Online journal ISSN - 1676-5680
GMR Genetics and Molecular Research
EVOLUTION AND TECHNOLOGY

Enhancer/Suppressor mutator (En/Spm)-like transposable elements of cassava (*Manihot esculenta*) are transcriptionally inactive

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Genet. Mol. Res. 9 (2): 639-650 (2010)
Received December 16, 2009
Accepted January 6, 2010
Published April 13, 2010
DOI 10.4238/vol9-2gmr713

ABSTRACT. Transposable elements contribute to the size, structure, variation, and diversity of the genome and have major effects on gene function. Sequencing projects have revealed the diversity of transposable elements in many organisms and have shown that they constitute a high percentage of the genome. PCR-based techniques using degenerate primers designed from conserved enzyme domains of transposable elements can provide quick and extensive surveys, making study of diversity and abundance and their applications possible in species where full genome sequence data are not yet available. We studied cassava (*Manihot esculenta*) *En/Spm*-like transposons (*Meens*) with regard to genomic distribution, sequence diversity and methylation status. Cassava transposase fragments characteristic of *En/Spm*-like transposons were isolated, cloned and characterized. Sequence analysis showed that cassava *En/Spm*-like elements are highly conserved, with overall identity in the range of 68-98%. Southern hybridization supports the presence of multiple copies of *En/Spm*-like transposons integrated in the genome of all cassava cultivars that we tested. Hybridization patterns of *Hpa*II- and *Msp*I-digested cassava genomic DNA revealed highly methylated sequences. There were no clear differences in hybridization

pattern between the cultivars. We did not detect RNA transcripts of *Meen*s by Northern procedures. We examined the possibility of recent transposition activities of the cassava *En/Spm*-like elements.

Key words: Cassava; Transposable elements; Transposon; *En/Spm*

INTRODUCTION

Transposable elements (TEs) have been classified into two major superfamilies according to their transposition intermediate and transposition mechanisms (Finnegan, 1992). Class I elements (retrotransposons or retroelements) move and amplify through RNA intermediates, which are reverse transcribed before their integration into the nuclear genome. They are the most widespread class of eukaryotic TEs (SanMiguel et al., 1996; Lander et al., 2001). Class II elements (DNA TEs) move by excision and reintegration via a DNA intermediate; they transpose by a "cut and paste" mechanism mediated by a transposase that recognizes their short terminal inverted repeated (TIRs) sequences. *Enhancer/Suppressor mutator (En/Spm*) TEs belong to this class.

Peterson (1953) and McClintock (1954) independently discovered the *En/Spm* TE system. Peterson named the autonomous element *Enhancer* (*En*), while McClintock called it *Suppressor-mutator (Spm)*. Autonomous TEs consist of sequences that are required *in cis* for transposition and express trans-acting proteins, whereas the non-autonomous elements do not express these proteins and can only transpose if the proteins are supplied by an autonomous element elsewhere in the genome. The non-autonomous element is called *Inhibitor/defective Suppressor-mutator* (*I/dSpm*). *En/Spm* is the most thoroughly studied member of the CACTA TE superfamily. The characteristic features of the CACTA elements are the presence of TIRs terminating with the sequence CACTA and the creation of 3-bp TSDs. Figure 1 summarizes the structural features of *En/Spm* of maize.

En/Spm transposons have proved to be useful in the isolation of many maize and *Antirrhinum* genes (Luo et al., 1991; Tissier et al., 1999). They have the advantage of exhib-

