HOSTED BY

ELSEVIER

Available online at www.sciencedirect.com

ScienceDirect

Characterization of the imprinting and expression patterns of *ZAG2* in maize endosperm and embryo

Chaoxian Liu[1], *Jiuguang Wang*[1], *Xiupeng Mei, Xiaojing Deng, Tingting Yu, Xiaoli Liu, Guoqiang Wang, Zhizhai Liu, Yilin Cai*[*]

Maize Research Institute, Southwest University, Chongqing 400715, China

ARTICLE INFO

Article history:
Received 28 January 2014
Received in revised form
10 October 2014
Accepted 3 December 2014
Available online 16 December 2014

Keywords:
Gene imprinting
ZAG2
Expression pattern
Endosperm
Embryo

ABSTRACT

ZAG2 has been identified as a maternally expressed imprinted gene in maize endosperm. Our study revealed that paternally inherited *ZAG2* alleles were imprinted in maize endosperm and embryo at 14 days after pollination (DAP), and consistently imprinted in endosperm at 10, 12, 16, 18, 20, 22, 24, 26, and 28 DAP in reciprocal crosses between B73 and Mo17. *ZAG2* alleles were also imprinted in reciprocal crosses between Zheng 58 and Chang 7-2 and between Huang C and 178. *ZAG2* alleles exhibited differential imprinting in hybrids of 178 × Huang C and B73 × Mo17, while in other hybrids *ZAG2* alleles exhibited binary imprinting. The tissue-specific expression pattern of *ZAG2* showed that *ZAG2* was expressed at a high level in immature ears, suggesting that *ZAG2* plays important roles in not only kernel but ear development.

1. Introduction

Genomic imprinting, the differential expression of alleles depending on parental origin, is an epigenetic phenomenon occurring in mammals as well as in angiosperms [1]. In mammals, imprinting usually occurs in embryonic and extra-embryonic tissues [2]. In flowering plants, the majority of imprinted genes are expressed in triploid endosperm [3–5]. *R1*, which regulates anthocyanin biosynthesis in maize endosperm, was the first-discovered gene showing genomic imprinting. When *R1* was inherited maternally, the full kernel was pigmented, whereas when *R1* was inherited paternally, the kernel exhibited mottled pigmentation [6]. Later, genomic imprinting was also discovered in mouse. A nuclear transplantation experiment showed that completion of mouse embryogenesis required both the maternal and paternal genomes, and that two copies of either the paternal or the maternal genome were not sufficient for normal development of mouse embryos [7,8]. Although imprinting was first discovered in plants, studies have been widely conducted in mammals. It is well established that imprinted genes regulate placenta development and fetal growth in mammals and that a change of methylation status of imprinted genes may cause human disease [9].

Imprinted loci are classified as paternally or maternally imprinted, depending upon which parental allele is expressed. In maize, paternally imprinted genes include *Meg1* [10], *nrp1* [11],

[*] *Corresponding author.*
E-mail address: caiyilin1789@163.com (Y. Cai).
Peer review under responsibility of Crop Science Society of China and Institute of Crop Science, CAAS.
[1] Chaoxian Liu and Jiuguang Wang contributed equally to this work.

http://dx.doi.org/10.1016/j.cj.2014.10.001

dzr1 [12], *Mez1* [13,14], *fie1*, and *fie2* [15], etc. By contrast, maternally imprinted genes are fewer [16]. With the development of modern sequencing technology, 111 maternally imprinted and 68 paternally imprinted genes were newly identified in 10 DAP endosperm from reciprocal crosses between inbred lines B73 and Mo17, according to whether the expression of a given allele was fivefold greater than that of the other allele [17]. A similar study was performed by Waters et al. [18], but employing different criteria: a gene designated as imprinted required 90% of reads to be from one allele. As a result, 100 putative imprinted genes were identified in endosperm 14 days after pollination (DAP) from reciprocal crosses between B73 and Mo17, including 54 maternally and 46 paternally expressed imprinted genes. Though only 50 genes were common to the 100 and 179 genes designated as imprinted by these two research groups, these studies still greatly expanded the number of imprinted genes in maize. *ZAG2* is one of the 50 imprinted genes identified in common. *ZAG2*, as well as the imprinted gene *OsMADS87* in rice [19] and *PHE1* in *Arabidopsis* [20], is also a MADS-box transcription factor. Here we report the imprinting characterization of *ZAG2* in three reciprocal crosses and evaluate the tissue-specific expression pattern of *ZAG2*, with the aim of elucidating the function of *ZAG2* in maize development.

