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Cloning and expression analysis of *RhHsf24* gene in Rose (*Rosa hybrida*)

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Rose (*Rosa hybrida*) is one of the most important ornamental and perfume industry crops worldwide, both economically and culturally. Abiotic stresses, such as high temperature and salt are crucial factors influencing the quality of roses. In this study, *RhHsf24* was isolated from rose (*R. hybrida* 'Samantha'), which encodes 295 amino acids (aa). Sequence comparison with members of *Arabidopsis Hsfs* family revealed that this gene is most closely related to *AtHsfB1*; phylogenetic tree analysis with proteins from other species showed that it clusters with *R. rugosa* (RrHSF24), *Fragaria vesca* (FvHSFB1a) and *Argentina anserina* (AaHSF24), which are the closest relatives and belong to the class B heat shock transcription factors. RhHsf24 was localized in the nucleus. The qRT-PCR results indicated that the gene was expressed in roots, stems, leaves, flowers and buds. Expression analysis of the gene in leaves subjected to various temperatures and durations of heat stress treatment demonstrated that *RhHsf24* gene expression is induced by heat stress. Under salt stress, the expression of the *RhHsf24* gene generally exhibited a high level of expression with increasing concentration. The above results preliminarily clarified the biological function of *RhHsf24*, and provide a genetic resource and theoretical reference for the resistance breeding of roses.

Keywords Heat shock transcriptional factor, Abiotic stress, Expression analysis, Heat stress

Instruction

Heat shock transcription factors (Hsfs) are key regulatory factors that play crucial roles in a multitude of biotic and abiotic stress responses[1–4]. They are particularly pivotal in plant tolerance to high temperature stress and other adversities[5,6]. *Hsfs* are extensively involved in responding to plant adversity, including biotic and abiotic stresses[7]. Their relationship with high temperature stress is particularly close. In response to high temperature stress, *Hsf* specifically binds to the heat shock element (HSE) located on the heat stress-responsive gene promoters, thereby inducing the expression of downstream responsive genes[8]. The expression of *Hsfs* varies in different plants under high temperature stress[7]. The plant *Hsf* is divided into three main categories, A, B, and C, each performing different functions[9]. Class A *Hsfs* are involved in the regulation of high-temperature stress response, while class B *Hsfs* lack transcriptional activity owing to the absence of activation domains[2,10–12]. Class C Hsfs may function as transcriptional repressors by means of protein interactions and other mechanisms[13], and there is a relative lack of research on this class. Abiotic stresses, such as drought and salt, have a profound impact on plant growth and development[14–17]. Studies have demonstrated that following exposure to a high temperature environment for an extended period, the majority of *MdHsfs* are induced to express in *Malus domestica*. And the expression of certain *Hsfs* has been observed to increase significantly after exposure to stress[18]. The expression of several class A *Hsf* genes in *Fragaria vesca* demonstrated a gradual increase in response to an environmental temperature of 42°C[10]. *VpHsf1*, a member of the *HsfB2* family of *Vitis pseudoreticulata*, was shown in overexpressing *Nicotiana benthamiana* to exert a dual regulatory function, acting as a positive regulator in acquired heat tolerance[19]. Furthermore, some *Hsf* genes have been reported to play a pivotal role in salt stress response. *Arabidopsis* plants overexpressing *AtHsfA2* and *AtHsfA7b* exhibited enhanced salt tolerance[20,21]. Additionally, numerous genes within the *Hsf* family demonstrated a notable response to salt stress. The enhanced expression of *DcHsf10*, *DcHsf01*, and *DcHsf16* was observed in the *Daucus carota* variety 'Jun-chuanhong'[22]. The *CsHsf* and *CsHsp*

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genes, which were found to be up-regulated by heat stress in cucumber (*Cucumis sativus* L.), were also induced by salt stress and regulated salt stress via a pathway common to heat stress[23].

The rose (*Rosa hybrida*) is a perennial woody plant in the genus rosa, family rosaceae, known as the "Queen of Flowers." It is the world's largest cut-flower and an important garden flower[24]. Additionally, it occupies an important position in terms of economic and ornamental value. The development of the rose industry has the potential to drive regional tourism development and promote rural revitalization[25]. However, rose planting is susceptible to abiotic stresses, and the intracellular ionic equilibrium is easily disrupted in high-temperature and high-salt environments, which shortens the flowering period and reduces the metabolism of roses, thus affecting the growth and development of roses and their ornamental effects. Concurrently, abiotic stress also constrains the quality and yield of cut flowers of rose and increases the cost of planting. Currently, the majority of studies on the isolation of genes related to abiotic stress in rose are focused on regulatory genes such as NAC and HB[26–29], while fewer reports on the *Hsfs* genes. In this experiment, an *Hsfs* gene, designated *RhHsf24*, was cloned for the first time from rose (*R. hybrida* 'Samantha'). The gene was then subjected to biological analysis, and transcriptional changes of the *RhHsf24* gene were analyzed under different parts of the rose. The basic role of the *RhHsf24* gene was investigated by qRT-PCR in rose samples subjected to heat stress treatments of different temperatures and durations, as well as treatments of different salt concentrations. The objective was to expand the gene reserve of the rose for resistance breeding and provide a theoretical reference for future studies.

Results

Cloning and bioinformatics analysis of the transcription factor gene *RhHsf24* in Rose

PCR amplification of *RhHsf24* gene was performed with the designed primers, and the amplified bands are presented in Fig. 1. After correction, the sequence of *RhHsf24* was obtained, and its CDS comprised 888 bp nucleotides, encoding 295 amino acids (aa).

The aa sequence of the rose *RhHsf24* gene was employed to construct a phylogenetic tree with 21 members of the Hsfs family of *Arabidopsis* using MEGA 11 (Fig. 2). The results of the analysis revealed that the proteins of *RhHsf24* and *AtHsfB1* exhibited the closest relationship (Fig. 2A). Furthermore, the aa sequence encoded by the *RhHsf24* gene was also used to construct an evolutionary tree with the Hsfs transcription factors of other species. The results indicated that the rose RhHsf24 fell within the same branch as *R. rugosa* (RrHSF24), *F. vesca* (FvHSFB1a) and *A. anserina* (AaHSF24), and displayed a closer relationship (Fig. 2B).

