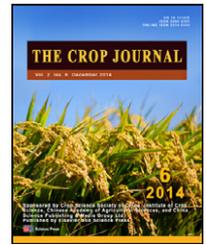


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# A MITE insertion into the 3'-UTR regulates the transcription of TaHSP16.9 in common wheat



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## ABSTRACT

Miniature inverted-repeat transposable elements (MITEs) are a type of DNA transposon frequently inserted into promoters, untranslated regions (UTR), introns, or coding sequences of genes. We found a 276-bp tourist-like MITE insertion in the 3'-UTR of a 16.9 kDa small heat shock protein gene (*TaHSP16.9-3A*) on chromosome 3A of common wheat. Haplotype analysis revealed two haplotypes, sHSP-W (wild type without MITE insertion) and sHSP-M (mutant with MITE insertion), present in wheat germplasm. Both semiquantitative PCR and quantitative real-time PCR analyses showed increased transcription levels of *TaHSP16.9-3A* in sHSP-M compared with those of sHSP-W after heat treatment at 42 °C. It appeared that the MITE insertion into the 3'-UTR enhances the transcription of *TaHSP16.9-3A*.

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## 1. Introduction

All living organisms can be induced to produce heat-shock proteins (HSPs) in response to heat stress. However, plant HSPs are more complex than HSPs in other organisms. Plant HSPs can be classified into five major classes: HSP60, HSP70, HSP90, HSP100, and small heat shock proteins (sHSPs) [1]. Plant sHSPs can also be divided into six classes according to their cellular localization and similarity. Three classes (CI, CII, and CIII) are present in the cytosol or in the nucleus, and the other three are present in the plastid, endoplasmic reticulum, and mitochondria, respectively [1–4]. *TaHSP16.9* in wheat belongs to class I and is the first eukaryotic sHSP for which a high-resolution structure has been described [5]. *In vitro* analysis of the chaperone activity of *TaHSP16.9* has indicated substrate

specificity for sHSP [6]. In plants, abundant sHSPs are induced to be synthesized in response to environmental stresses and developmental stimuli, whereas most sHSPs are restricted to low expression levels at certain development stages under normal environmental conditions, indicating that sHSPs play an important role in stress tolerance [2,3]. It has been suggested that sHSPs act as molecular chaperones that selectively bind non-native proteins to prevent aggregation in an ATP-independent manner [3].

Transposable genetic elements (TEs) are repeated DNA sequences that are able to move from one site in the genome to another and replicate during mobilization. TEs are essential components of many eukaryotic genomes and may play important roles in size, structure, polymorphism, genome evolution, and regulation of gene expression [7–9]. According to

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their mode of transposition, TEs are divided into two classes, retrotransposons and DNA transposons. Retrotransposons transpose indirectly through an RNA intermediate and consist of two principal groups, long terminal repeat (LTR) and non-LTR retrotransposons. DNA transposons transpose directly as DNA that is excised from the original site in the genome and inserted into a new site. DNA transposons may also be divided into two classes. Autonomous DNA transposons contain genes encoding transposases that mediate DNA transposition, such as *Ac* transposons in maize. Although nonautonomous DNA transposons lack genes encoding transposases, they can transpose by mediation of transposases encoded by autonomous transposons such as the *Ds* transposons in maize [7,8,10].

Miniature inverted-repeat transposable elements (MITEs) are a particular class of DNA transposons that have typical structures of non-autonomous DNA transposons, containing target site duplications (TSD) and terminal inverted repeats (TIR) [10,11]. In plant, MITEs are divided into tourist-like, stowaway-like and pogo-like groups, according to the similarity of their terminal inverted repeat and target site duplication sequences [11–13]. However, MITEs differ from classic DNA transposons by their small size (usually less than 500 bp), large copy number (usually hundreds or thousands), and consistency of related elements, which are the features of retrotransposons. The small size and large copy number of MITEs lead to their frequent insertion into promoters, untranslated regions, introns, or coding sequences of plant genes [14–18]. The proximity between MITEs and adjacent genes promotes the hypothesis that MITEs play an important role in regulating gene expression.

