig. 9C-G). Hence, we presumed that *RcHsfA6* overexpression increased the ABA

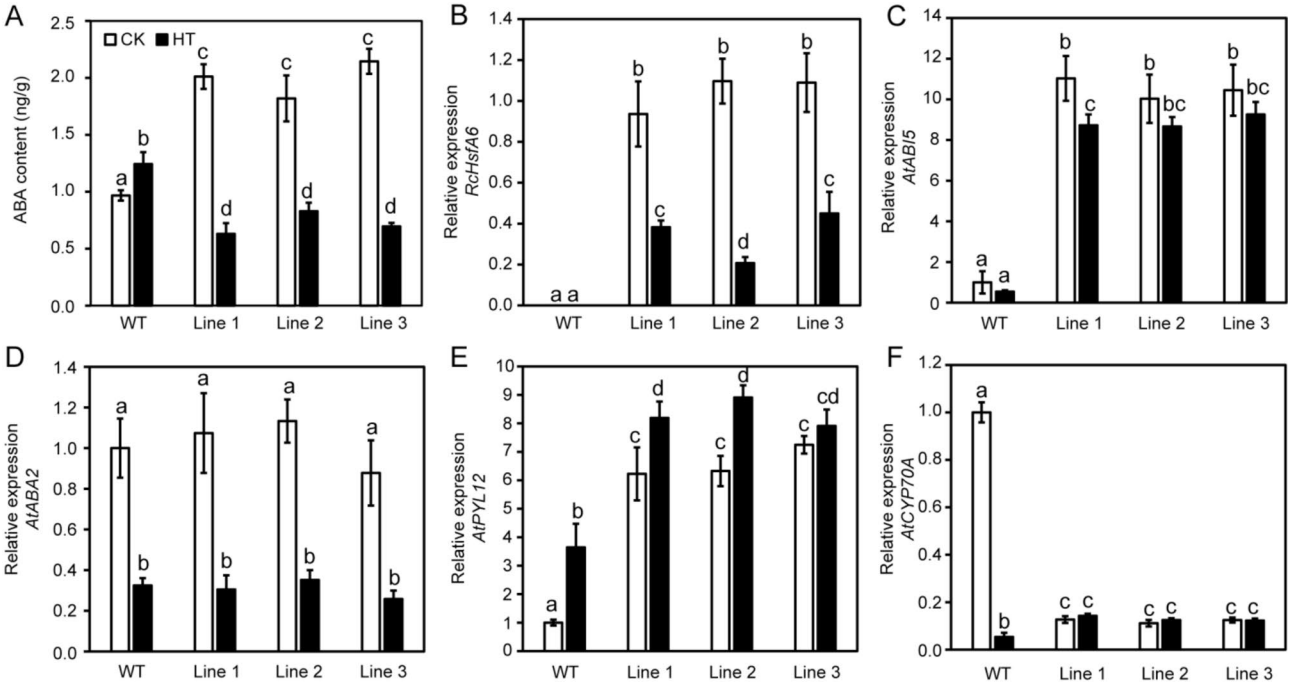


**Fig. 8** Expression levels related to ABA synthesis and regulation in *RcHsfA6* transgenic lines. (**A**) ABA content in the wild-type and transgenic lines under control (CK) and high-temperature (HT) treatments. (**B**) Expression of *RcHsfA6* in the wild-type and transgenic lines under control (CK) and high-temperature (HT) treatments. (**C**-**F**) Expression of ABA biosynthetic genes *AtABI5*, *AtABA2* and signaling transduction gene *AtPYL12* and catabolic gene *AtCYP707A* in the wild-type and *RcHsfA6* transgenic lines. The letters indicate significant differences. $p < 0.05$

content by regulating the expression of *AtABI1*, *AtABI5*, and *AtPYL12* and *AtCYP707A*, thereby inhibiting the germination of *Arabidopsis* seeds under ABA treatment.

