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Isolation and expression analysis of genes encoding DNA methyltransferase in wheat (*Triticum aestivum* L.)

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Abstract

DNA methylation of cytosine residues, catalyzed by DNA methyltransferases, is suggested to play important roles in regulating gene expression and plant development. In this study, we isolated four wheat cDNA fragments and one cDNA with open reading frame encoding putative DNA methyltransferase and designated *TaMET1*, *TaMET2a*, *TaMET2b*, *TaCMT*, *TaMET3*, respectively. BLASTX searches and phylogenetic analysis suggested that five cDNAs belonged to four classes (*Dnmt1*, *Dnmt2*, *CMT* and *Dnmt3*) of DNA methyltransferase genes. *TaMET2a* encoded a protein of 376 aa and contained eight of ten conserved motifs characteristic of DNA methyltransferase. Genomic sequence of *TaMET2a* was obtained and found to contain ten introns and eleven exons. The expression analysis of the five genes revealed that they were expressed in developing seed, during germination and various vegetative tissues, but in quite different abundance. It was interesting to note that *TaMET1* and *TaMET3* mRNAs were clearly detected in dry seeds. Moreover, the differential expression patterns of five genes were observed between wheat hybrid and its parents in leaf, stem and root of jointing stage, some were up-regulated while some others were down-regulated in the hybrid. We concluded that multiple wheat DNA methyltransferase genes were present and might play important roles in wheat growth and development.

Keywords: DNA methylation; Wheat; Methyltransferase gene; Expression; Heterosis

1. Introduction

In the genome of higher plants, up to 30% of the total cytosine are methylated [1]. Cytosine methylation occurs predominantly in the CG and CNG sites, and the levels change in a tissue- and/or development-specific pattern. Although the physiological functions of DNA methylation in plants are not fully understood, it has been implicated in a number of biological processes, such as gene expression [2], embryo development, cell differentiation, genomic imprinting [3], X chromosome inactivation [4] and cell memory [5].

The presence of 5-methylcytosine in genomic DNA is the result of enzymatic activity of the DNA methyltransferase (MET), which transfers a methyl group from *S*-adenosyl-L-methionine to the fifth position of a cytosine residue. Genes encoding DNA methyltransferase have been isolated in *Arabidopsis* [6], rice [7], carrot [8] and maize [9]. Sequence analysis revealed that DNA methyltransferase genes can be grouped into at least four distinct classes, namely *Dnmt1/MET1*, *Dnmt2*, *CMT* and *Dnmt3*, respectively [10]. The *Dnmt1/MET1* class acts primarily as maintenance methyltransferase, which transfers methyl groups to cytosine in hemi-methylated CpG sites after DNA replication [11], resulting in stable patterns of methylation that are maintained throughout development or, in many cases, between generations. The *Dnmt2* class does not appear to play a significant role in establishing or maintaining DNA methylation patterns, and the function of

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these *Dnmt2* methyltransferase-like proteins is unclear. *Dnmt3* acts as de novo methyltransferase that transfers methyl group to unmethylated DNA templates. The chromomethylases (*CMTs*) represent a class of DNA methyltransferases that was found only in plants and catalyze CNG methyltaion.

Previous studies in carrot and *Arabidopsis* indicated that DNA methyltransferase genes are abundantly expressed in meristematic cell and could be related to cell division [8]. *METIII* in *Arabidopsis* was expressed at a high level in floral tissue [12]. It was also suggested that DNA methyltansferase genes of different class showed different expression patterns. For example, *Arabidopsis METI* transcripts are at least 10,000-fold more abundant than those of *METII*. It was reported that maize *ZmMETI* was down-regulated by cold treatment [9].

DNA methyltransferase genes have been isolated and characterized in both dicot (*Arabidopsis*) and monocots (maize and rice) species. However, to our knowledge, no genes encoding DNA methyltransferase has been reported in wheat. In this study, five cDNA sequences with high homology to genes encoding different DNA methyltransferase were cloned, and their expression patterns were analyzed. The expression profiles of DNA methyltransferase gene between wheat hybrid and its parents were also investigated in order to gain insight into the understanding of the regulatory mechanisms of differential gene expression observed between hybrid and parents, and possibly heterosis.