Researchers continue to investigate the function of MITEs in gene regulation. Earlier studies discovered two types of rice *ubiquitin2* (*rubq2*) promoter in rice lines, with two nested MITEs (*Kiddo* and *MDM1*) inserted in IR24 and only *MDM1* inserted in T309 [15]. The insertion of *Kiddo* increased the transcription rate of *rubq2* in rice, but methylation of *Kiddo* neutralized this enhancement effect [19]. MITE insertion into the coding region of an oleoyl-PC desaturase gene (*ahFAD2B*) resulted in a premature stop codon with a putatively truncated protein, leading to a reduced transcript level of *ahFAD2B* and high oleate content [18]. A MITE-like insertion close to the start codon of the water-stress tolerance gene *Hsdr4* in barley regulated the transcription of *Hsdr4* by forming a hairpin-like secondary structure [20]. The size of a repeated structure harboring a tourist-like MITE insertion in the upstream region of the *SbMATE* gene (multidrug and toxic compound extrusion) positively corresponded with aluminum tolerance in sorghum. The results suggested that the MITEs act as cis-acting elements to multiplicatively enhance the expression of *SbMATE*, explaining the positive correlation between the repeat structure and aluminum tolerance [21]. Recent research in the Solanaceae showed that MITEs generated small RNA by a TE-derived siRNA pathway as described in *Arabidopsis*, and supported the hypothesis that a MITE-derived siRNA targeted the gene with MITE insertion in post-transcriptional silencing pathway [22]. In rapeseed (*Brassica napus* L.), haplotype analysis revealed a tourist-like MITE insertion/deletion polymorphism in the upstream region of *BnFLC.A10* distinguishing most winter types (insertion) from spring types (deletion). The polymorphism was positively associated with the difference in *BnFLC.A10* expression between Tapidor (insertion) and Ningyou 7 (deletion). Association analysis among two types

of rapeseed showed that the MITE insertion was significantly associated with vernalization requirement [23].

Common wheat (*Triticum aestivum* L.) is one of the most important food crops. Owing to its large genome size, hexaploidy, and highly repetitive DNA sequence, regulation by MITEs of wheat gene expression has been poorly investigated. Here we report the identification and transcription regulation effects of a 276-bp tourist-like MITE insertion into the 3'-UTR of *TaHSP16.9*, a 16.9-kDa small heat shock protein in common wheat.

## 2. Materials and methods

### 2.1. Plant materials

Two hexaploid bread wheat genotypes, the heat-tolerant cultivar TAM107 and the heat-susceptible landrace Chinese Spring [24], were used as materials to clone the promoter, 5'-UTR, coding region, and 3'-UTR of *TaHSP16.9-3A*. Also, 17 common wheat cultivars and 23 Chinese landraces were selected for haplotype analysis of the MITE insertion (Table 1). Chinese Spring and its nullisomic-tetrasomics and ditelosomics were kindly provided by Drs. W. J. Raupp and B. S. Gill, Wheat Genetics Resource Center, Kansas State University, USA.

### 2.2. High temperature stress treatments

Seeds were surface-sterilized with 1% sodium hypochlorite for 30 min, rinsed with distilled water, and soaked in the dark overnight at room temperature. After germination, seeds were planted in flasks (10 seedlings per flask) containing 1% agar culture medium and grown in a climate-controlled incubator (16 h day/8 h night, 22 °C day/18 °C night, 60% humidity) [24]. Ten days later, seedlings were transferred to another incubator (16 h day/8 h night, 42 °C day/18 °C night, 80% humidity) for heat treatment at 42 °C for 0.5 h, 1 h, 2 h, and 3 h. A total of 30 seedlings in three flasks were subjected to each treatment, and three independent biological replications were prepared. At the end of each treatment, leaf samples from three flasks were immediately frozen in liquid nitrogen and stored at –80 °C until total RNA extraction.