The protein structural domains of *RhHsf24* were analyzed, and a search using the NCBI (National Center for Biotechnology Information) website revealed that the aa sequences of the *RhHsf24* gene were similar to those of eight plant species, namely *R. rugosa*, *F. vesca*, *A. anserina*, *Prunus dulcis*, *P. persica*, *P. mume*, *Ziziphus jujuba*, and *Theobroma cacao*. The aa sequence comparison maps were constructed using ClustalX and BioEdit software (Fig. 3). It was found that *RhHsf24*, like the Hsfs of the other species, contains the DNA binding domain (DBD), oligomerization domain (OD or HR-A/B), nuclear localization signal (NLS). Additionally, it has a repress domain (RD) at its C-terminus with the core sequence -LFGV-, which indicates that *RhHsf24* belongs to the class B heat shock transcription factors.

Subcellular localization of RhHsf24 protein in Rose

In order to further study the function of the RhHsf24 protein, *N. benthamiana* was transiently transformed with pSuper::1300-GFP-RhHsf24 recombinant vector and control vector pSuper::GFP and subsequently observed by confocal laser scanning microscopy. The results, as shown in Fig. 4, indicated that the control pSuper::GFP showed green fluorescence signals in the nucleus and plasma membrane, while the green fluorescence of the pSuper::1300-GFP-RhHsf24 recombinant vector was only present in the nucleus. These results indicate that the protein encoded by the *RhHsf24* gene is localized within the nucleus. Among the genes from other species that are most closely to *RhHsf24*, the subcellular localization of strawberry FvHSFB1a is known to reside in the nucleus[9], which is consistent with the localization of the RhHsf24 protein in this study. Similarly, *Arabidopsis* AtHsfB1 is also localized in the nucleus[30].

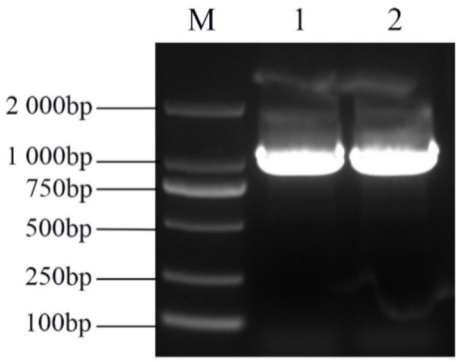

Fig. 1. PCR amplification of *RhHsf24*. (M: BM2000 DNA Marker; Lane 1, 2: Amplification product of *RhHsf24* gene. Original gels are presented in Supplementary Fig. 1.).

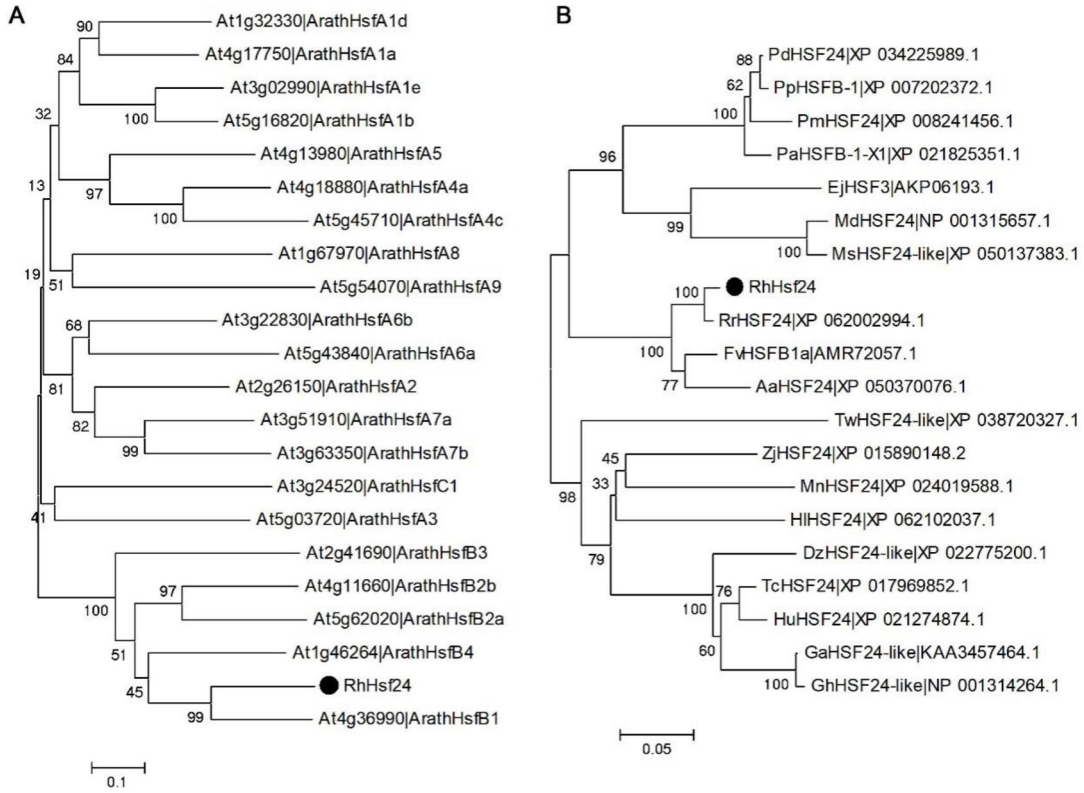

Fig. 2. Phylogenetic tree analysis. (**A**) Phylogenetic tree of RhHsf24 and all HSFs in *Arabidopsis*. (**B**) Phylogenetic analysis of RhHsf24 and related proteins from other species. This tree was constructed by MEGA 11. The dark dot indicates an RhHsf24 protein.

Expression characterization of Rose *RhHsf24* under different treatments

In order to clarify the expression pattern of *RhHsf24*, qRT-PCR detection was performed. The results of qRT-PCR analysis showed that *RhHsf24* gene was expressed in roots, stems, leaves, flowers and buds, with predominant expression in stems and the lowest level of expression observed in buds (Fig. 5).

After examining the expression of *RhHsf24* under different thermal treatment temperatures, it was found that the expression of *RhHsf24* exhibited an upward trend as the treatment temperature increased (Fig. 6). To further simulate outdoor high-temperature stress, thermal treatments at 42 °C were conducted for various durations, and the results revealed that the expression level of *RhHsf24* gradually increased as the duration of the heat treatment was extended (Fig. 7).