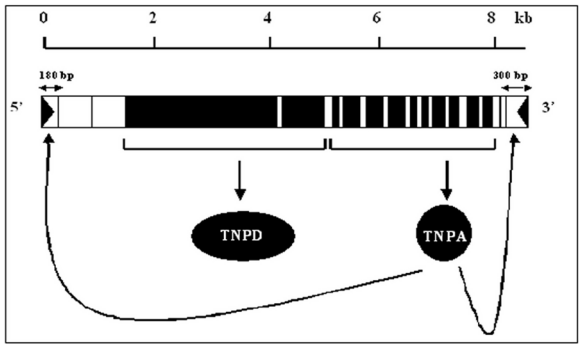

Figure 1. Structural organization of *Enhancer/Suppressor mutator* (*En/Spm*) of maize. *En/Spm* is an ~8.3-kb DNA transposon flanked at the termini by terminal inverted repeats (TIRs) shown as triangles. Exon sequences for the two genes of *En/Spm* are shown as shaded boxes: *TnpD* codes for the gene product TNPD or the transposase, while *TnpA* codes for TNPA. The open boxes represent the introns. TNPA protein binds to the sub-terminal sequence motifs (each 12 bp), which are scattered within ~180 bp at the 5' end (nine) and ~300 bp at the 3' end (fifteen) shown. At the 5' end, the most internal of the TNPA binding motifs overlaps with the TATA box, which exists as TATGAA in *En/Spm*.

iting frequent reversion of the disrupted gene to wild-type due to excision of the transposon. Suppression, a phenomenon characterized by the ability of an active transposon to change the degree of the phenotype produced by an insertion without excision, is another attribute of *En/Spm*, which may contribute to tagging effectiveness. This depends on the *TnpA* gene product of *En/Spm*, which acts by binding at the sub-termini of *En/Spm* or *I/dSpm* elements and interfering with transcription of the host gene (Grant et al., 1990, 1993).

En/Spm has successfully been exploited as a tool for functional genomics in *Arabidopsis* by using it to generate mutant lines (Wisman et al., 1998; Tissier et al., 1999; Speulman et al., 1999). The present study assessed the presence and activity of *En/Spm*-like transposons in cassava. *En/Spm*-like transposons could be used to elucidate the apparently subtle actions of genes and might have potential as a tool for genomic analysis and biotechnological development of cassava. To date, there has not been any published information as to the presence and nature of *En/Spm*-like transposons in cassava.

MATERIAL AND METHODS

Plant material

Young leaf samples for the isolation of DNA and total RNA were obtained from various cassava cultivars. Cassava plants were grown in the tropical glasshouse at the University of Bath at 22-28°C, with a relative humidity of 40-80% and a minimum light period of 12 h per day under daylight, supplemented with 400 W Phillips high-pressure sodium lights when necessary.

PCR amplification of transposase fragment of cassava *En/Spm*-like sequences and cloning

The polymerase chain reaction (PCR) method used was as described by Staginnus et al. (2001), with some modifications as follows: 50- or 100-µL PCR mixes contained 50 pmol of each primer, 0.5 mM dNTPs, 3 mM $MgCl_2$, and 250 ng template DNA, using a PCR cycle of 94°C (2 min), 35 times [at 94°C for 1 min, 55°C for 1 min and 72°C for 1 min], and at 72°C for 5 min. Amplified DNA bands were gel purified (Qiagen, Qiaquik), ligated into pGEM®-T Easy vector (Promega) and used to transform competent *Escherichia coli* DH5α according to standard procedures (Sambrook et al., 1989).

DNA gel blot analysis

Cassava genomic DNA was isolated from young leaves by the method of Dellaporta et al. (1983). Restriction digestions of genomic DNA (5 µg each) were carried out using buffer and reaction conditions specified by the manufacturer (Promega). Blotting and hybridization were performed using standard procedures (Sambrook et al., 1989).

DNA sequencing and sequence analyses

DNA molecules were sequenced on an ABI 337 automated dye primer sequencer using universal primers for the cloning vector. Initial confirmation of sequence identity was by BLASTN and TBLASTX searches against the GenBank non-redundant database

using the default parameters (Altschul et al., 1990). Sequence data were aligned using CLUSTAL W (version 1.82) (Thompson et al., 1994).