### 2.3. DNA extraction and DNA amplification

Genomic DNA was extracted from 10-day-old seedling leaves by the CTAB method [25]. DNA amplification reactions were performed in a 20- $\mu$ L volume containing 100 ng DNA, 0.2 mmol L<sup>-1</sup> dNTPs, 0.3  $\mu$ mol L<sup>-1</sup> of each primer, 1 U *Taq* polymerase, and 1 $\times$  assay buffer. The amplification parameters were as follows: 94 °C for 5 min; 40 cycles of 94 °C for 45 s, 53–60 °C (depending on primers used) for 45 s, 72 °C for 1 min; and 72 °C for 10 min. The PCR products were checked on 8% nondenaturing polyacrylamide gels or 2% agarose gels.

### 2.4. Genome walking

Genomic DNA extracted from wheat genotypes TAM107 and CS was purified with phenol/chloroform after RNase (TaKaRa, Japan) treatment at 37 °C for 1 h. DNA samples were evaluated by electrophoresis on a 1% agarose gel in

**Table 1 – Common wheat cultivars and landraces used for MITE haplotype analysis.**

Name	Cultivar/landrace	Haplotype	Name	Cultivar/landrace	Haplotype
TAM107	Cultivar	sHSP-M	Chinese Spring	Landrace	sHSP-W
Huangmaizi	Landrace	sHSP-M	Huangkexiaomai	Landrace	sHSP-W
Huangxiaomai	Landrace	sHSP-M	Huangmai	Landrace	sHSP-W
HuoshaoMai	Landrace	sHSP-M	Huixianhong	Landrace	sHSP-W
HuoshaoMai	Landrace	sHSP-M	Huilibaimai	Landrace	sHSP-W
Jiyumai	Landrace	sHSP-M	Huixianmai	Landrace	sHSP-W
Jingdong 8	Cultivar	sHSP-M	Huohuaxiaomai	Landrace	sHSP-W
Xiaoyan 88	Cultivar	sHSP-M	Huoliaoyan	Landrace	sHSP-W
Shi 4185	Cultivar	sHSP-M	Huomai	Landrace	sHSP-W
Jingdong 6	Cultivar	sHSP-M	Huomai	Landrace	sHSP-W
Zhoumai 18	Cultivar	sHSP-M	Huomai	Landrace	sHSP-W
Nongda 3338	Cultivar	sHSP-M	Huomaier	Landrace	sHSP-W
Lankao 906	Cultivar	sHSP-M	HuoshaoTian 57	Landrace	sHSP-W
Wanmai 38	Cultivar	sHSP-M	Honghua	Landrace	sHSP-W
Bainong AK58	Cultivar	sHSP-M	Jiyupo	Landrace	sHSP-W
			Jianzitou	Landrace	sHSP-W
			Jianermai	Landrace	sHSP-W
			Jianmai	Landrace	sHSP-W
			Shijiazhuang 8	Cultivar	sHSP-W
			Triumph	Cultivar	sHSP-W
			Nongda 189	Cultivar	sHSP-W
			Jing 411	Cultivar	sHSP-W
			Handan 6172	Cultivar	sHSP-W
			Yanda 1817	Cultivar	sHSP-W
			Shi 9036	Cultivar	sHSP-W

Tris-acetate ethylenediaminetetraacetic acid (TAE) buffer containing ethidium bromide ( $0.5 \mu\text{g mL}^{-1}$ ) for DNA integrity and quantified by spectrophotometry. A genome walker library was constructed using the Genome Walker Universal Kit (Clontech, Japan) according to the manufacturer's protocol. The cycling conditions were as follows: 94 °C for 3 min; 7 or 5 cycles of 94 °C for 25 s, 72 °C for 3 min; 32 or 25 cycles of 94 °C for 25 s, 67 °C for 3 min; with a final extension of 67 °C for 7 min.