Normal-growing rose 'Samantha' plants were subjected to salt stress treatment by watering with solutions containing salt concentrations of 0%, 0.20%, 0.40%, 0.60%, 0.80%, as well as 1.00%, respectively. After 30 days, leaves from the treated plants were harvested for RNA extraction and subsequent reverse transcription into cDNA, followed by the analysis of *RhHsf24* expression at each concentration. *RhHsf24* expression changes with treatment. As shown in Fig. 8, the expression of *RhHsf24* in leaves showed an increasing and then decreasing trend. This suggests that *RhHsf24* is capable of responding to salt stress in roses, with its induction gradually intensifying as the salt concentration rises, peaking at the 0.80% salt concentration treatment.

Discussion

Heat shock transcription factors are important regulators that control the expression of heat shock proteins (HSPs) in cells[31,32], acting as molecular chaperones of the proteins. Heat shock proteins are induced by various abiotic stresses in plants and exhibit a wide range of responses by reducing protein damage from abiotic stresses through protein folding and processing in cellular responses[33,34]. In this study, we cloned the CDS sequence of the heat shock transcription factor *RhHsf24* gene from *R. hybrida* 'Samantha' and obtained a CDS sequence of 888 bp in size encoding a total of 295 aa after PCR amplification. Relevant studies have shown that HSFs can be categorized according to the length of the helical linkage between the N-terminal conserved DNA-binding domain and the oligomerization domain structure and the number of aa inserted between HR-A/B[35]: 9–39 for class A, 50–78 for class B, and 14–49 for class C[35,36], it can be clearly stated that *RhHsf24* belongs to the B class of heat shock transcription factors. Class B HSFs are generally regarded as repressors of the heat stress response and function by regulating class A HSFs[37]. In tomato (*Lycopersicon peruvianum*), HsfB1 acts as a coactivator and is involved in the regulation of high-temperature stress together with members of the HsfA family[38], HsfB2a and HsfB2b in *Arabidopsis* can improve the heat tolerance of *Arabidopsis* by binding to HsfA1a and HsfA1b[39]. Therefore, it is hypothesized that *RhHsf24* interacts with class A family members to participate in abiotic stress

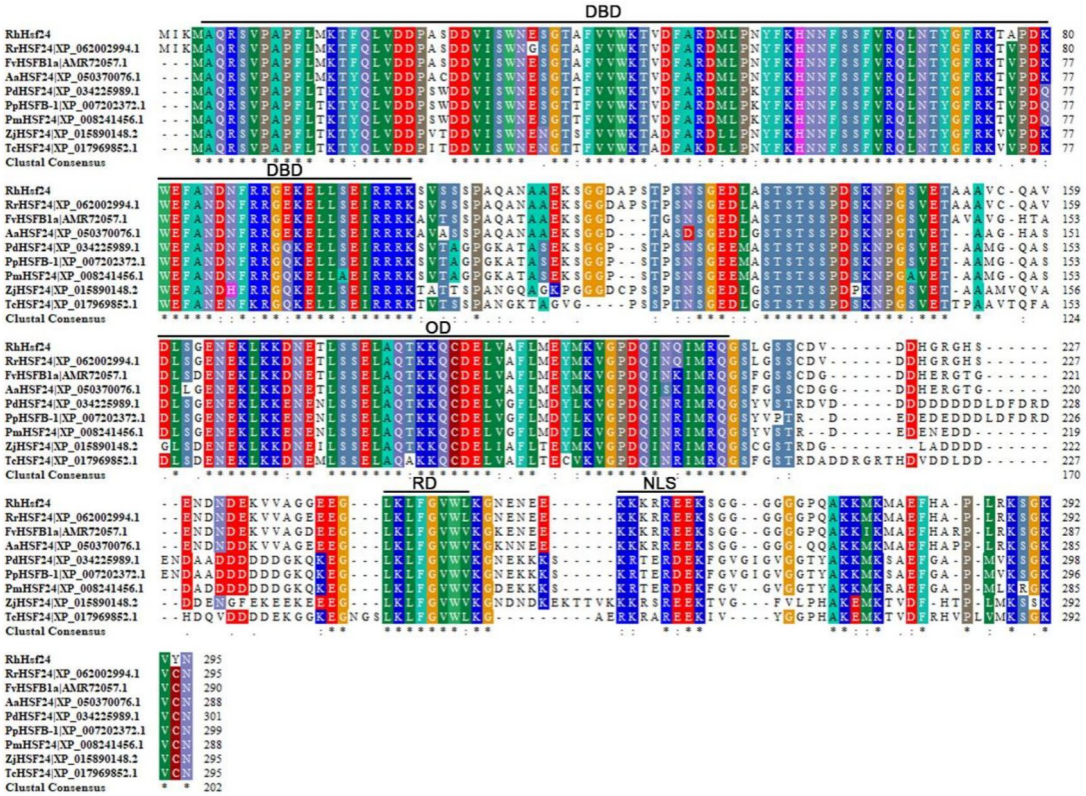

Fig. 3. Alignment of amino acid sequence of Hsf24 in different plants. Rr: *Rosa rugosa*; Fv: *Fragaria vesca* ; Aa: *Argentina anserina*; Pd: *Prunus dulcis*; Pp: *Prunus persica*; Pm: *Prunus mume*; Zj: *Ziziphus jujuba*; Tc: *Theobroma cacao*.

