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RESEARCH PAPER

Genome-Wide Analysis of *MuDR*-Related Transposable Elements Insertion Population in Maize

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Abstract: Insertional mutagenesis has now been widely used to knockout genes for functional genomics. The maize *Mutator* transposons hold an advantage of high activity to construct large mutant libraries. In this study, a *MuDR* line was used to cross with an elite Chinese maize inbred line Z31. A total of 1000 M_1 individuals were planted and self-pollinated to generate their M_2 families. Experiments were conducted to investigate the insertion specificity of *MuDR* related transposable elements. Six hundred and ninety-five *MuDR* inserted flanking sequences were isolated with a modified MuTAIL-PCR method and analyzed with bioinformatics. Three hundred and seventy-four non-redundant insertion sites were identified and 298 of them were mapped to a single locus on the integrated maize map. The results revealed some prominent features of the *MuDR*-related insertions of maize: random distribution across the 10 chromosomes, preferential insertion into genic sequence and favoring some classes of functional genes.

Keywords: *Zea mays*; *Mutator* (*Mu*) transposons; *MuDR* elements; flanking sequence; insertion sites; *Mu*TAIL-PCR

Gene knockout has become a powerful and indispensable tool in molecular genetics and functional genomics. A comprehensive collection of gene knockouts allows us to understand the relationship between the phenotypes and mutations of genes [1].

Diverse approaches have been used to develop comprehensive gene knockout resources, which are necessary for forward and reverse genetic analysis in plants. *Arabidopsis thaliana* has provided a good model using flanking sequence tags (FSTs) approach via insertional mutagenesis populations [2, 3]. Several methods were primarily applied to systematically amplify and sequence the genomic DNA flanking the T-DNA tags from each mutant. Then these FSTs were searched against the public DDBI/EMBL/GenBank GSS database to obtain the genome annotations. An improved FST approach was adopted in rice (*Oryza sativa* L.) [4–6] and the latest release of OryGenesDB database contained 171,000 FSTs (http://orygenesdb.cirad.fr/index.html), which greatly accelerated rice functional genomic research [7].

Maize (*Zea mays* L.) is comparable to rice as the model genetic system for genome studies [8, 9]. Since the *Ac/Ds* transposable elements were discovered [10], the transposon mutagenesis approaches have been widely used in disrupting genes, isolating mutants, and studying gene function in maize [11, 12].

Transposon insertional mutagenesis can be classified into low-copy and high-copy strategies [13]. The former contains *Ac* and *Spm*, which produce low genome-wide mutation rates and transpose preferentially to linked sites [14–16]. These populations cannot remove the interference of the background mutations due to the non- autonomous *Ds* elements in the genome. The high-copy system most commonly used in maize is *Mutator* [17].

Up to date, *Mutator* is the most active and mutagenic plant transposon discovered in maize [18–20]. Its properties of high copy numbers and high transposition frequency make it suitable for forward and reverse genetic analysis [21]. The *Mutator* transposon family is a two component system [22]. All the maize *Mutator* elements contain conserved −220 bp terminal inverted repeats (TIRs), but each class of elements

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contains specific and unrelated internal sequences [18, 22]. Liu et al. [23] defined 21 novel *Mu* TIRs using a DLA-454 strategy, different from the TIRs reported previously. This system is regulated by autonomous *MuDR* elements, which control the transposition of themselves and the other classes of the non-autonomous *Mu* elements [24, 25]. Besides, studies have shown that *Mu* insertions are heavily biased for transcribed regions of the genome [9, 26–28].

The successful use of transposon tagging lies in the identification and isolation of the genomic sequences flanking the insertion sites. Many PCR-based methods have been developed and some are optimized for *Mu* elements according to the conserved sequences in the inverted terminal repeats. Amplification of insertion mutagenized sites (AIMS) was a ligation-mediated method with the *Mu* primer biotinylated [29]. *Mu*TAIL was an adaptation of thermal asymmetrically interlaced (TAIL) PCR [30] to amplify flanking fragments in a complex pool of *Mu*-induced mutants [28]. Yi et al. [31] combined elements of both *Mu*TAIL and AIMS into a procedure called *Mu*TA for cosegregation analysis. Besides, an adaptor-mediated PCR-based method, Digestion-ligation-amplification (DLA), was developed to overcome difficulties of amplifying unknown sequences flanking known DNA sequences in large genomes [32]. Furthermore, an improved draft nucleotide sequence of the 2.3-gigabase maize genome has been released in 2009. All of these would greatly facilitate the *Mutator* tagging strategy in maize functional genomic research.