## Discussion

With global warming, the effects of high temperatures on plant growth and development are becoming increasingly significant. Rose often experiences high temperatures during the growing season. Therefore, a complex regulatory mechanism is needed to cope with high temperatures. In this study, a total of 2519 DEGs were identified in rose petals in the control and high-temperature treatments (Tab. S3). GO and KEGG enrichment analyses revealed that these DEGs were both significantly enriched in the plant hormone signal transduction pathway (Fig. 2). Transcription factors play a central regulatory role in enhancing plant protection mechanisms [54]. At high temperatures, 528 TFs were annotated as DEGs, among which the bHLH, MYB-related, WRKY, and NAC transcription factor families were the most abundant (Fig. 2A). HSF transcription factors are central regulators of the high-temperature response and play key roles in thermotolerance [55]. In our research, five *RcHsf* genes were upregulated among the DEGs according to RT–PCR, among which the *RcHsfA6* (LOC112172194) gene was significantly highly expressed at high temperatures (Fig. 2B). In *Arabidopsis*, HsfA6a has been reported to be required for heat stress resistance [56]. On the basis of the expression of the *RcHsf* genes and the enrichment analysis of the DEGs, we found that *RcHsfA6* appears to be an important gene in response to high-temperature stress in rose and thus warrants further examination.

Hsfs are composed of five major structural domains, in which CTAD is the least conserved region in the Hsfs sequence and contains a short peptide AHA motif and a large number of aromatic amino acids, hydrophobic amino acids, and acidic amino acid residues [57]. Although the conservation of CTAD is relatively low in heat shock transcription factors, it also participates in the induced activation and expression of Hsfs and endows them with the function of transcriptional activators. RcHsfA6 is highly conserved in the DBD, OD and NLS structures with AtHsfA6 and SlHsfA6 in *Arabidopsis* and tomato but differs greatly in the AHA domain. In contrast, RcHsfA6, AtHsfA6 and SlHsfA6 all have an extended insertion of 21 (class A) and 7 (class C) amino acid residues between the HR-A and HR-B parts (Fig. 3). The CTAD domain is the least conserved region, and not all Hsfs contain the CTAD motif [58–60]. Most class A Hsfs have one or multiple AHA motifs at the C-terminus and these function as trans-activators, whereas class B and class C Hsfs do not and are generally considered to lack a transcriptional activation function [61–63]. However, some class A Hsf genes do not contain the AHA motif, but they contain tryptophan residues with activation functions, such as HsfA3 [64, 65]. As an essential