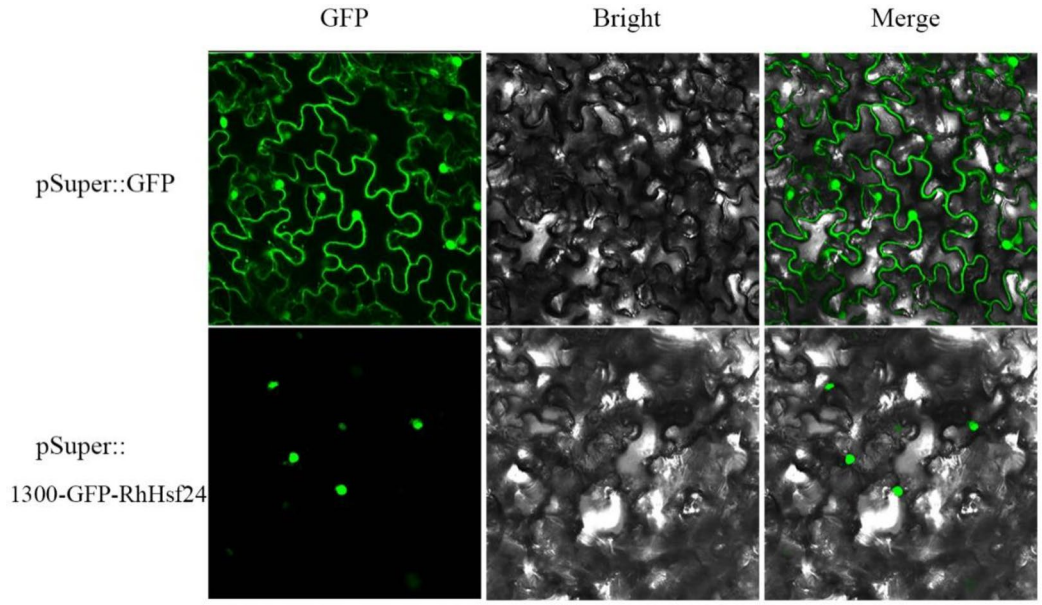

Fig. 4. Subcellular localization of RhHsf24. (The pSuper::GFP vector served as the control).

response in plants, the principle of which is not yet clear. The phylogenetic tree results showed that *RhHsf24* of rose was in the same branch with RrHSF24, FvHSFB1a and AaHSF24 with close affinity, and compared with 21 genes of *Hsfs* family of *Arabidopsis*, it was closest in affinity to AtHsfB1, which was hypothesized to have a similar role with AtHsfB1 of *Arabidopsis*. The subcellular localization results showed that the *RhHsf24* gene was

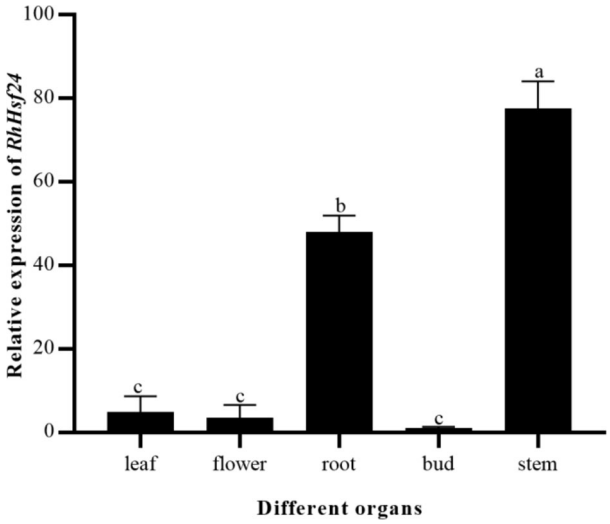

Fig. 5. Expression analysis of *RhHsf24* in different organs by qRT-PCR. (Bars are means ± SD of three independent experiments, with different letters indicating statistically significant difference. $p < 0.05$.).

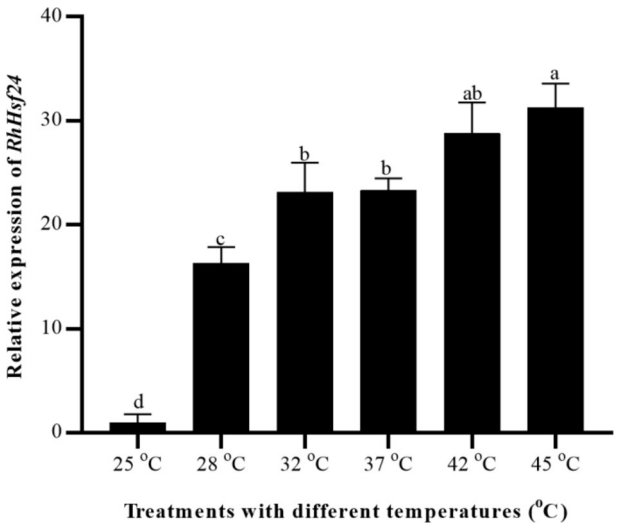

Fig. 6. Expression analysis of *RhHsf24* under different temperature treatments by qRT-PCR. (Bars are means ± SD of three independent experiments, with different letters indicating statistically significant difference. $p < 0.05$.)

localized to the nucleus, indicating that the *RhHsf24* gene exerts transcriptional translational functions in the nucleus, consistent with most class B heat shock transcription factors.

The qRT-PCR results showed that the expression of *RhHsf24* was generalized in different parts of the rose. *RhHsf24* was expressed in roots, stems, leaves, flowers and buds, and the highest expression was found in stems, followed by roots, leaves and flowers, and the lowest expression was found in buds, which may be related to the reproductive period of the rose at the time of sampling. During the growth of the rose, the leaves undergo photosynthesis and transpiration to regulate growth, while the leaf cytoplasmic membrane adapts to adversity through ion transport. The expression of *RhHsf24* in rose can be studied in more detail by applying different temperatures and different times of heat treatment. The vast majority of *HSF* genes in *Hypericum perforatum* were up-regulated and expressed under heat-excitation treatments[40], in *Cymbidium ensifoliu*, the relative expression level of *CeHSF1* peaked at 24 h of high-temperature treatment[41]. In this study, *RhHsf24* in leaves showed up-regulated expression with increasing treatment temperature, and the expression of *RhHsf24* at 24 h was significantly higher than that of 0 h time treatment under heat-excited treatment at 42 °C. Consistent with previous studies, it further indicated that *RhHsf24* was sensitive to high-temperature stress response.