2. Materials and methods

2.1. Plant material and growth conditions

Maize inbred lines including B73, Mo17, Zheng 58, Chang 7-2, Huang C, and 178 were planted in spring at Beibei (29°76′N, 106°37′E), Chongqing, China. Among these inbred lines, B73 and Mo17 were the most widely used elite inbred lines worldwide. Zheng 58 and Chang 7-2 and Huang C and 178 were the respective parents of Zhengdan 958 and Nongda 108, well-known corn hybrids in China. Endosperm tissues were collected at 10, 12, 14, 16, 18, 20, 22, 24, 26, and 28 DAP. Embryos were harvested at 14 and 18 DAP. Tissues at the same developmental stage derived from different ears were pooled prior to RNA or DNA extraction. Additionally, the root, stem, leaf, immature tassel (3–4 cm), and ear (3–4 cm) were collected from B73.

2.2. Total RNA extraction and cDNA synthesis

Samples were ground in liquid nitrogen. Ground tissue (200 mg) was treated with 1 mL Trizol (Invitrogen). Total RNA was isolated and then digested with DNaseI to remove genomic DNA before use.

Approximately 1 mg of total RNA was used as a template for cDNA synthesis, using an oligo(dT) primer according to the specifications of RevertAid First Strand cDNA Synthesis Kit (Fermentas). The quality of the synthesized cDNA was evaluated by *actin* amplification.

2.3. Development of cleaved amplified polymorphic sequence (CAPS) markers

Gene specific primers (GSP) were designed with Primer-BLAST (http://www.ncbi.nlm.nih.gov/tools/primer-blast/index. cgi?LINK_LOC = BlastHome) and used to amplify the target sequence of *ZAG2* cDNA for CAPS marker development. The main parameters were as follows: primer size of about 22 bp, primer GC content of 40–60%, and no more than 0.7 °C difference melting temperature between forward primer and reverse primer. The PCR products were cloned and sequenced for identifying single-nucleotide polymorphisms. SNPs that created restriction sites were selected for development of CAPS markers with SNP2CAPS [21]. To test the CAPS markers, PCR products were digested with an appropriate FastDigest enzyme (Fermentas) for 5 min with incubation at the corresponding temperature, and then resolved on 1% agarose stained with GoodView (SBS).

2.4. Tissue-specific gene expression analysis of the ZAG2 gene

Tissue-specific expression of *ZAG2* was assessed by real-time quantitative RT-PCR, performed on a CFX96 Touch Cycler (Bio-Rad). The detection of amplification rates was performed using THUNDERBIRD qPCR Mix (TOYOBO). *ZAG2* mRNA expression was normalized against *actin*. Every sample had three technical replicates. The cycle parameters were as follows: an initial denaturation step of 60 s at 95 °C, and then denaturation at 95 °C for 15 s and annealing and extension at 64 °C for 60 s, for 45 cycles of PCR.

3. Results

3.1. Characterization of ZAG2

According to the putative cDNA sequence (GRMZM2G160687), the gene-specific primer GSP-1 (Table 1) flanking the open reading frame (ORF) was designed to amplify the target gene from cDNA of 14 DAP maize kernels. The PCR products were sequenced and BLASTed against the Reference RNA Sequences database and High Throughput Genomic Sequences database of *Zea mays* (http://www.ncbi.nlm.nih.gov/BLAST). The amplified sequence showed 99% identity with *Zea mays AGAMOUS homolog2* (*ZAG2*). *ZAG2* putatively encoded a protein composed of 269 amino acids and contained a typical MADS-box domain and K-box domain (Fig. 1), indicating that *ZAG2* was a member of the MADS-box gene family.

3.2. ZAG2 is a maternally expressed imprinted gene

A CAPS marker, GSP-2 (Table 1) located at the 3′ terminus of the *ZAG2* cDNA, was developed and used for amplifying the 14 DAP endosperm cDNAs of the reciprocal hybrids between B73 and Mo17. DNA sequencing showed that SNPs were present between B73 and Mo17 alleles and could be recognized by the enzyme *Sph* I. The digestion profile of the PCR products digested by *Sph* I indicated that the expression of *ZAG2* from the maternal allele was greater than that from the paternal allele in B73 × Mo17 (Fig. 2). Thus, *ZAG2* was a maternally expressed imprinted gene. Extremely weak expression of *ZAG2* from the paternal allele was detected in B73 × Mo17, indicating that *ZAG2* also displayed differential imprinting. However, the case was very different in Mo17 × B73, in which *ZAG2* was thoroughly imprinted, indicating that *ZAG2*