2. Material and methods

2.1. Plant materials

One highly heterotic interspecific hybrid 3338/2463 and its female parent 3338 (common wheat) and male parent 2463 (spelt wheat) were used for this study. For the greenhouse-grown plants, seeds were germinated in the filter paper and were then transplanted into pots. They were cultivated in vermiculite that was watered by nutrient solution and grew in a growth chamber at a relative humidity of 75% and 26/20 °C day and night temperature. Leaves and roots of different development stage (seedling stage, tillering stage, jointing stage and heading stage) were collected. In addition, embryos isolated from the seeds which were imbibed in the moist filter paper for 4 to 28 h with 4 h interval were collected. cDNAs from dry seeds and developing seeds 6 and 12 days after pollination (DAP) were also used for RT-PCR analysis.

2.2. Identification of DNA methyltransferase ESTs and in silico cloning of a complete open reading frame (ORF)

The cDNA sequences encoding known DNA methyltransferase of maize (Accession numbers: AF063403, AAK15805, AAF68437 and AAK40306) and rice (Accession numbers: AF462029 and BK001405) were used as query sequences to search against wheat expressed sequence tag (EST) database (http://www.ncbi.nlm.gov/dbEST/) using the BLAST program (http://www.ncbi.nlm.nih.gov/blast/). Four ESTs (Accession numbers: BQ166624, CA496954, BE445315 and CD867242), as putative wheat DNA methyltransferase cDNA clones of different classes, were selected for further study. Three overlapping ESTs (Accession numbers: CD872573, BE492805 and BJ284697) were assembled into a contig with an open reading frames (ORF).

2.3. RNA and DNA extraction

The frozen samples were ground to fine powder under liquid nitrogen and transferred into 1.5 ml Eppendorf tubes. 800 μl of prewarmed (90 °C) extraction buffer (containing 100 mM LiCl, 1% SDS, 10 mM EDTA, 100 mM Tris–HCl, pH 9.0) was added to each tube and mixed with equal volume of phenol (pH 8.0). Tubes were then incubated in 90 °C water bath for 2 min, and cooled on ice. After adding 300 μl chloroform, the samples were shaken for 10 min and centrifuged at 12,000 rpm for 10 min at 4 °C. The supernatant was removed into clean tubes and extracted twice by equal volume of chloroform. The supernatant was collected and precipitated with one-third volume of 8 M LiCl for 16 to 18 h at 4 °C. The samples were then centrifuged at 12,000 rpm for 10 min at 4 °C and the supernatant was removed. The precipitate was washed in 2 M LiCl and 80% ethanol, and redissolved in double distilled water treated by DEPC. Total RNA was digested with DNaseI (Promega, Madison, USA) for 30 min and extracted twice by chloroform to eliminate residual DNA. Nucleic acids were precipitated with 0.3 M NaAc (pH 5.2) and 2 volumes of ethanol, collected by centrifugation (13,000 rpm, 15 min at 4 °C).

Total DNA was extracted from the seedling leaves by the CTAB method [13] but with minor modifications.

2.4. Cloning and sequencing

Specific primers were designed based on the four EST sequences and the assembled contig (Table 1). PCR was carried out using aliquots of 2 μl of the obtained cDNA and 125 pmol of specific primers in a 20 μl reaction volume containing 0.2 mM of each dNTPs, 1.5 mM $MgCl_2$ and 1 U Tag polymerase. The PCR conditions for the specific primers were 5 min at 94 °C followed by 40 cycles of 1 min at 95 °C, 1 min at 60 °C, and 2 min at 72 °C, and a final extension of 7 min at 72 °C. PCR products were separated on 1% agarose gels and the single specific band of PCR products was obtained and cloned into the pGEM-Teasy vector (CLONTECH) for sequencing.

2.5. Semi-quantitative RT-PCR analysis

Two microgram total RNA of each sample was used for first-strand cDNA synthesis in 20 μl reactions containing