Currently, there are not many studies on the response of rose *Hsfs* to salt stress. In white clover, expression of the *TrHSFB2* gene in roots and shoots showed a trend of increasing and then decreasing after treatment with 200 mM NaCl. The expression gradually increased at 0, 3, 6 and 12 h of treatment and decreased at 24 h

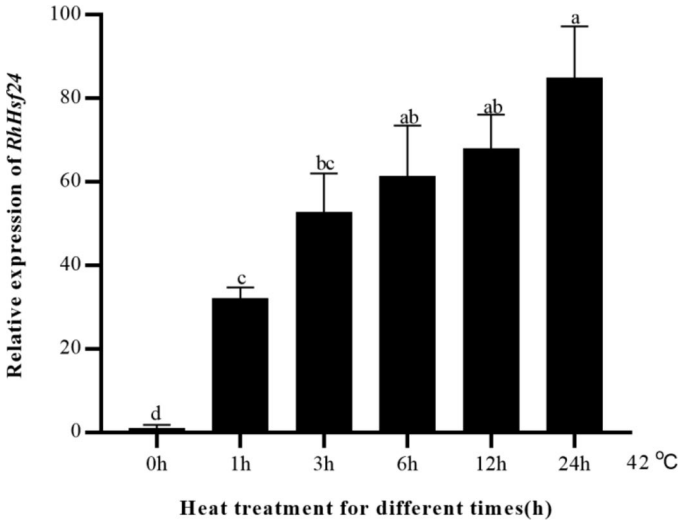

Fig. 7. Expression analysis of *RhHsf24* under 42 °C heat stress treatment at various time points by qRT-PCR. (Bars are means ± SD of three independent experiments, with different letters indicating statistically significant difference. $p < 0.05$.).

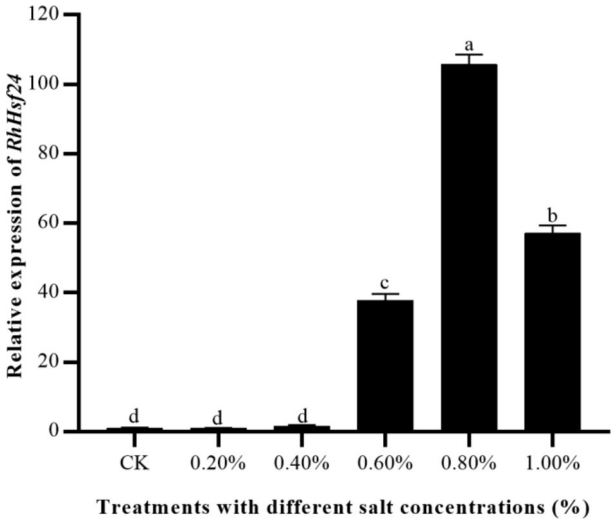

Fig. 8. Expression analysis of *RhHsf24* under different salt concentration treatments by qRT-PCR. (Bars are means ± SD of three independent experiments, with different letters indicating statistically significant difference. $p < 0.05$.)

of treatment[42]. Since the expression of *RhHsf24* in leaves showed a trend of first increasing and then decreasing when treated with salt concentrations of 0, 0.20%, 0.40%, 0.60%, 0.80%, and 1.00% in this experiment, the preliminary study indicated that the rose *RhHsf24* has a certain response to salt stress.

Numerous studies of overexpressed *Arabidopsis* plants have shown that Hsf family genes act in interaction with other transcription factors (TFs) to initiate physiological adaptations to salt stress in plants by initiating in vivo adaptation to salt stress[20,43]. Overexpression plants constructed from the *ThHsfA1* gene of *Tamarix hispida* and *Arabidopsis* showed that the ThHsfA1 protein can regulate salt stress by mediating ThWRKY4[44], resulting in lower levels of reactive oxygen species (ROS), higher levels of antioxidant enzyme activities, and plants showing greater salt tolerance[20]. Similarly, *AeHsfA2b* transgenic *Arabidopsis* outperformed wild-type *Arabidopsis* under salt stress by binding directly to the AeRFS4 promoter using *Actinidia eriantha* as a material[45], thereby increasing raffinose levels and enhancing plant tolerance to salt stress[46]. In addition, the function of class B heat shock transcription factors is different from that of class A. It has been found that some class B Hsf with a repressor domain at the C-terminus can interact with class A Hsf or other protein factors[47]. In this experiment, the significant response of *RhHsf24* to different salt concentrations suggests that this gene is also involved in the salt stress response process in rose plants, but whether it interacts with related protein factors and what the response mechanism is still needs further investigation.

Usage	Forward primer (5′→3′)	Reverse primer (5′→3′)
qRT-PCR of *RhUBI2*	GCCCTGGTGCGTTCCCAACTG	CCTGCGTGTCTGTCCGCATTG
RhHsf24 ORF amplification	GGAGTTCCTCACTGCCCTTC	GCCGATCCATAGGCCTTACC
qRT-PCR of *RhHsf24*	GATCAGATCAATCAGATTATGCGGC	CATTATCATTCTCACTGTGA CCACG
Used for pSuper::1300-GFP-RhHsf24 vector Construction	GACTCTAGTCTAGAAAGCTTATGATCAAAATGGCTCAAAG	CCCTTGCTCACCATGGTACCG TTGTACACCTTCCCGCTCT
RT-PCR of *GFP*	GCCATTTCGCCTTTTCAG	TGATAATCATCGCAAGACCG

Table 1. The primers used in this study.

In conclusion, by cloning the sequence of the *RhHsf24* gene in rose and analyzing the expression status of *RhHsf24* in different organs, different heat stress treatments as well as different concentrations of salt stress, it helps to find out the expression pattern of *RhHsf24* and provides theoretical references for the study of gene expression regulation mechanism under the adversity of rose.

Materials and methods

Plant materials and treatments

Rose (*R. hybrida* 'Samantha') was grown in greenhouse at (24 ± 1) °C with appropriate photoperiod, and the plants were selected to be of similar fertile period, good growth, free from pests and diseases and uniform in size.

The selected rose was sampled in different parts of roots, stems, leaves, flowers and buds, and other rose plants were selected to be treated under different heat stress conditions for five time periods of 1 h, 3 h, 6 h, 12 h and 24 h at 42 °C and for 3 h at five temperatures of 28 °C, 32 °C, 37 °C, 42 °C and 45 °C. Salt concentrations of 0%, 0.20%, 0.40%, 0.60%, 0.80% and 1.00% were also treated for 30 d. Immediately after the end of the treatment, samples were taken from the leaves of the rose, and the samples were wrapped in tin foil and put into liquid nitrogen, and stored in a refrigerator at -80 °C.

