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Epigenetic inheritance and variation of DNA methylation level and pattern in maize intra-specific hybrids

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Abstract

Studies on transmission genetics of cytosine methylation in plants are important for elucidating the biological roles of this epigenetic modification. Using three sets of reciprocal maize hybrids and their inbred parents, we compared level and pattern of cytosine methylation in each of the hybrids and their corresponding parental inbred lines by the methylation-sensitive amplified polymorphism (MSAP) method. We found that whereas a great majority of cytosine methylation sites manifested faithful epigenetic inheritance, from 6.59% to 11.92% of the sites showed altered parental patterns in hybrids, with the extent of deviation being depending on both direction of the cross and parental combinations. DNA gel blot analysis demonstrated that the great majority of MSAP profiles in a hybrid, both inheritance and alteration, can be validated. The changes in cytosine methylation level and pattern were not caused by parental heterozygosity, and they could be either directed or stochastic among individual hybrid plants. Homology analysis of isolated MSAP profiles indicated that diverse sequences underlie methylation alterations, including known-function genes, ESTs and uncharacterized sequences. We discuss possible relevance of methylation-pattern remodeling to heterosis in a maize hybrid relative to its parental inbred lines.

Keywords: Cytosine methylation; Epigenetic inheritance; Heterosis; Hybrid; Methylation remodeling; Maize

1. Introduction

Cytosine DNA methylation is an important epigenetic modification that increase the information content of many eukaryotic genomes including those of higher plants. DNA methylation plays important roles in fundamental cellular processes, particularly in repressive control of genome expression over development and in maintenance of the overall genomic integrity [1–3]. Consequently, disturbance of intrinsic DNA methylation patterns may lead to functional and phenotypic abnormality or evolutionary opportunity [4,5].

In spite of their common existence, several distinct differences characterize cytosine DNA methylation in animals from that in plants, including substrate specificity, genomic distribution and epigenetic inheritance [4,6,7]. Among these, the meiotic heritability of methylation levels and patterns in plants versus their often ‘‘erasure and reset’’ dynamics in each organismal generation in animals [8,9] (but also see [10]) is particularly intriguing, given the proposed common origin of this epigenetic marker as an ancient genome defense system [3,11].

It has been demonstrated in *Arabidopsis* that the induced genomic hypomethylation state by the *ddm1* (decrease in DNA methylation) gene mutation can be stably transmitted over generations [12]. More recently, it was uncovered that the naturally occurred differential cytosine methylation states at certain genomic regions including two major ribosomal loci in different ecotypes of *Arabidopsis* also largely exhibit faithful epigenetic inheritance from parents to inter-strain hybrids [7]; nevertheless, apparent non-inheritance or modification of parental methylation patterns sometimes occur in certain combinations, suggesting *trans*-acting modifier factors that are different in different ecotypes are involved in the control of fidelity of methylation inheritance [13]. In rice, genome-wide

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analysis of cytosine methylation patterns between a single inter-strain hybrid and its parents indicated that 96% methylation sites showed stable inheritance while about 4% sites showed alteration [14]. In contrast, in certain inter-specific allopolyploid hybrids, like those in the *Triticum–Aegilops* complex, parental cytosine methylation patterns often underwent rapid and dramatic remodeling, which may effect up to 15% of the genomic loci [15,16], suggesting intrinsic genome instability in these plants under hybrid conditions involving divergent genomes.

Maize has a large and complex genome with more than 80% constituents being repetitive retrotransposons or their derivatives [17]. Moreover, in this plant, genes and repetitive sequences are differentially methylated [18]. Therefore, it can be envisioned that the methylation status of the maize genome in both level and pattern might be different from that of compact-genome plants like *Arabidopsis* or rice.

Given the importance of heterosis or hybrid vigor in maize production, and the suggested possible role played by epigenetic regulation on allelic gene expression [19], it will be interesting to investigate the inheritance or variation of cytosine methylation level and pattern from inbreds to hybrids. The present study was aimed to address this issue from both a genome-wide perspective and locus-specific patterns.

