MUTATOR-LIKE ELEMENTS IN THE GENOME OF CASSAVA (*MANIHOT ESCULENTA* CRANTZ)

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Introduction

Transposable elements (TEs) constitute a large portion of eukaryotic genomes. Their contribution to genetic variability and genomic restructuring for development and evolution, as agents for response to genomic stress and as originator of ecotypes and cultivated plants and animals have recently gained increased attention (Bennetzen 1996; Fedoroff 1999). The genome sequencing projects are enabling a wide variety of these elements to be analysed in details using computer-based sequence searches.

Cassava is an important staple crop for more than half a billion people worldwide. It is a hardy crop with many advantages to the small-scale farmer and potentials for industrial applications. Understanding of the genome of this important crop plant could be a step in the direction of addressing some of the many problems including diseases, cyanogenesis, and post-harvest physiological deterioration, which limit its production, exploitation, utilisation and acceptance. Cassava is presently understudied and there is no extensive genomic sequence data. Our study shows that cassava genome does contain many *Mutator*-like elements, MULEs.

Results

Identification and confirmation of Cassava MULE transposase cDNA clone (Me-cTP1)

The cDNA recombinant plasmid in pBluescript was cloned in DH5α *E. coli* and high quality plasmids were isolated using Qiagen 'QIAprep Miniprep kit' and sequenced. The sequence data was subjected to blastn and blastx searches using the NCBI database (www.ncbi.nhm.nih.gov). Blastn search revealed that the cDNA encodes a cassava transposase predicted mRNA sequence with a 79 % pairwise similarity to that from *Arabidopsis* MULE (not shown) while blastx search showed that the c *Arabidopsis* MULE (Figure 1).

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Me:1    LQRDDGPPGNMAVLPCLKSMTWVMENKNTTPGNRVAVINLKLQDYSKTPSTEFEVKFQLS
        L RDD  P NM  LPCLKS+TW ME+KNT PG RVAVINLKL DY K PS + +VKFQLS
At 708  LHRDDTAPENMVALPCLKSLTWGMESKNTMPGGRVAVINLKLHDYRKFPSADMDVKFQLS

Me:181  RVTLEPMLRSMAYISEQLSTPANRVAVINLKLQDTETTSGESEVKFQVSRDTLGAMLRSM
         VTLEPMLRSMAYISEQLS+PANRVAVINLKLQDTETT+GESEVKFQVSRDTLGAMLRSM
At:768  SVTLEPMLRSMAYISEQLSSPANRVAVINLKLQDTETTTGESEVKFQVSRDTLGAMLRSM

Me:361  AYIREQLS 367
        AYIREQLS
At:828  AYIREQLS 835
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Figure 1: Alignments of cassava transposase (*Me*) predicted amino acids sequence with that from Arabidopsis MULE(*At*),GI number 18401324

Assessment of the diversity of mutator–like element transposase in cassava

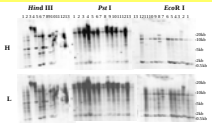

Figure3: DNA gel blots of 13 cassava cultivars: 1,SM1088; 2,MDOM5; 3,CM2177-2; 4,NGA-2; 5,SM627-5; 6,CM7033; 7, NGA19; 8,CG402; 9,SM524-1; 10,NGA1; 11,MVENN77; 12,SM985-9; 13,MCOL22 10 µg genomic DNA from each of the cultivars was digested with *Hin*dIII, *Pst*I or *Eco*RI and probed with cassava transposase cDNA. Washing was at high (**H**) or medium (**L**) stringency

Identification and isolation of cassava MULE clones from genomic library

For first round high-density screening, duplicate filters were prepared from four plates with each plate containing ~10^5 plaques. The membranes were then screened by hybridisation overnight with the radiolabelled *Me-TP1* probe. The membranes were washed to a final stringency equivalent to 0.2 X SSC at 60 °C. Up to fifty total duplicate positive plaques were found from the plates when the autoradiographic films were developed (figure 4a). Forty-seven duplicate positive plaques were cored out and eluted in 500 µl of SM buffer with 2 drops of HLPC grade chloroform. Each of these was used to prepare second round screening plates at lower density (~ 500 plaques per plates) Duplicate filters were prepared as before and screened by hybridisation with the radiolabelled probe. After autoradiography (figure4b), at least 1 duplicate positive isolated plaque was cored out from each of the low-density secondary screening.