Total RNA extraction and cDNA synthesis

RNA was extracted using the FastPure Plant Total RNA Isolation Kit (RC401), The samples were subjected to lysis, DNA removal, transfer to an adsorption column for centrifugation, deproteinization, rinsing, and elution. And cDNA synthesis was performed with reference to the instruction manual of the SPARKscript II 1st Strand cDNA Synthesis Kit (AG0302). A total of 9.0 μL of RNA (0.1-1 ng) were added to the RNase-free tube, along with 20.0 μl of gDNA Eraser. The volume of RNase-free water was adjusted to 10.0 μl. The solution was transferred gently and blown into the PCR instrument, which should then be set to 42 °C for 5 min. Upon completion of the reaction, the solution was transferred to an ice bath. An additional 10.0 μL of Master Mix was added, mixed thoroughly, and then subjected to centrifugation. Subsequently, the incubation programme was conducted at 50 °C for 30 min using a PCR instrument, and the solution should be heated to 85 °C for a period of 5 min. Subsequently, the sample was placed in a -20 ℃ freezer for further use.

Cloning of *RhHsf24* gene

To clone the *RhHsf24* gene, firstly, the sequence of the rose *RhHsf24* gene was obtained from the NCBI website. Two specific primers (Table 1) were designed according to the results, and then PCR amplification was performed using the cDNA sequence of rose as a template. The PCR reaction system consisted of 2.0 μl cDNA, 1.5 μl upstream and downstream primers, and 25.0 μl Taq enzyme (2×M5 HIPer plus Taq HiFi PCR mix (with blue dye) (MF002-plus-100)). PCR amplification was performed after brief centrifugation. The reaction program was 95.0 °C for 3 min, 95.0 °C for 15 s, 57.0 °C for 15 s, 72.0 °C for 2 min, with a total of 34 cycles. After the reaction was completed, the PCR amplification products were detected by agarose gel electrophoresis, and after confirming that the bands were correct, the target fragment was recovered by using a Gel DNA Purification Kit (Bejing Zoman Biotechnology Co., Ltd.) (ZPN202-3), and the recovered product was ligated with the vector 5×ZTOPO Mix and then transformed into *Escherichia coli*, and then cultured overnight, a single colony was picked for transient amplification. After overnight incubation, single colonies were picked and subjected to PCR identification. Positive colonies were selected for sequencing verification by Sangon Biotech (Shanghai) Co., Ltd. For those colonies with correct sequencing results, the plasmid was extracted using the FastPure Plasmid Mini Kit (DC201-01), and the bacterial cultures were stored in glycerol at -80 °C for future use.

Bioinformatics analysis of RhHsf24 gene in Moonflower season

The correctly calibrated *RhHsf24* gene sequence was searched for several plants with high aa sequence similarity using the NCBI website, and aa sequence comparison maps were constructed using Clustal X[48] and BioEdit[49], phylogenetic trees were constructed using MEGA 11.0[50] for 21 genes of *Arabidopsis* Hsfs families and the *RhHsf24*, proteins of RhHsf24 and other species.

Construction of overexpression vectors for RhHsf24

To construct the overexpression vector, two primers should be designed (Table 1), and the plasmid extracted from the correctly sequenced *E. coli* liquid of *RhHsf24* gene was used as the template for PCR amplification, and the system was as follows: PrimeSTAR DNA polymerase 25.0 ul, plasmid 1.0 ul, two primers (24-1300-HindIII-F, 24-1300- KpnI-R) 1.5 μl each, ddH_2O 21 μl, the amplification program was 95 °C for 3 min, 95 °C for 15 s, 59 °C

for 15 s, 72 °C for 120 s, and 34 cycles, and the correct bands were selected for recovery after gel electrophoresis. The PCR-amplified fragments were digested with Kpn I and Hind III double digests system, and then loaded into the 1300-GFP-C expression vector, and the ligation system was as follows: recovered product 1.0 μl, 5×CE II Buffer 2 μl, Enzyme-cut vector 3.0 μl, Exnase II 1.0 μl, ddH2O 3.0 μl. 37 °C PCR was incubated for 1 h to obtain the recombinant plasmid pSuper::1300-GFP-RhHsf24. The ligated product was transformed into *E. coli*, single colony PCR was performed, and positive clones were selected for sequencing verification.

Subcellular localization of RhHsf24 protein

In order to investigate the localization of *RhHsf24* gene expression protein in cells, the binary vector was transformed into *Agrobacterium* EHA105 strains and were injected into leaves of *N. benthamiana*. The treated tobacco (*Nicotiana benthamiana*) seedings were then placed in a controlled environment chamber with a 16 h light/ 8 h dark light photoperiod at a constant temperature of 24 ℃. After 48 h, images were captured using confocal laser scanning microscopy.

Gene expression assay

The primers for quantitative real-time PCR of *RhHsf24* were designed using SnapGene and DNAman software, and the primer sequences are shown in Table 1. The reaction system for qRT-PCR consisted of a total of 20 μl: cDNA template 1 μl, upstream and downstream primers 0.5 μl each, the fluorescent dye (2× RealStar Fast SYBR qPCR Mix) 10.0 μl, ddH2O 8.0 μl. The reaction program was: pre-denaturation at 95 °C for 3 min, denaturation at 95 °C for 10 s, annealing at 60 °C for 30 s, for a total of 39 cycles. *RhUBI2* was used as internal controls for *R. hybrida*[51]. The experiments were performed on three or four independent biological replicates of each sample. Relative expression was calculated using the $2^{-\Delta\Delta ct}$ method, and the data obtained were analyzed for significance using Prism 8.0 software.

Conclusion

In this study, the *RhHsf24* gene with a CDS length of 888 bp and encoding 296 aa, was cloned from the *R. hybrida* 'Samantha'. Sequence comparison with *Arabidopsis* Hsfs family genes and phylogenetic tree analysis with proteins from other species revealed that this gene is the closest relative to *AtHsfB1*, and is in the same branch as *R. rugosa* (RrHSF24), *F. vesca* (FvHSFB1a) and *A. anserina* (AaHSF24), which belongs to the class B heat shock transcription factors. The results of subcellular localization showed that RhHsf24 protein was localized to the nucleus. qRT-PCR indicated that *RhHsf24* responded to high-temperature and salt stress. It may play an important role in the perception, transmission, and amplification of stress signals and the response of regulatory factors and functional proteins in rose. This study provides an important gene source and theoretical basis for further research on the stress tolerance mechanism of the rose and for expanding the cultivation of rose germplasm resources.