### 2.5. RNA extraction and reverse transcription

Three sHSP-W (without MITE insertion) and three sHSP-M (with MITE insertion) cultivars or landraces were randomly chosen and subjected to heat treatment and total RNA was extracted from 10-day-old seedlings using TRIzol reagent (Tiangen, Beijing) according to the manufacturer's instructions. Total RNA samples were treated with DNase I (Promega, USA) at 37 °C for 1 h and subjected to phenol/chloroform extraction to eliminate residual DNA. RNA was precipitated with  $0.3 \text{ mol L}^{-1}$  NaAc (pH 5.2) and two volumes of ethanol, collected by centrifugation ( $12,000 \times g$ , 15 min at 4 °C). RNA concentration was determined by measuring absorbance at 260 nm. First-strand cDNA was synthesized from 2  $\mu\text{g}$  of total RNA using (dT)<sub>18</sub> primer with MMLV reverse transcriptase (Promega, USA) following the manufacturer's instructions. The reverse transcription reaction conditions were as follows: 37 °C for 60 min, 95 °C for 5 min. The product of reverse transcription was examined by amplifying *Tabeta-actin* (300 bp), which was used as the endogenous control in semi-quantitative PCR.

### 2.6. Quantitative real-time PCR

Quantitative real-time PCR was performed on a CFX96 real-time system (Bio-Rad, USA) using SYBR premix Ex Taq mixture

(TaKaRa, Japan) with cycle conditions as follows: 95 °C for 5 min; 38 cycles of 95 °C for 10 s, 56 °C for 10 s, 72 °C for 20 s; melt curve from 65 °C to 90 °C, increment 0.3 °C for 0.03 s, plate read. All reactions were run in triplicate and included no template and no reverse transcription controls. Quantification results were expressed in terms of the cycle threshold (CT) value according to the baseline adjusted to 0.04. The comparative CT method (PE Applied Biosystems, USA) was used to quantify relative gene expression compared with *Tabeta-actin* (120 bp). Briefly, the CT values were averaged for each triplicate. Differences between the mean  $C_T$  values of specific genes and those of *Tabeta-actin* were calculated as  $\Delta C_T^{\text{sample}} = C_T^{\text{gene}} - C_T^{\text{Tabeta-actin}}$ . In the final results, the relative expression levels were determined by the  $2^{-\Delta C_T}$  method. Statistical significance was tested using Student's *t* test ( $P < 0.05$ ) [25]. Each PCR product was evaluated in at least two independent experiments. The primers used for semiquantitative PCR and quantitative real-time PCR are listed in Table 2.

## 3. Results

### 3.1. Identification of a MITE insertion in the 3'-UTR of TaHSP16.9 in common wheat

A 276-bp tourist-like MITE insertion was identified in the 3'-UTR of a small heat shock protein gene, *TaHSP16.9*, in wheat during annotation of the *TaHSP16.9* sequence from a public database. Two haplotypes, sHSP-W (wild type without MITE insertion) and sHSP-M (mutant with MITE insertion), were found in public NCBI wheat EST data sets and then were confirmed in wheat germplasm by specific primers (CJD/MITER1 and GSP3/MIDE) flanking the MITE (Table 2; Fig. 1). A set of common wheat cultivars and landraces were

**Table 2 – Primers used for semiquantitative PCR, quantitative real-time PCR, chromosome assignment, and haplotype analysis.**

Primer pairs	Purpose	Forward primer (5'–3')	Reverse primer (5'–3')	Annealing temperature (°C)	Product size (bp)
BD	Semiquantitative PCR and chromosome assignment	AGTTCGTCAGGGCTTCCG	CGCAATACAGAGATGGCTCGTC	58	245
SSD	Quantitative real-time PCR	CGAGGTCAAGAAGCCTGAGG	CGTCAGACTCGGAAGAACA	58	130
<i>Tabeta-actin</i>	Internal standard in semiquantitative PCR	GACCCAGACAACCTCGCAAC	GGAATCCATGAGACCACCTAC	56	300
<i>Tabeta-actin</i>	Internal standard in quantitative real-time PCR	ACTCATCATACTCGCCCTTCG	CAAGCAGCATGAAGATCAAGGT	56	120
CJD/MITE-R1	Haplotype analysis	GCTGAGTATTCGGGTCTGGTTC	AGGTAGTAACTTAGGTGCCACAT	58	400

then selected for MITE insertion polymorphism analysis. The sHSP-M haplotype was detected in 5 of 23 wheat landraces and 10 of 17 wheat cultivars. Interestingly, the MITE insertion was present in a heat-tolerant wheat cultivar, TAM107, but absent from a heat-sensitive wheat landrace, Chinese Spring (Table 1).

