Recovering flanking sequence tags of a miniature inverted-repeat transposable element by thermal asymmetric interlaced-PCR in peanut

Transposable elements are widely distributed among animal and plant genomes. Based on the transposition mechanism, two classes of transposable elements are recognized. Class I-type elements transpose via RNA and cDNA intermediates from one location to another, whereas class II type elements disintegrate from their present location and move to a new location in the genome[1,2]. Therefore, class I and class II type transposable elements are compared to 'copy and paste' and 'cut and paste' mechanisms respectively[3]. Transposable elements in each class can be subdivided into autonomous and non-autonomous based on whether the enzyme required for transposition is produced by the same or different element respectively[1].

Miniature inverted-repeat transposable elements (MITEs) belong to non-autonomous class II type[4]. MITEs are commonly distributed in animal and plant genomes[5]. In plants, they were first discovered in grass genomes[6,7]. Many genomes are now known to harbour MITEs. Their copy number varies from 3000 to 10,000 per genome. Elements of the MITE family are related by structure rather than by sequence similarity. MITEs are structurally characterized by relatively small (generally 80–500 bp long) size, AT-rich and non-coding, interspersed elements with a terminal inverted repeat of ~25 bp. Evidences suggest that MITEs are activated by chemical mutagen treatment[8] and *in vitro* tissue culture stresses[9]. They have a preference for target sites containing AT repeats. Many MITEs are known to be preferentially placed in the low copy genes and their promoters, indicating their potential role in gene regulation, defining chromatin domains and genome organization. Therefore, MITEs have been implicated in the evolution of gene structure and function[10]. Recent sequence evolution in the *indica* and *japonica* subspecies of rice has been largely due to MITE insertions[11].

In peanut, though extensive variation exists at the morphological level, limited polymorphism at DNA level has been a major constraint in elucidating its genome[12]. Moreover, transposons were implicated in the hyper-mutability of Dharwad Early Runner (DER), leading to generation of extensive variability[13–15], which could not be fully explained by genetic analysis. Recently, two independent high oleate mutations induced by diethyl sulphate (DES) were shown to be due to MITE (205 bp) insertion in the *ahFAD2B* gene encoding oleoyl-PC desaturase[8]. Since MITEs have a preference for genic and regulatory regions, it is interesting to analyse various mutants for MITE transposition. As the peanut genome sequence is not available, tracking MITE transposition by simple PCR-based techniques would be useful. Thermal asymmetric interlaced-PCR (TAIL-PCR) is widely used for recovering the unknown genomic sequences flanking a known sequence. With this background, a well-characterized peanut MITE was considered for recovering the 5′ genomic flanking sequence tag using TAIL-PCR to know its location in the genome, so that MITE can be tracked during mutation breeding. To the best of our knowledge, this is the first report on recovering a flanking sequence tag to find the genomic location of the MITE using TAIL-PCR in peanut.

An elite tetraploid peanut variety, TMV2 was grown under controlled conditions in the greenhouse. DNA was isolated using GenElute™ Plant Genomic DNA miniprep kit (Sigma Aldrich) from young leaves collected at 3–4 leaf stage of the seedling. TAIL-PCR was done essentially by following the protocol of Liu *et al.*[16], with necessary modifications[17]. The arbitrary primers used were: RB9_AD2, 5′STTGNTASTNCTNTGC3′; RB10_AD5, 5′RCAGNTGWTNGTNCTG3′; RB11_AD7, 5′NTCGASTWTSGWGTT3′; RB12_AD8, 5′NGTCGASWGANAWGAA3′; RB13_AD11, 5′NCASGAWAGNCSWCAA3′, and RB14_AD13, 5′NTSGASNTCNGAATCA3′. Considering the available sequence of MITE inserted in *ahFAD2B* gene[8], peanut MITE-specific nested primers were designed to recover the 5′ genomic flanking sequence tag. The nested primers were: RB18_MITETAIL1, 5′ATCTTCTCATGAAGATGCTTTGGT3′; RB19_MITETAIL2, 5′TGTGGTTTATTTATTTAGCCACACTT3′, and RB20_MITETAIL3, 5′ACCATACAATCCATCATCCAA3′. The specific primers were designed to have *Tm* higher than those of arbitrary primers. The primary TAIL-PCR products, when separated on 1.5% gel, formed a smear without any specific bands. However, when a diluted product of the primary TAIL-PCR was used as the template in the secondary TAIL-PCR along with a nested primer (RB19_MITETAIL2), four bands, two each with RB10_AD5 (~120 and 650 bp) and RB11_AD7 (~230 and 650 bp) were observed. However, the product specificity did not improve further in the tertiary TAIL-PCR with another nested primer (RB20_MITETAIL3). These products (Figure 1) from the tertiary TAIL-PCR were run on 1.5% agarose gel. However, only two bands, one amplified with RB10_AD5 (~230 bp) and another with RB11_AD7 (~120 bp) were eluted and cleaned using MinElute™ Gel Extraction kit (QIAGEN). These PCR products were sequenced with the same nested primer, RB20_MITETAIL3 (at Bangalore Genei, Bangalore). The flanking sequence tags were BLAST searched to find out MITE locations in the genome.