Wounding and incubation procedures

Wounding stress was induced in cassava leaves with a modified method of Takeda et al. (1998). In one set of experiments, leaf discs were prepared by cutting young cassava (cultivar MCOL22) leaves into discs of approximately 1 cm^2. In a second, the leaf segments prepared from young leaves were stabbed with the points of a forceps about twenty times. The leaf segments (stabbed or unstabbed) were then incubated on 0.05% 2-[N-morpholino] ethane sulfonic acid (MES)-KOH buffer, pH 5.7, at 25°C under ambient light. MES is generally used in tissue culture to prevent oxidative degradation of biomolecules. Leaf samples were taken at 2, 4, 6, 8, 12, 24, and 48 h from the incubation mix, rapidly wrapped with aluminum foil and submerged in liquid nitrogen. These were immediately ground to a fine powder with a mortar and pestle and the powdered leaves stored at -70°C until required. Total RNA was isolated from the powdered samples of leaves as below. For the root, the conditions for deterioration experiments were as described by Han et al. (2001).

RNA extraction and Northern blot procedures

Using 2-mL nuclease-free microfuge tubes, RNA was extracted from the homogenized tissue using the Promega SV total RNA isolation system with some modifications to the specifications of the manufacturer as follows: 350 µL lysis buffer was added to 0.09 g of the ground tissue in the microfuge tube and mixed by inversion. RNA dilution buffer (700 µL) was then added and the contents again mixed by inversion before centrifugation at 13,000 rpm for 10 min. The cleared lysate solution was then transferred to a fresh microfuge tube by pipetting without disturbing the pelleted debris, and the centrifugation step was repeated. A volume of 400 µL 95-100% ethanol was added to the clear lysate and mixed by pipetting four times. The mixture was then transferred to the spin column assembly and spun at 13,000 rpm for 1 min. The rest of the protocol followed procedure E (RNA purification by centrifugation) of the kit manufacturer (Promega). Total RNA was extracted from cassava storage roots using the method of Chang et al. (1993).

Ten micrograms total RNA per lane was electrophoresed on a 1.5% agarose gel containing formaldehyde and blotted onto a nylon membrane (Hybond N+, Amersham), according to standard procedures (Sambrook et al., 1989). Hybridizations were carried out at 65°C overnight, after which membranes were washed at a final wash stringency of 0.2X SSC, 0.2% SDS for 20 min at 65°C. The membranes were then exposed to Fuji X-ray film and incubated at -70°C, and the autoradiograph was then developed.

The nucleotide sequences described here have been submitted to the GenBank database and given the accession numbers AY946045-AY946084.

RESULTS

PCR amplification of cassava *En/Spm* transposase fragment, cloning and sequence analysis

PCR was carried out as described in the Material and Methods section above. The

amplified products were analyzed by electrophoresis on ethidium bromide stained 1.0% agarose gels (Figure 2).

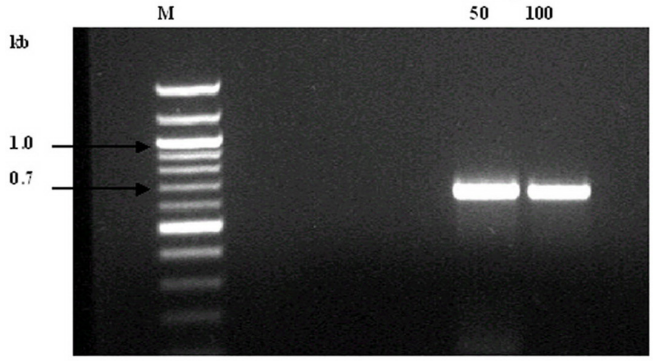

Figure 2. Polymerase chain reaction (PCR) amplification of *En/Spm*-like transposase fragment from cassava genomic DNA. The PCR product was run on a 1.0% agarose gel stained with ethidium bromide. The size marker (lane M) is Bioline DNA 100-bp ladder while the PCR product for 50- and 100-µL reaction mixes are shown in the right lanes.

Approximately 650-bp putative fragments of the transposase domain were amplified in PCR. The DNA band was gel purified, and the purified DNA was sequenced directly using the PCR primers. The PCR product sequence was then submitted to BLASTN and TBLASTX searches. These confirmed that an *En/Spm*-like transposon fragment had been amplified in the PCR experiments. The cassava element was 67% identical (within the region of the alignment) to the *En/Spm*-like transposon of *Daucus carota* (accession number AB071202) at the amino acid sequence level (Figure 3). The cassava sequence (*Me*) had unresolved ambiguous nucleotides within the overall sequence. This ambiguity suggested that the PCR band represents a population of diverse individual members of *En/Spm*-like transposons. Subsequent to cloning, randomly selected clones were fully sequenced. These sequences showed clear homology to the transposase of *En/Spm*-like transposons (in most cases E-values from BLAST searches were in the region of E^{-63}).