Data availability

All data generated in this study is included in this article. The nucleotide and protein sequence of RhHsf24 is available at in the National Centre for Biotechnology Information (NCBI) GenBank repository [Accession number: LOC112187155].

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References

1. Yoshida, T. et al. *Arabidopsis* HsfA1 transcription factors function as the main positive regulators in heat shock-responsive gene expression. *Mol. Genet. Genomics*. **286**, 321–332 (2011).
2. Haider, S., Raza, A., Iqbal, J., Shaukat, M. & Mahmood, T. Analyzing the regulatory role of heat shock transcription factors in plant heat stress tolerance: a brief appraisal. *Mol. Biol. Rep.* **49**, 5771–5785 (2022).
3. Guo, M. et al. The plant heat stress transcription factors (HSFs): structure, regulation and function in response to abiotic stresses. *Front. Plant. Sci.* **7**, 114 (2016).
4. Tiwari, M., Kumar, R., Min, D. & Jagadish, S. V. K. Genetic and molecular mechanisms underlying root architecture and function under heat stress-A hidden story. *Plant. Cell. Environ.* **45**, 771–788 (2022).
5. Deng, C., Chen, Y., Wei, W., Chen, X. & Jiang, J. Genome-wide identification of heat shock transcription factor family and key members response analysis to heat stress in *Loquat*. *Horticulturae* 10, 1195 (2024).
6. You, Y. J., Ahn, S. Y. & Yun, H. K. Heat shock transcriptional factors (HSFs) are expressed in response to hydrogen peroxide production in grapevines inoculated with *Colletotrichum* species. *Hortic. Environ. Biote.* **63**, 735–745 (2022).
7. Majee, A. et al. Elucidation of heat shock transcription factor family (Hsfs) postulates significant insights for the identification of their putative roles in root development and hormonal regulation in tomato. *J. Plant. Growth Regul.* **42**, 2327–2344 (2023).
8. [8]Mallick. Genome-wide analysis and characterization of heat shock transcription factors (Hsfs) in common bean (*Phaseolus vulgaris* L). *Funct. Integr. Genomic.* **22**, 743–756 (2022).
9. Tan, B. et al. Genome-wide identification of HSF family in Peach and functional analysis of *PpHSF5* involvement in root and aerial organ development. *PeerJ* **9**, e10961 (2021).
10. Hu, Y. et al. Identification, isolation, and expression analysis of heat shock transcription factors in the diploid woodland strawberry *Fragaria vesca*. *Front. Plant. Sci.* **6**, 736 (2015).
11. Kotak, S., Port, M., Ganguli, A., Bicker, F. & von Koskull-Döring, P. Characterization of C-terminal domains of *Arabidopsis* heat stress transcription factors (Hsfs) and identification of a new signature combination of plant class A Hsfs with AHA and NES motifs essential for activator function and intracellular localization. *Plant. J.* **39**, 98–112 (2004).
12. Bharti, K. et al. Tomato heat stress transcription factor HsfB1 represents a novel type of general transcription coactivator with a histone-like motif interacting with the plant CREB binding protein ortholog HAC1. *Plant. Cell.* **16**, 1521–1535 (2004).
13. Goel, K. et al. Functional divergence of heat shock factors (Hsfs) during heat stress and recovery at the tissue and developmental scales in C4 grain Amaranth (*Amaranthus hypochondriacus*). *Front. Plant. Sci.* **14**, 1151057 (2023).