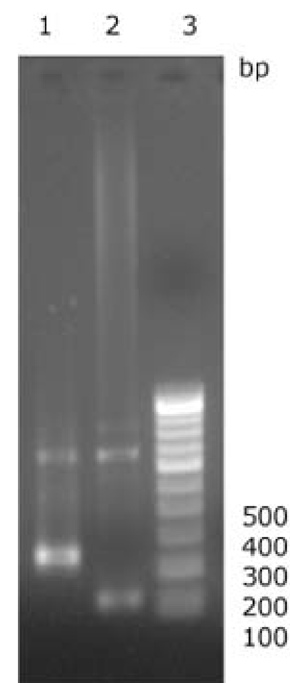

Figure 1. Tertiary TAIL-PCR products. Lane 1, With RB11_AD7 primer; lane 2, RB10_AD5 primer, and lane 3, Molecular weight marker (100 bp ladder).

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FST1  (1) TTGCCATCTTCATTGTAGTATCCACCTATTTATATAACCTCGTAATCCAA
          ---------------------------------------->***********
     (51) TCTCACCTCTCCATTCTCTGAAACAAATCTCATCCTTTTCTTCTCCCTCT
    (101) TCCTCTTCTTCAATTTTTTTAATTATTAATAAAATCTTGATTTAATTCTT
    (151) AAATTTTTTAAAAACTACAATCAAACACGAAACTCGAA

FST2  (1) ATCTTCATTGTAGTATCCACCTAATTATTTATTTATGCAGCACCAACACC
          -------------------------------->***************
     (51) TGA
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Figure 2. 5′ end of MITEs and their flanking sequence tags. Arrow shows the inverted terminal repeat and AT repeat is indicated by asterisk.

The 5′ flanking sequence tags (FSTs) were 151 (FST1) and 17 bp (FST2) long. An AT repeat, a characteristic feature of MITE in peanut was found next to MITE in the genomic sequence (Figure 2). It was 11 and 15 bp long in FST1 and FST2 respectively. However, variants of this AT repeat are known in DES-induced mutants of peanut[8]. Mutant M2-225 and MF (Mycogen-Flavo) had seven and eight ATs out of 9 bp repeat. An inverted terminal repeat, yet another structural feature of MITE, was also observed in these sequences, indicating that the FSTs recovered in this study are MITE-specific. This would also indicate the efficiency of TAIL-PCR in selectively amplifying genomic sequences flanking the known sequence of MITE. TAIL-PCR has been widely used to recover flanking sequences of T-DNA and/or transposon insertion for functional genomics, cloning such insertion-tagged genes, recovering upstream regulatory sequences of tagged gene, cloning genes from large molecules such as P1 phage, yeast and bacterial artificial chromosomes, etc. Like TAIL-PCR, many other PCR-based techniques such as inverse PCR[18], gene walking PCR[19] and single primer PCR[20] can be used for the same purpose, but they employ special steps before PCR such as Southern analysis, restriction digestion and ligation, making the task laborious, and they also tend to generate non-specific products. However, a different approach was used in rice to find out the copy number of *mPing*, an active MITE using transposon display (TD)[21], which is a modification of the amplified fragment-length polymorphism technique.

The BLAST search of 151 bp FST1 obtained in this study showed homology to a small region (23/24 bp) of genomic survey sequence of *Arachis batizocoi* (GenBank acc. no. DX508954) methylation filtered library (LibID703). *A. batizocoi* being the putative donor of B genome of cultivated peanut[22], the sequence homology is expected. However, 17 bp FST2, being too short, matched to several clones of *Arachis* taxa. Nevertheless, no homology was found with the clone DX508954, indicating that FST2 is a putative flanking sequence, but clearly independent of FST1. Hence they are the flanking sequence tags of MITE at two different locations in the genome. Once more genes get annotated or the peanut genome sequence is available, these FSTs would find a completely homologous region based on which the MITE location can be traced. At the moment, these FSTs could be useful for tracking these two copies of MITE under various conditions favouring its transposition and also to implicate its role in peanut mutations.

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R. S. BHAT[1,*]
V. U. PATIL[1]
T. M. CHANDRASHEKAR[1]
V. SUJAY[2]
M. V. C. GOWDA[2]
M. S. KURUVINASHETTI[1]

[1]*Department of Biotechnology, and*
[2]*Department of Genetics and Plant Breeding,*
University of Agricultural Sciences,
Dharwad 580 005, India
**For correspondence.*
e-mail: bhatramesh12@rediffmail.com