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Me: 103 ICRRPEIKKDPISGKYPKACYCLDNQSKMILCDWLKTXKFPDGYVSNIGRCVDSRKLRLF 282
        +CRRPEL  D  + KYPKACY LD + K  +C WL+  KFPDGYVSN+GRC+D +K +LF
Dc: 132 ICRRPELAIDESTRKYPKACYSLDKKGKEAVCKWLQDLKFPDGYVSNMGRCIDMKKYKLF 311

Me: 283 GMKSHDCHVFMQR 321
        GMKSHDCHVFMQR
Dc: 312 GMKSHDCHVFMQR 350
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Figure 3. Alignment of amino acid sequence of cassava *Meen* (*Me*) polymerase chain reaction product with that of *Daucus carota* (*Dc*) *En/Spm*-like transposon-transposase (accession number AB071202). The two sequences share 67% identity.

Phylogenetic analysis

The nucleotide sequences of 40 unique clones representing cassava *En/Spm*-like transposons (*Meen*s) were aligned using CLUSTAL W and highlighted in GENDOC version 2.3 (Figure 4). The alignment showed that there is a high degree of nucleotide sequence conservation among the cassava *En/Spm*-like transposons with overall identity in the range of 68-98%.

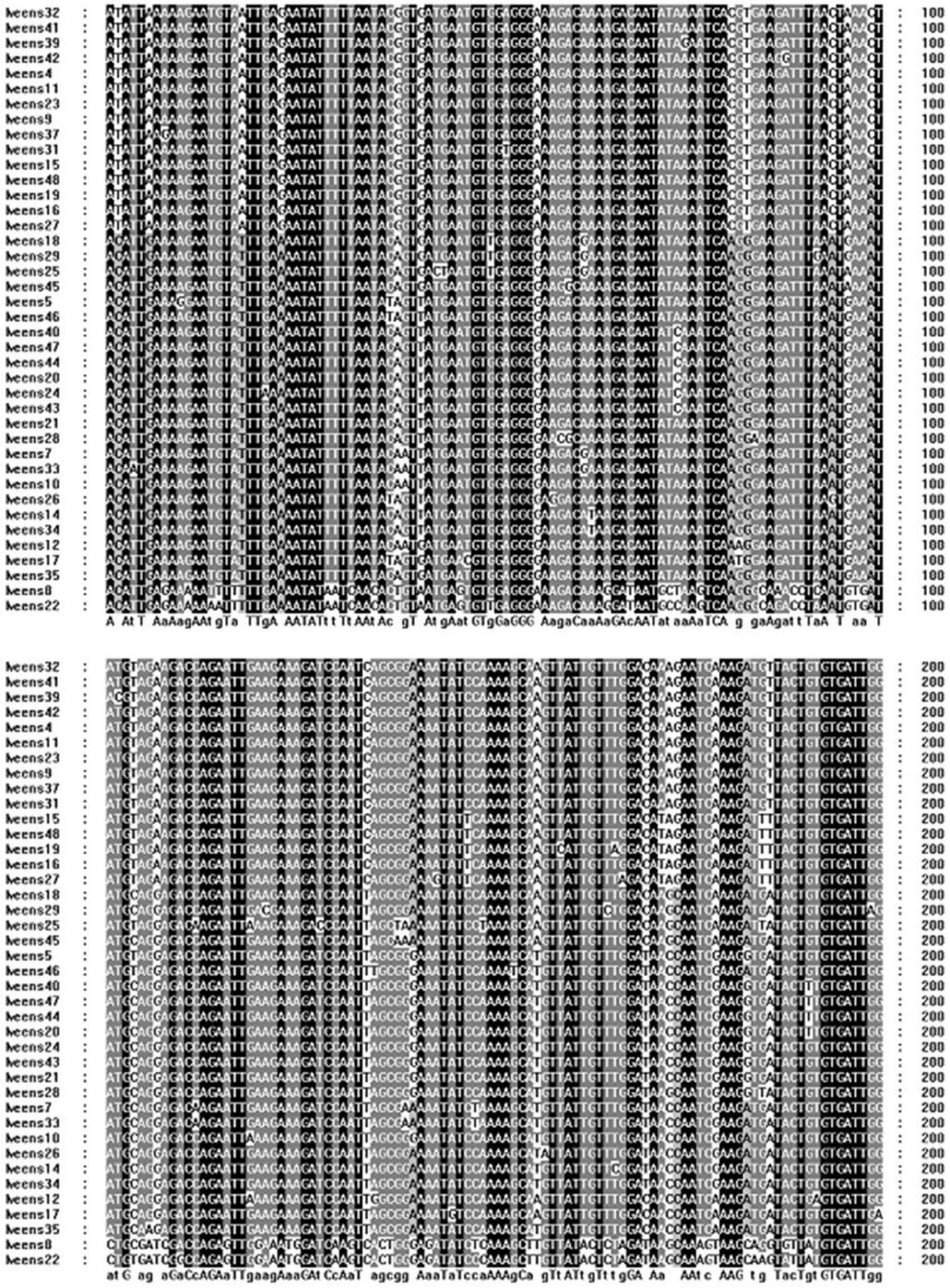

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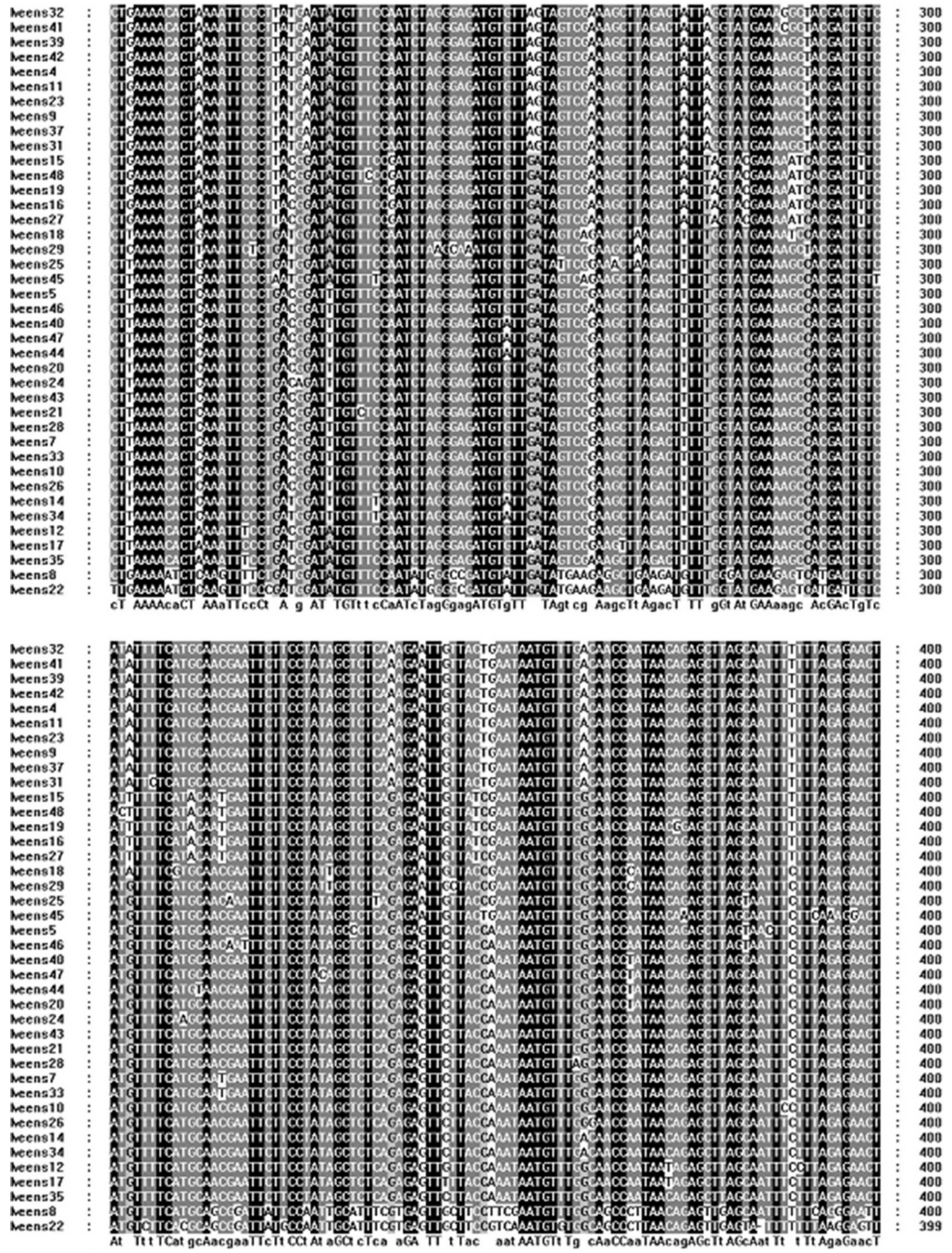
Figure 4. Continued.