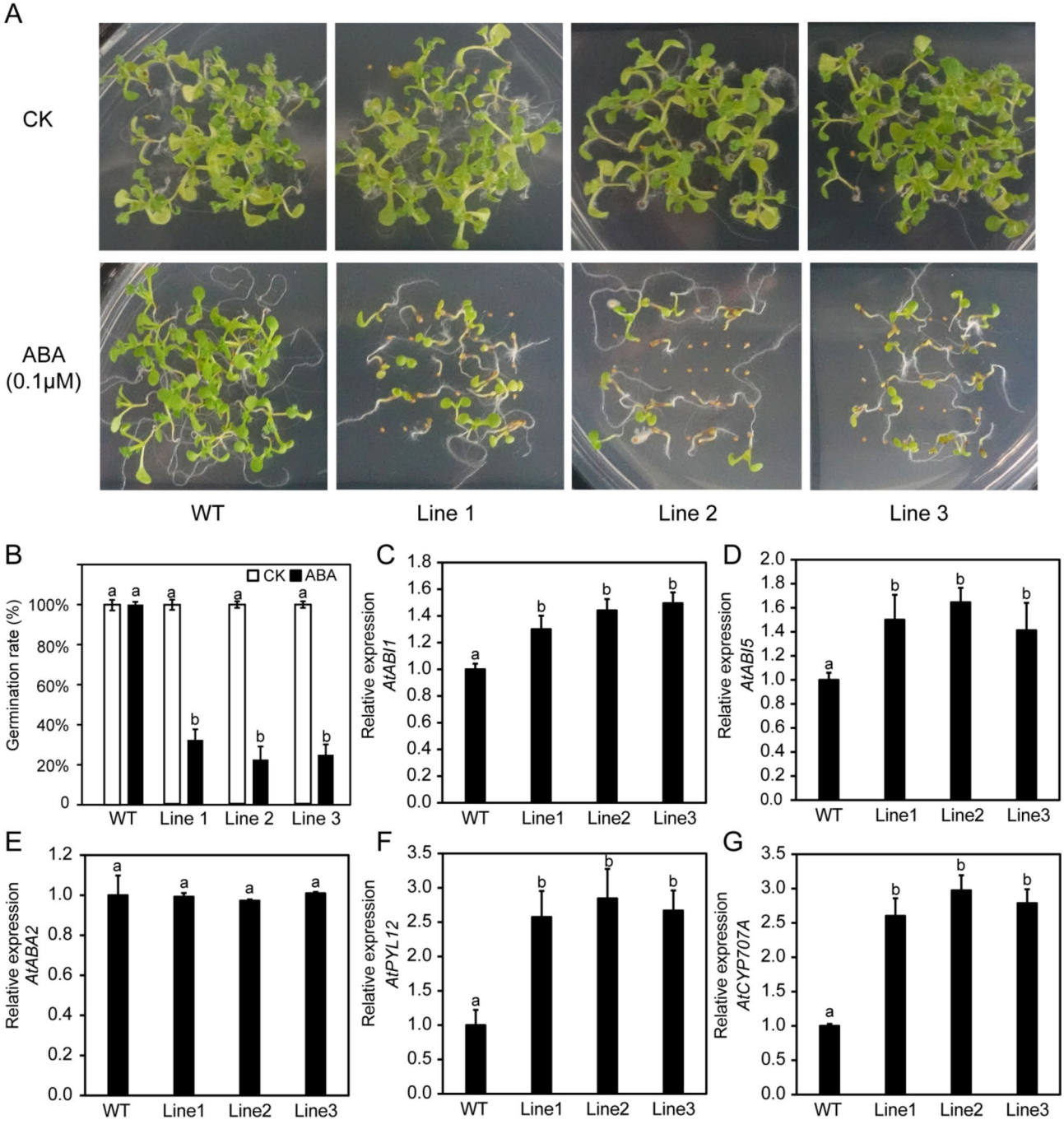


**Fig. 9** ABA treatment of *RcHsfA6* transgenic lines. (**A**) Phenotypes of wild-type and *RcHsfA6* transgenic seedlings under CK and 0.1 μM ABA treatment. (**B**) Germination rates of *RcHsfA6* transgenic lines and wild-type seeds under CK and 0.1 μM ABA treatment. (**C**-**G**) Expression of the ABA pathway genes *AtABI1*, *AtABI5*, *AtABA2*, *AtPYL12*, and *AtCYP707A*. The letters indicate significant differences. $p < 0.05$

component of HS memory in *Arabidopsis*, HsfA3 binds to HsfA2 to form heteromeric complexes that are highly effective at promoting HS memory. Except for preferred binding partner HsfA2, AtHsfA3 forms multimeric complexes with other Hsf proteins, such as AtHsfA6 and AtHsfA1, which providing a tentative explanation for the residual activity of HsfA3 [4]. In lily, HsfC2 could interact with multiple Hsf (HsfA1, HsfA2, HsfA3A, and HsfA3B) to accelerate their transactivation ability and act as a transcriptional coactivator [66]. In this study, the RcHsfA6 protein lacked the AHA motif, and transcriptional activation analysis further verified that it did not have self-activation activity. Therefore, we speculated

that *RcHsfA6* may play a role by interacting with other transcription factors.