The chromosomal location of *TaHSP16.9* was determined using nullitetrasonic and ditelosomic lines of Chinese Spring. *TaHSP16.9* was assigned to chromosome 3AL (Fig. 2).

### 3.2. Genomic structure of *TaHSP16.9-3A* between TAM107 and Chinese Spring

The promoter, 5'-UTR, coding sequences, and 3'-UTR of *TaHSP16.9-3A* were amplified from genomic DNA of TAM107 and Chinese Spring by genome walking. *TaHSP16.9-3A* is a single-exon gene without introns. Two SNPs (G/C and A/G) in the coding region, 27 SNPs in the promoter region, and a 276-bp MITE insertion and deletion (InDel) in the 3'-UTR were observed in the *TaHSP16.9-3A* between TAM107 and Chinese Spring. The MITE was located 142 bp downstream of the stop codon (TGA) and upstream of the polyadenylation termination sequence (AATAAAA) signal site in the 3'-UTR with typical tourist-like structure of TAA as target site duplication (TSD) and 5'-GGCTGCTCATAGTGG-3' as terminal inverted repeat (TIR) (Fig. 1).

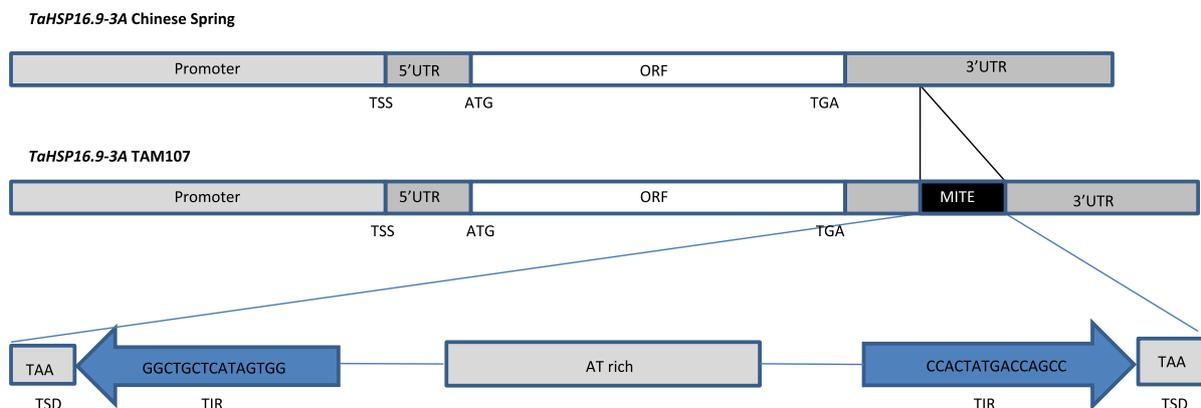
The promoter regions of *TaHSP16.9-3A* between TAM107 and Chinese Spring were scanned in the PlantCARE web site (<http://bioinformatics.psb.ugent.be/>) to predict putative

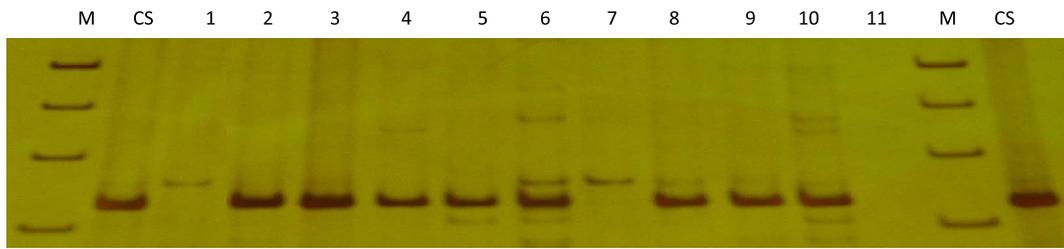
regulatory motifs, some of which were stress responsive. No significant differences in stress-response elements were identified between TAM107 and Chinese Spring (data not shown). The G/C and A/G SNPs in the coding regions resulted in two amino acid polymorphisms (N/K; E/K). The N/K amino acid polymorphisms were located in the  $\beta$ -strand 5 within the conserved  $\alpha$ -crystallin domain of *TaHSP16.9* [5].