In our study, the M_1 population was generated by crossing the active *Mutator* transposon line as donor parent with the recipient parent Z31. The number of copies of the *MuDR* elements per mutagenic plant is one or two in the population. We observed the phenotypes of the M_1 and the M_2 generations and amplified the flanking sequences of the *MuDR* with a modified *Mu*TAIL-PCR method. The TAIL products were cloned and sequenced for further bioinformatics analysis. We attempt to construct our own *Mutator* insertional mutant populations of maize in China and create more novel mutants for the maize functional genomic studies.

1 Materials and methods

1.1 Plant materials

MuDR-active line was used as the pollen donor in a cross with the maize inbred line Z31 (yellow kernel). The progenies of the cross were self-pollinated to produce the *Mutator* mutant stocks. The resulted kernels were planted and young leaves were harvested for genomic DNA extraction based on the protocol described by Settles [28].

1.2 Isolation of flanking sequences

Mu flanking sequences were amplified via a modified *Mu*TAIL-PCR [28]. Two nested specific primers were modified according to the *MuDR* TIR sequence reported as follows: TIR9-1: 5′-ATAGAAGCCAACGCCATGGCCTCCATTTCG TC-3′; TIR9-2: 5′-GGCCTCCATTTCGTCGAATCCCTT-3′. The 12 arbitrary primers used in our experiment were designed.

Either 5% DMSO or glycerin was added to the PCR mixture. TAIL-PCR products were purified with QIA-quick Gel Extraction Kit (QIAGEN, Germany) based on the manufacturer's instructions. The confirmed products were ligated to the pGEM-T Vectors (Promega, USA). Then the ligation products were transformed into the *E. coli* DH5α by electronic transformer (Electroporator 2510, Eppendorf), and plated onto the LA medium containing Ampicillin, X-gal and IPTG (40 mg μL^{-1}). Cloned inserts corresponding to the size of the fragment from the PCR were full-pass sequenced by an ABI3730XL sequencing facility (Applied Biosystems, CA, USA) and the sequences were analyzed bioinformatically.

1.3 Sequence analysis

A homology search was first performed against the vector sequences of the plasmids using the NCBI VecScreen tool (http://www.ncbi.nlm.nih.gov/VecScreen/VecScreen.html). The masked sequences were then analyzed for the presence of conserved *MuDR* TIR sequence using a BLAST program. A BLASTN was consequently conducted against the B73 RefGen_v1 database to get the predicted location hits using the B73 Genomic Browse Tools with the expectation score cut-off < 10^{-12} (http:// www.maizegdb.org/).

2 Results

2.1 *MuDR* elements insertion sites

Mu-specific fragments were identified and cloned by comparing its parental band patterns. Not all the 12 arbitrary primers were found to be suitable in our materials. Only four of them, BAD5, CTG1, SAD11, and SW41 yielded large products from *Mutator* lines (Fig. 1).

Totally, 695 specific *Mu*TAIL-PCR fragments were obtained and sequenced from about 5000 M_2 individuals. Theoretically, a

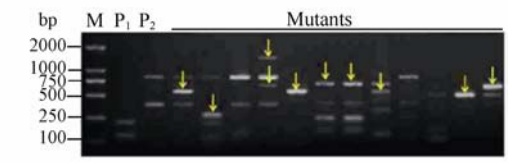

Fig. 1 *Mu*TAIL products from the DNA of parents and mutants using CTG1 arbitrary primer

M is DNA ladder marker DL2000. P_1 and P_2 are Z31 and *Mutator* active parent. The other 12 lanes show the secondary products of the TAIL-PCR. Arrows indicate the specific fragments.

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