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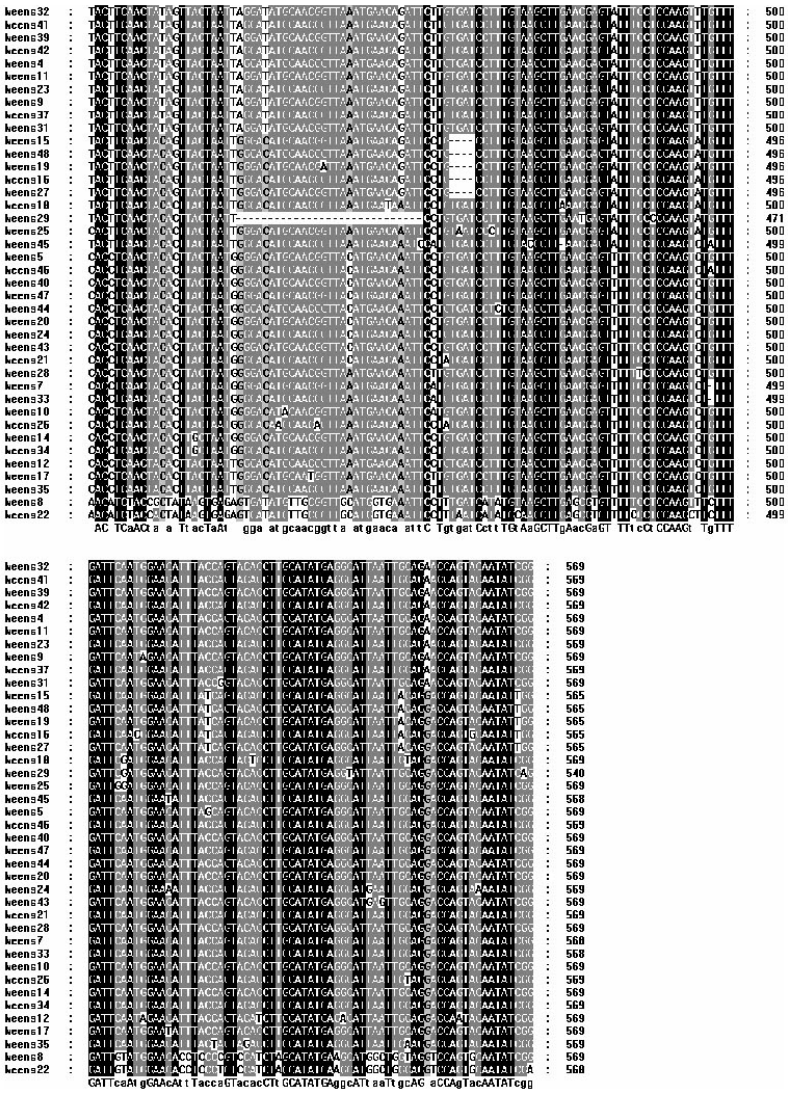

Figure 4. Multiple sequence alignment of the nucleotide sequences for 40 transposase fragments of cassava *En/Spm*-like transposons (*Meens*). The sequences were aligned using the CLUSTAL W program. Gaps (indicated with dashes) represent deletions in the sequence. Color blocking (done in GENDOC) indicates sequence conservation: Black = 100% identity, grey = 60-80% and non-shaded = <60% identity.