### 3.3. Expression pattern of *TaHSP16.9-3A* under high temperature stress

Semiquantitative PCR and quantitative real-time PCR analysis showed that *TaHSP16.9-3A* was expressed in a heat-inducible fashion and that the expression increased significantly in both TAM107 and Chinese Spring with increasing seedling exposure time to high temperature stress (42 °C). However, the expression levels were higher in TAM107 than in Chinese Spring at each time point. No *TaHSP16.9-3A* expression was detected under the control condition (22 °C day/18 °C night) in either TAM107 or Chinese Spring (Fig. 3).

To test whether the regulatory effects of MITE insertion in the 3'-UTR on *TaHSP16.9-3A* were universal, semiquantitative PCR and quantitative real-time PCR analysis were performed to evaluate the transcription of *TaHSP16.9-3A* in 6 Chinese wheat landraces under 42 °C heat-treatment for 2 h. The results revealed an increased transcript level of *TaHSP16.9-3A* in the sHSP-M haplotype (Huangmaizi, Huangxiaomai, and Jiyumai) compared with the sHSP-W haplotype (Chinese Spring, Jianermai, Jianmai) (Fig. 4).

**Fig. 1 – Structure comparisons of the *TaHSP16.9-3A* gene between Chinese Spring and TAM107.**



**Fig. 2 – Chromosome arm assessment of *TasHSP16.9* in Chinese Spring homoeologous group 3 nullisomic-tetrasomics and ditelosomics. M: marker; CS: Chinese Spring; 1: N3A-T3B; 2: N3B-T3A; 3: N3B-T3D; 4: N3D-T3A; 5: N3D-T3B; 6: Dt3AS; 7: Dt3AL; 8: Dt3BL; 9: Dt3DS; 10: Dt3DL; 11: ddH<sub>2</sub>O.**

#### 4. Discussion

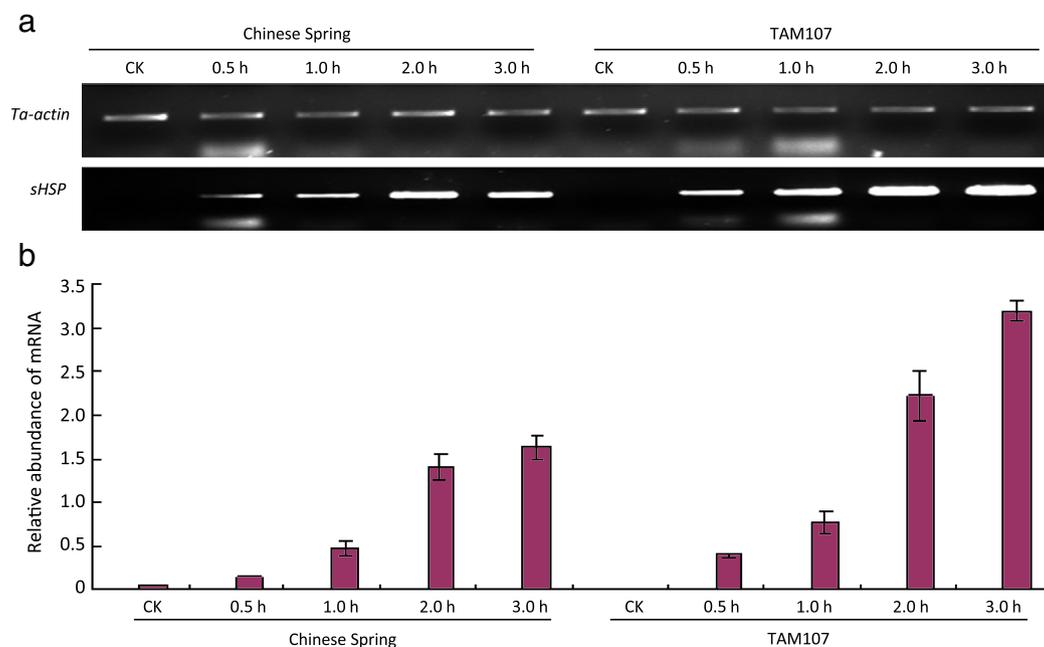
Wheat is one of the most important food crops, and is grown under various environmental conditions worldwide. High temperature is one of its key limiting factors, causing wheat yield loss and reducing wheat quality. Plants are induced to synthesize abundant low molecular weight HSPs (sHSPs) in response to heat stress, suggesting that plant HSPs play a role in tolerance to heat shock.