14. dos Santos, T. B., Ribas, A. F., de Souza, S. G. H., Budzinski, I. G. F. & Domingues, D. S. Physiological responses to drought, salinity, and heat stress in plants: a review. *Stress* **2**, 113–135 (2022).
15. Zhang, H., Zhu, J., Gong, Z. & Zhu, J. K. Abiotic stress responses in plants. *Nat. Rev. Genet.* **23**, 104–119 (2022).
16. Kopecká, R., Kameniarová, M., Černý, M., Brzobohatý, B. & Novák, J. Abiotic stress in crop production. *Int. J. Mol. Sci.* **24**, 6603 (2023).
17. Zhang, Y. et al. Plants' response to abiotic stress: mechanisms and strategies. *Int. J. Mol. Sci.* **24**, 10915 (2023).
18. Giorno, F., Guerriero, G., Baric, S. & Mariani, C. Heat shock transcriptional factors in *Malus domestica*: identification, classification and expression analysis. *BMC Genom.* **13**, 639 (2012).
19. Peng, S. et al. A novel heat shock transcription factor, *VpHsf1*, from Chinese wild *Vitis pseudoreticulata* is involved in biotic and abiotic stresses. *Plant. Mol. Biol. Rep.* **31**, 240–247 (2013).
20. Zang, D., Wang, J., Zhang, X., Liu, Z. & Wang, Y. *Arabidopsis* heat shock transcription factor HSFA7b positively mediates salt stress tolerance by binding to an E-box-like motif to regulate gene expression. *J. Exp. Bot.* **70**, 5355–5374 (2019).
21. Ogawa, D., Yamaguchi, K. & Nishiuchi, T. High-level overexpression of the *Arabidopsis HsfA2* gene confers not only increased themotolerance but also salt/osmotic stress tolerance and enhanced callus growth. *J. Exp. Bot.* **58**, 3373–3383 (2007).
22. Huang, Y. et al. Heat shock factors in Carrot: genome-wide identification, classification, and expression profiles response to abiotic stress. *Mol. Biol. Rep.* **42**, 893–905 (2015).
23. Chen, X. et al. Genome-wide identification and expression analysis of *Hsf* and *Hsp* gene families in cucumber (*Cucumis sativus* L). *Plant. Growth Regul.* **95**, 223–239 (2021).
24. Meyer, P. W. The return to China, mother of gardens. *Arnoldia* **68**, 4–11 (2010).
25. Shi, L. Y. et al. Research progress on the effect of abiotic stress on the growth and development of Moonflower. *J. Henan Agric. Univ.* **58**, 1–14 (2023).
26. Dai, F. et al. RhNAC2 and RhEXPA4 are involved in the regulation of dehydration tolerance during the expansion of Rose petals. *Plant. Physiol.* **160**, 2064–2082 (2012).
27. Geng, L. et al. Genome-wide analysis of the Rose (*Rosa chinensis*) *NAC* family and characterization of *RcNAC091*. *Plant. Mol. Biol.* **108**, 605–619 (2022).
28. Geng, L. et al. Transcription factor RcNAC091 enhances Rose drought tolerance through the abscisic acid-dependent pathway. *Plant. Physiol.* **193**, 1695–1712 (2023).
29. Fan, Y. et al. The *RhHB1*/*RhLOX4* module affects the dehydration tolerance of Rose flowers (*Rosa hybrida*) by fine-tuning jasmonic acid levels. *Hortic. Res-England.* **7**, 74 (2020).
30. Scharf, K. D. et al. The tomato Hsf system: HsfA2 needs interaction with HsfA1 for efficient nuclear import and May be localized in cytoplasmic heat stress granules. *Mol. Cell. Biol.* **18**, 2240–2251 (1998).
31. Tokunaga, Y., Otsuyama, K. I. & Hayashida, N. Cell cycle regulation by heat shock transcription factors. *Cells* **11**, 203 (2022).
32. Jiang, C. et al. A cytosolic class I small heat shock protein, RcHSP17.8, of *Rosa chinensis* confers resistance to a variety of stresses to *Escherichia coli*, yeast and *Arabidopsis thaliana*. *Plant. Cell. Environ.* **32**, 1046–1059 (2009).
33. Lubkowska, A., Pluta, W., Strońska, A. & Lalko, A. Role of heat shock proteins (HSP70 and HSP90) in viral infection. *Int. J. Mol. Sci.* **22**, 9366 (2021).
34. Jacob, P., Hirt, H. & Bendahmane, A. The heat-shock protein/chaperone network and multiple stress resistance. *Plant. Biotechnol. J.* **15**, 405–414 (2017).
35. Baniwal, S. K. et al. Heat stress response in plants: a complex game with chaperones and more than Twenty heat stress transcription factors. *J. Biosci.* **29**, 471–487 (2005).
36. Sakuma, Y. et al. Dual function of an Arabidopsis transcription factor DREB2A in water-stress and heat-stress-responsive gene expression. *Proc. Natl. Acad. Sci. U S A.* **103** (49), 18822–18827 (2006).
37. Czarnecka-verner, E., Pan, S., Salem, T. & Gurley, W. B. Plant class B HSFs inhibit transcription and exhibit affinity for TFIIB and TBP. *Plant. Mol. Biol.* **56**, 57–75 (2004).
38. Baniwal, S. K., Chan, K. Y., Scharf, K. D. & Nover, L. Role of heat stress transcription factor HsfA5 as specific repressor of HsfA4. *J. Biol. Chem.* **282**, 3605–3613 (2007).
39. Zhao, L., Pan, H., Sun, M., Zhang, Q. & Molecular cloning of *Arobidopsis thaliana HSFA2* gene and *Agrobacterium* mediated genetic transformation of *Chrysanthemum morifolium Ramat*. *Adv. Intel. Soft Compu.* 134, 827–834 (2012).
40. Zhou, L. et al. Genome-wide identification, classification and expression profile analysis of the HSF gene family in *Hypericum perforatum*. *PeerJ* **9**, e11345 (2021).
41. Zheng, R. et al. General analysis of heat shock factors in the *Cymbidium ensifolium* genome provided insights into their evolution and special roles with response to temperature. *Int. J. Mol. Sci.* **25**, 1002 (2024).
42. Iqbal, M. Z. et al. A heat shock transcription factor TrHSFB2a of white clover negatively regulates drought, heat and salt stress tolerance in Transgenic *Arabidopsis*. *Int. J. Mol. Sci.* **23**, 12769 (2022).
43. Yang, J. et al. HY5-HDA9 orchestrates the transcription of HsfA2 to modulate salt stress response in *Arabidopsis*. *J. Integr. Plant. Biol.* **65**, 45–63 (2023).
44. Sun, T. T. et al. Confers salt stress tolerance through modulation of reactive oxygen species scavenging by directly regulating *ThWRKY4*. *Int. J. Mol. Sci.* **22**, 5048 (2021).
45. Ling, C. et al. Genome-wide identification of *HSF* gene family in Kiwifruit and the function of *AeHSFA2* Bin salt tolerance. *Int. J. Mol. Sci.* **24**, 15638 (2023).
46. Yang, J. et al. Genome-wide expression profiling analysis of Kiwifruit *GolS* and *RFS* genes and identification of *AcRFS4* function in raffinose accumulation. *Int. J. Mol. Sci.* **23**, 8836 (2022).
47. Ikeda, M., Mitsuda, N. & Ohme-Takagi, M. *Arabidopsis* HsfB1 and HsfB2b act as repressors of the expression of heat-inducible Hsfs but positively regulate the acquired thermotolerance. *Plant. Physiol.* **157**, 1243–1254 (2011).
48. Thompson, J. D., Gibson, T. J., Plewniak, F., Jeanmougin, F. & Higgins, D. G. The CLUSTAL_X windows interface: flexible strategies for multiple sequence alignment aided by quality analysis tools. *Nucleic Acids Res.* **25**, 4876–4882 (1997).
49. Hall, T., Biosciences, I. & Carlsbad, C. J. G. B. B. BioEdit: an important software for molecular biology. *GERF Bull. Biosci.* **2**, 60–61 (2011).
50. Tamura, K., Stecher, G. & Kumar, S. MEGA11: molecular evolutionary genetics analysis version 11. *Mol. Biol. Evol.* **38**, 3022–3027 (2021).
51. Cheng, C. et al. Ethylene-regulated asymmetric growth of the petal base promotes flower opening in Rose (*Rosa hybrida*). *Plant. Cell.* **33**, 1229–1251 (2021).

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Author contributions

C.P.W conceived and designed the experiments. S.D.L., Y.Q.S., Z.X.H., F.D., J.Z., and M.Q.C., performed the experiments and analyzed the data; S.D.L., Y.Q.S., and C.P.W., wrote the article.

Declarations

Competing interests

The authors declare no competing interests.

Additional information

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