Although evolutionary analysis revealed that RcHsfA6 and *Arabidopsis* HsfA6 are highly similar in structure, RcHsfA6 and AtHsfA6 have significant differences in heat resistance functions. *AtHsfA6a* expression was not induced by high temperature (37 °C) but was clearly induced by ABA, NaCl and drought treatment in *Arabidopsis* [67, 68]. Transgenic barley lines overexpressing *TaHsfA6b* presented improved thermotolerance at 35 °C [69]. In this study, we found that high temperatures can rapidly induce the expression of *RcHsfA6* in petals. When the temperature reaches 31 °C, treatment for 30 min can induce high expression of *RcHsfA6*, and when the temperature is higher than 33 °C, treatment for 15 min can induce *RcHsfA6* expression (Fig. 5). The expression of *RcHsfA6* was highest in old leaves, followed by roots and stems, and lowest in young leaves and petals, which was consistent with its heat resistance (Fig. 4B). When flowering roses are exposed to high temperatures, flowers and young leaves firstly experience symptoms of high-temperature stress, such as curled petals and wilting young leaves. Plants perceive high-temperature signals and upregulate the expression of heat resistance genes to initiate internal defense mechanisms to cope with high temperatures [55, 70–72]. Compared with petals and young leaves, old leaves, roots and stems have no obvious symptoms in rose under high-temperature stress. The low expression of heat tolerance-related genes, such as Hsf genes, in petals may be related to the intolerance of petals to high temperatures [12].

The significant difference in germination rate between *RcHsfA6* transgenic lines and wild-type seeds under heat stress (42 °C for 2 h) indicated that heterologous expression of *RcHsfA6* plays a role in enhancing *Arabidopsis* heat resistance. The wild-type seeds had a low germination rate of approximately 25%, demonstrating their vulnerability to high temperature stress (Fig. 7B). In contrast, the germination rate of *RcHsfA6* transgenic lines exceeded 70%, highlighting the positive impact of *RcHsfA6* overexpression on the ability of seeds to withstand heat and germinate normally (Fig. 7B). This suggested that *RcHsfA6* may play a crucial role in protecting the seed germination process from heat-induced damage. Hsps are known to function as molecular chaperones, assisting in protein folding and preventing protein aggregation under heat stress conditions. In the *RcHsfA6* transgenic lines, *AtHsp18*, *AtHsp25*, *AtHsp26*, and *AtHsp70* were significantly induced at high temperatures (Fig. 7C-F), which indicated that *RcHsfA6* overexpression likely promotes the activation of these Hsp genes, enhancing the ability of *Arabidopsis* to cope with heat stress.

ABA is a well-known phytohormone that enhances thermotolerance by stabilizing cellular membranes, regulating stomatal closure, and activating stress-responsive genes [38]. The increased ABA content in transgenic lines under normal conditions likely contributes to their improved thermotolerance. In this study, We found that overexpression of *RcHsfA6* significantly increased ABA content in transgenic lines compared to the wild-type under normal conditions (Fig. 8A), likely through the upregulation of ABA biosynthetic and signal transduction genes (*AtABI5* and *AtPYL12*) and the downregulation of ABA catabolic gene (*AtCYP707A*) (Fig. 8C, E, and F). However, under high temperature stress, the ABA content in transgenic lines decreased. So we speculated that *RcHsfA6* may play a dual role in ABA regulation: promoting ABA accumulation under normal conditions but modulating the dynamic balance of ABA through complex mechanisms under stress conditions, rather than simply increasing ABA content. The downregulation of *RcHsfA6* expression at high temperatures further supports its role as a temperature-sensitive regulator of ABA homeostasis (Fig. 8B).

