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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*.

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]. *Ta*HSP16.9 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 *Ta*HSP16.9 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-μL volume containing 100 ng DNA, 0.2 mmol L^{-1} dNTPs, 0.3 μmol L^{-1} of each primer, 1 U *Taq* polymerase, and 1× 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