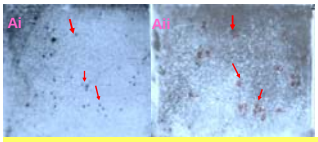

Figure4a: Typical high density duplicate autoradiographs. Corresponding duplicate positive clones are indicted with arrows

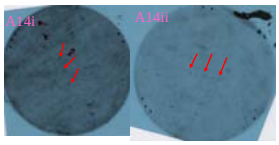

Figure 4b: Typical low density secondary screening autoradiographs

Diversity of cassava MULEs: Restriction enzymes sites analysis

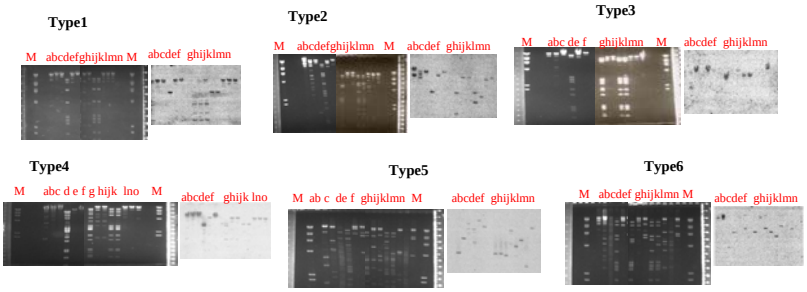

Figure6: Ethidium bromide stained 0.8% agarose gels and the corresponding hybridised Southern. M is λ *Hin*d III marker while other alphabets represent enzyme(s) used in the digestion as follows:**a=*Eco*R I ; b= *Hin*D III; c=*Kpn* I; d=*Pst* I; e=*Sal* I;f= *Xho* I; g=a+b; h=a+c; i=a+d; j=d+e; k=d+f; l=e+b; n=e+c** and **o=e+f**

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Comparative analysis of cassava MULE transposase with transposases from other plant species

The nucleotide sequences of *mudrA (Zea mays*) and Arabidopsis *mudrA*-related ORFs were downloaded from the NCBI Genbank database. These and the cassava MULE transposase cDNA sequenced were compared by pairwise alignment using GCG Clustalw (1.60)(see figure2)

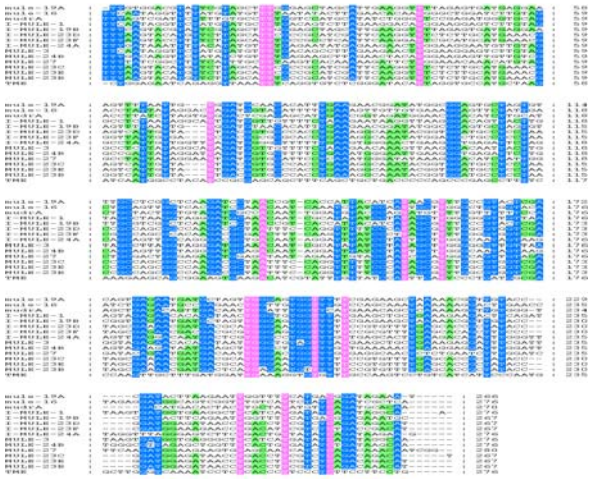

Figure 2: Multiple alignment of the most conserved region between cassava transposase, *Arabidopsis mudrA*-related ORFs and the Maize *mudrA*. Nucleotide sharing smilarity >60 are shaded. The corresponding GI numbers for each of the MULEs are as follows: **I-MULE-1**, 3510344; **MULE-3**, 2832639; **mule-16**, 2443899; **mule-19A**, 5041971; **I-MULE-19B**, 4585891; **MULE-23B**, 6007863; MULE-**23C**, 3063438; **I-MULE-23D**, 3980374**; MULE-23E**, 5041964; **I-MULE-23F**, 4519197; **I-MULE-24A**, 2760316; **MULE-24B**, 3319339; **MULE-27,** 4388816**. *TME***, cassava *mudrA*-like sequence; ***mudrA***, maize gene

PCR confirmation of clones

Using individual isolation clones as template and primers designed from Me-TP1 sequence, PCR was occasionally used to confirm clones identity.The gel purified product was then sequenced

Figure5a: Ethidium bromide stained 1%TAE agarose gel of the PCR products using *Me*-TP1 plasmid as positive control. M is 100bp marker

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Me: 01LQRDDGPPGNMAVLPCLKSMTWVMENKNTTPGNRVAVINLKLQDYSKTPSTEFEVKFQLS180
pcrMe:          15 KSMTWVMENKNTTPGNRVAVINLKF*DYSKTPSTEFEVK 131
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Figur5b: Alignment of cassava clones PCR product predicted amino acid sequence with cassava transposase, *Me-TP1*

Conclusion

- Cassava genome does contain diverse types of MULEs
- There appears to be less stringently related family members of MULE or its transposase in the cassava cultivars.
- Diversity of MULEs in cassava could be useful in assessment of genetic diversity

References

- Bennetzen JL (1996) The contribution of retroelements to plant genome organisation, function and evolution. Trends Microbiol **4**: 347-353
- FAO.FAOSTAT Database (1998) http://apps.fao.org/
- Fedoroff NV (1999) The *suppressor-mutator* element and the evolutionary riddle of transposons. Genes Cells **4**: 11-19