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 originators 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 pHBluescript was cloned in DH5a. 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.nlm.nih.gov). BlastoX 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 blasts search showed that the cDNA is very similar to Arabidopsis MULE (Figure 1).

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

For first round high-density screening, duplicate filters were prepared from four plates with each plate containing ~105 plaques. 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 core out and slotted at 500 µl of SM buffer with 2 drops of HLPc chloride chromofrom. Each of these was used to prepare second round screening plates at lower density (~500 plaques per plate).

Identification and isolation of cassava MULE clones from genomic library

Type 1 MULE clones were isolated by screening plates using the radiolabelled probe. The gel purified product was then sequenced. Multiple alignment of the most conserved region between cassava transposase, Arabidopsis mudrA-related ORF and the Maize mudrA sequence (Figure 5) revealed that the cassava ORF sequences had a 79% pairwise similarity to that from Arabidopsis MULE (not shown) while blastx search showed that the cDNA is very similar to Arabidopsis MULE (Figure 1).

Diversity of cassava MULEs: Restriction enzymes sites analysis

For each cassava type, multiple restriction enzyme digestions were performed in order to identify the characteristic fragment patterns. The membrane blots were hybridized using the radiolabelled probe.

PCR confirmation of clones

Using individual isolation clones as template and primers designed from Me-cTP1 sequence, PCR was occasionally used to confirm clones identity. The gel purified product was then sequenced.

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.

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References