The complex expression patterns of ABA synthesis and catabolic genes under heat stress indicate that RcHsfA6 may interact with a broader regulatory network to fine-tune ABA levels and thermotolerance. Transcriptome analysis revealed that *ABI5*, key genes in the ABA signaling pathway, were significantly upregulated in rose petals under high temperatures, while the expression of *PYL12* was reduced (Table 2). In addition, overexpression *RcHsfA6* in *Arabidopsis* significantly upregulated *AtABI5* and *AtPYL12*, which was significantly greater than the effect of high temperature on the expression of *AtABI5* and *AtPYL12* (Fig. 8). *ABI5* has a synergistic inhibitory effect on seed germination [73]. The overexpression of *RcHsfA6* increased the germination rate of transgenic *Arabidopsis* seeds and significantly reduced the expression of *AtABI5* (Fig. 9). In *RcHsfA6* overexpression transgenic lines, the expression of ABA biosynthesic gene *AtABA2* did not change significantly under normal condition, but significantly decreased under high temperature (Fig. 8). *CYP707A* is the key enzyme in ABA catabolic, and its expression level significantly decreased in *RcHsfA6*-overexpressing transgenic lines [74]. The expression of *AtCYP707A* did not differ between the *RcHsfA6*-overexpressing transgenic lines and the wild type under high temperatures but significantly increased under ABA treatment, indicating that *AtCYP707A* was involved in the regulation of ABA metabolism by *RcHsfA6*, but this process was not affected by high temperatures (Fig. 9). The overexpression of *RcHsfA6* increased the expression of genes involved in ABA synthesis under normal conditions. However, the reduction in ABA content in transgenic lines under high temperatures stress, despite the overexpression of *RcHsfA6*, suggested the involvement of additional genes or pathways,

such as those related to ROS scavenging, HSPs, or other phytohormones, may interact with RcHsfA6 to modulate ABA levels. These findings provide valuable insights into the molecular mechanisms of high temperature responses and highlight the potential of *RcHsfA6* as a target for improving thermotolerance in horticultural plants. Further research is needed to explore the broader regulatory network involving *RcHsfA6* and its potential applications in horticultural plants improvement.

## Conclusion

In this study, we provided transcriptomic data on the response of rose to high temperatures. A total of 2519 DEGs were identified, including 1491 upregulated DEGs and 1028 downregulated DEGs at high temperatures. Among the upregulated DEGs, 318 TFs were annotated, including 5 Hsf genes. *RcHsfA6* (LOC112172194) was significantly highly expressed at high temperatures. *RcHsfA6* overexpression increased the ABA content and the expression of ABA biosynthetic gene *AtABI5* and signal transduction gene *AtPYL12*, thereby increasing thermotolerance in *Arabidopsis* at high temperatures.

### Abbreviations

| | |
|---|---|
| HSF | Heat stress transcription factor |
| HSP | Heat shock proteins |
| HS | Heat stress |
| HSE | HS elements |
| DEGs | Diferentially expressed genes |
| GO | Gene Ontology |
| KEGG | Kyoto Encyclopedia of Genes and Genomes |
| FPKM | Fragments per kilobase of transcript per million fragments mapped |
| DBD | DNA-binding domain |
| OD | Oligomerization domain |
| AHA | Aromatic, hydrophobic and acidic amino acid residues |
| NLS | Nuclear localization signal |
| NES | Nuclear export signal |
| CTAD | C-terminal activator domain |
| ABA | Abscisic acid |
| MeJA | Methyl Jasmonate |
| ROS | Reactive oxygen species |
| GFP | Green fluorescent |
| cDNA | Complementary DNA |
| qRT | PCR-Quantitative real-time PCR |

## Supplementary Information

The online version contains supplementary material available at https://doi.org/10.1186/s12870-025-06652-1.

Supplementary Material 1

### Acknowledgements

Not applicable.

### Author contributions

W.J. designed the research; Y.K., M.L., H.W., Y.Y., X.S. and P.S. performed the experiments; Y.K. analyzed the data; W.J. and Y.K. wrote the paper. All the authors have read and agreed to the published version of the manuscript.

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### Data availability

Sequence data that support the findings of this study have been deposited in the China National GenBank DataBase with the primary accession code CNP0006864.

## Declarations

### Ethics approval and consent to participate

Not applicable.

### Consent for publication

Not applicable.

### Competing interests

The authors declare no competing interests.

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