2. Materials and methods

2.1. Plant materials

Three sets of reciprocal maize hybrids (designated as 8M, M8, MD, DM, D8 and 8D) and their inbred parental lines [7**8**599 (8), **M**o17 (M) and **D**an340 (D)] were used in this study. All three inbred lines have been maintained in our hands by strict self-pollination for many generations, whilst the hybrids were made by careful manual emasculation and pollination.

2.2. DNA isolation

Genomic DNA was first isolated from expanded leaves at the 9–10th leaf-stage, of pooled maize plants of the various inbred lines and hybrids by a modified CTAB method [20]. For the purpose of analyzing uniformity or variation of methylation alterations among different hybrid individuals, and to detect possible heterozygosity in the parental inbred lines, genomic DNA was also isolated from expanded leaves of the same stage individual plants of hybrids and parental inbred lines.

2.3. MSAP analysis

The methylation-sensitive amplified polymorphism analysis (MSAP) method essentially as reported [14,21–26] was used. The restriction enzymes *Hpa*II and *Msp*I were purchased from the New England Biolabs Inc. (Beverly, Mass.). In total, one pair of pre-selective primers and 43 pairs of selective primers were used for amplifications (Supplementary Table 1). Silver stained sequencing gel was used to resolve and visualize the amplification products. Only clear and reproducible bands that appeared in two independent PCR amplifications (starting from the digestion–ligation step, i.e., the first step of MSAP) were scored.

The scored MSAP bands were transformed into a binary character matrix, ‘‘1’’ for presence and ‘‘0’’ for absence of a band, at a particular position. For statistical analysis of the differences between the mid-parental value and the reciprocal hybrids, the following formula was used:

$$p = \frac{y_1 + y_2}{n_1 + n_2}, \qquad q = 1 - p, \qquad \sigma_{p_1 - p_2} = \sqrt{pq\left(\frac{1}{n_1} + \frac{1}{n_2}\right)}, \qquad U = \frac{p_1 - p_2}{\sigma_{p_1 - p_2}}$$

where n_1 is the total methylation sites (of CCGG) for a given sample (herein refers to the mid-parent values); n_2 the total methylation sites for another sample (herein refers to a hybrid); y_1 the total methylation sites, hemimethylation sites (outer Cs) or fully methylation sites (inner Cs) for a given sample (herein refers to the mid-parent values); y_2 the total methylation sites, hemimethylation sites or fully methylation sites for another sample (herein refers to a hybrid); p_1 the percentage of total methylation sites, hemimethylation sites or fully methylation sites for a given sample (herein refers to the mid-parent values); p_2 is the percentage of total methylation sites, hemimethylation sites or fully methylation sites for another sample (herein refers to a hybrid).

2.4. Recovery and sequencing of MSAP bands

Representative bands showing methylation alteration in the silver-stained MSAP gels were eluted and re-amplified with the appropriate selective primer combinations. Sizes of the PCR products were verified by agarose gel electrophoresis, and then cloned into the AT cloning vector (Takara Biotech. Inc., Dalian). The cloned DNA segments were sequenced with vector primers by automatic sequencing. The Advanced BlastN and BlastX programs at the NCBI website (http://www.ncbi.nlm.nih.gov/) were respectively used for homology analysis of the cloned DNA sequences that gave quality-reads.

2.5. DNA gel blot analysis

Genomic DNA (10 μg per sample) was digested by either of the pair of methylation-sensitive isoschizomers, *Hpa*II or *Msp*I. To ensure complete digestion, an excess of enzymes (10 units enzyme per μg DNA) was used and the incubation time was extended to ~48 h. Digested DNA was fractionated by running through 1% agarose gels and transferred onto Hybond N+ nylon membranes (Amersham Pharmacia Biotech) by the alkaline transfer recommended by the supplier. Cloned DNA segments representing different methylation patterns in the MSAP profile were selected as hybridization probes. Hybridization signal was detected by the Gene Images CDP-Star detection module (Amersham Pharmacia Biotech) after washing at a stringency of 0.2× SSC, 0.1% SDS for 2× 50 min. The filters were