In the present study, a miniature inverted-repeat transposable element (MITE) insertion in the 3'-UTR of *TaHSP16.9-3A* resulted in two haplotypes (sHSP-W and sHSP-M) in wheat germplasm. An increased transcript level of *TasHSP16.9-3A* was detected in sHSP-M haplotypes compared with sHSP-W haplotypes under high-temperature stress. Comparative analysis of the promoter sequences revealed no recognizable cis-acting stress-response elements differing between TAM107 and Chinese Spring. The nucleotide diversity in the promoter sequence may not contribute to the increased transcript level of *TaHSP16.9-3A* under heat stress treatment (Fig. S1). The G/C

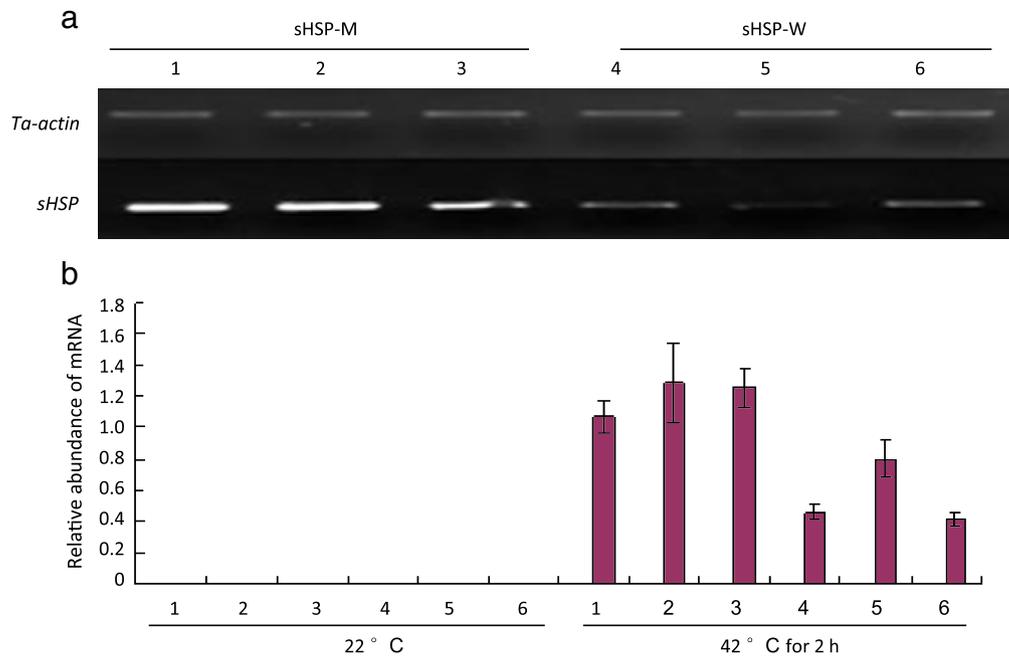
and A/G SNPs in the coding region resulted in two amino acid polymorphisms (Fig. S2). The N/K and E/K amino acid polymorphisms may influence the 3-D structure of the sHSP protein, but are unlikely to influence the transcription level. It appeared that the MITE insertion into the 3'-UTR could be a key factor enhancing the transcription of *TaHSP16.9-3A*.

Previous studies showed that increased HSP gene expression was positively correlated with genetic differences in cellular thermal tolerance in winter wheat. Earlier synthesized and higher levels of sHSP mRNAs were found in the heat-tolerant wheat cultivar Mustang than in the heat-susceptible wheat cultivar Sturdy under heat stress [26,27]. We propose that MITE insertion into the 3'-UTR of *TaHSP16.9-3A* improves the heat tolerance of wheat genotypes by increasing the transcription of *TaHSP16.9-3A* under heat stress. Association analysis of heat tolerance with the MITE insertion using a diversity panel of wheat germplasm and over-expression of the *TaHSP16.9-3A* in heat-sensitive wheat varieties should allow testing this hypothesis.