Study on the genomic organization and diversity of *En/Spm*-like transposons in cassava cultivars

In order to gain an insight into the genomic organization of the cassava *En/Spm*-like transposons, a representative cassava element, *Meens 5*, was used to probe a Southern blot of the DNA from 10 different cassava cultivars separately digested with *Bgl*II, *Hin*dIII and *Eco*RI

(see Figure 5). Strong signals were obtained, revealing the presence of multiple bands after a short exposure time, which suggests that many copies of *Meens* and relatives were integrated in the genome. Incomplete digestion of the genomic DNA was ruled out because the same gel blot probed with a well-characterized sequence showed only few distinct hybridizing bands (data not shown). The data also suggest that *Meens* 5 cross hybridizes with sequences highly homologous to the probe, represented by the strong major bands, as well as related diverged fragments, seen as weak signals. The digests did not reveal clear differences in the hybridization pattern between the cultivars tested (Figure 5, Panels a, b and c).

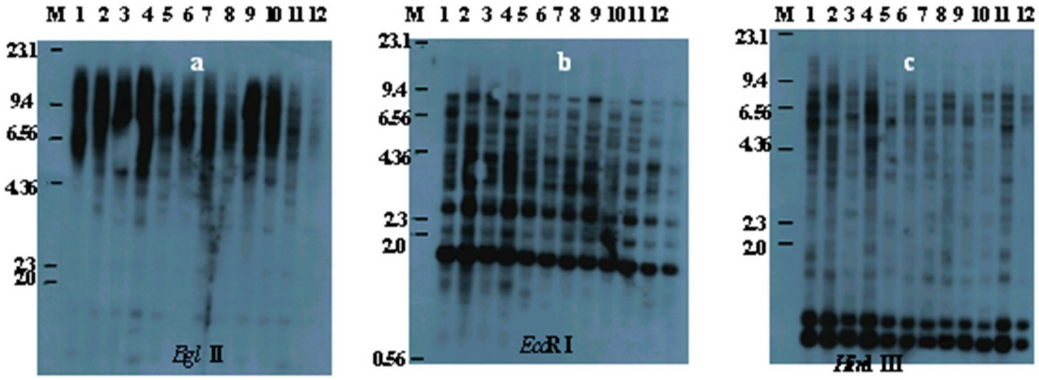

Figure 5. Southern blot analysis of *En/Spm*-like transposase of 12 cassava cultivars. Each cassava cultivar was carried out with 10 µg genomic DNA. *Lane 1* (MGA1), *lane 2* (MNGA2), *lane 3* (MDOM5), *lane 4* (MNGA19), *lane 5* (MCOL22), *lane 6* (CMC40), *lane 7* (MVEN77), *lane 8* (CG402), *lane 9* (SM627), *lane 10* (SM985), *lane 11* (SM1088), and *lane 12* (CM2177) were digested with *Bgl*II (a), *Eco*RI (b) or *Hin*dIII (c). The digested DNAs were separated on 0.8% agarose gels, transferred to a nylon membrane and hybridized with the *Meens* 5 probe. *Hin*dIII-digested lambda DNA was used as a DNA size marker (M).