The 3'-UTR could play an important role in gene regulation by controlling mRNA stability and translation efficiency. The



**Fig. 3 – The *TaHSP16.9-3A* transcription pattern of TAM107 and Chinese Spring under 42 °C heat treatment for different time intervals (0 h, 0.5 h, 1 h, 2 h, 3 h) detected by semi-quantitative PCR (a) and quantitative real-time PCR (b).**



**Fig. 4 – The *TaHSP16.9-3A* transcription pattern in Chinese wheat landraces detected by semiquantitative PCR (a) and quantitative real-time PCR (b). 1: Huangmaizi; 2: Huangxiaomai; 3: Jiyumai; 4: Chinese Spring; 5: Jianermai; 6: Jianmai. Heat treatment was at 42 °C for 2 h. MITE insertions were present in the 3′-UTRs of Huangmaizi, Huangxiaomai, and Jiyumai but absent from Chinese Spring (CS), Jianermai, and Jianmai.**

3′-UTR may act as a binding site of *trans*-acting factors such as proteins and miRNA. A recent study showed that the binding of miRNA23b to the 3′-UTR of mouse  $\mu$ -opioid receptor (MOR1) suppressed MOR1 mRNA translation by inhibiting mRNA interaction with polysomes [28]. Detailed analysis of SAUR-AC1 expression in *Arabidopsis* showed that the 3′-UTR acted as an mRNA instability determinant [29]. The highly conserved DST (downstream element) sequence in the 3′-UTR may contribute to the mRNA instability and mediate mRNA decay [30]. The mRNAs of heat shock genes have structures like the regions in the 3′-UTR that allow their selective translation in stressed cells by increasing mRNA stability [31]. Studies have suggested that the abundance of gene expression is often regulated at the post-transcriptional level like mRNA degradation, which is associated with mRNA stability [29,30]. In this study, our results suggested that the MITE insertion enhanced the transcription of *TaHSP16.9-3A* mRNA in wheat lines containing MITE insertion in the 3′-UTR. Further analysis is needed to determine whether the MITE insertion improved the stability of *TaHSP16.9-3A* mRNA.

In plants, recent studies have suggested that MITEs contribute to genome structure and gene regulation [16,17,32–34]. TEs are usually silenced by methylation, but they may affect the expression of adjacent genes under certain stresses. In other words, various environmental stresses are associated with the activation of TEs. The contribution of the MITE (*Kiddo*) insertion in the promoter to the enhancement of *rubq2* promoter activity in rice was neutralized by methylation of *Kiddo* [19]. The DNA transposon *mPing* acts as an enhancer to up-regulate the expression of nearby genes under cold stress regardless of the site of TE insertion in rice [16]. In sorghum, MITEs may act as *cis*-acting elements to multiplicatively enhance expression of

*SbMATE* to induce aluminum tolerance [21]. In this study, we speculate that either heat stress activates the MITE to enhance the expression of *TaHSP16.9-3A*, or the MITE acts as a *cis*-acting element interacting with *trans*-acting elements to enhance the expression of *TaHSP16.9-3A* in wheat lines (sHSP-M), resulting in heat tolerance.

## 5. Conclusions

Annotation of *TaHSP16.9* sequence from a public database revealed the presence of a tourist-like MITE insertion in the 3′-UTR of *TaHSP16.9* on chromosome 3A in wheat. Haplotype analysis of a set of common wheat cultivars and landraces revealed two haplotypes, sHSP-W and sHSP-M. Transcription analysis suggested that MITE insertion is the key factor up-regulating the expression of *TaHSP16.9-3A* in the sHSP-M haplotype after heat treatment at 42 °C, indicating a possible role of the MITE in gene regulation in wheat.

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## Supplementary material

Supplementary material to this article can be found online at <http://dx.doi.org/10.1016/j.cj.2014.07.001>.

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