Methylation status and transcriptional activity of *En/Spm*-like transposons of cassava

To determine the methylation status of cassava *En/Spm*-like transposons, *Meens 5* was used to probe Southern blots of the DNA from 10 different cassava cultivars digested with *Hpa*II or *Msp*I. The hybridization pattern in most cases showed a strong smear over a range of low to high molecular weights (Figure 6). These sequences were probably highly methylated as revealed by minor differences between the hybridization pattern of the *Hpa*II and *Msp*I digests. These two enzymes share the recognition nucleotide base sequence CCGG, but *Hpa*II is blocked by methylation at either C, while *Msp*I is blocked by methylation at the external C only. The finding here suggests the presence of only few unmethylated external cytosine residues in the sequence CCGG of cassava *En/Spm*-like transposons, and therefore completely or near completely methylated sequences. In addition, when *Meens* 5 was used to probe the Northern blot of total RNA prepared from cassava leaves and roots, as described in the Material and Methods section, there were no detectable signals in the hybridizations.

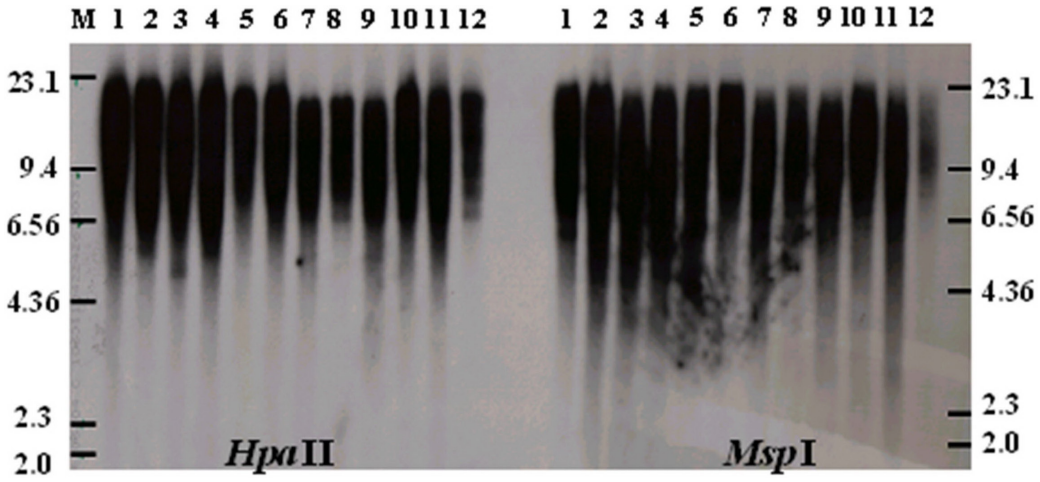

FIGURE 6. Methylation status of *En/Spm*-like transposase of 12 cassava cultivars. A total of 10 µg genomic DNA from each of the cassava cultivars (as in Figure 5) was digested with *Hpa*II or *Msp*I. The digested DNAs were separated on 0.8% agarose gels, transferred to a nylon membrane and hybridized with the *Meens* 5 probe. *Hin*dIII-digested lambda DNA was used as a DNA size marker (M).

DISCUSSION

Using PCR degenerate primers, cassava transposase fragments characteristic of *En/Spm*-like transposons were isolated, cloned and sequenced. Alignment of the nucleotide sequences of cassava *En/Spm*-like transposon clones (*Meens*) showed that there was a high level of nucleotide sequence conservation among the cassava elements. The alignment of the putative peptide sequence of representative *Meens* with known *En/Spm*-like transposons of other plants (data not shown) revealed identity at most positions that were conserved in the majority of the elements compared.

These findings for *Meen*s are similar to those observed for the *En/Spm* transposons of *Arabidopsis thaliana*, *Solanum lycopersicum* and *Elaeis guineensis*. However, more diverged sequences of *En/Spm*-like transposons were reported for *Cicer arietinum* (Staginnus et al., 2001). An extreme case of divergence of *En/Spm* transposons within a plant has been reported for the nucleotide sequences of carrot (*Daucus carota*), where the *Tdc C* element was found to be highly divergent from the other two families, *Tdc A* and *Tdc B* (Itoh et al., 2003).

Our Northern blot hybridizations of total RNA did not produce detectable signals. This suggests that the cassava *En/Spm*-like elements are transcriptionally inactive or their transcripts were produced at such a low level that they were not detectable here. We found, from the deduced translations of the cassava *En/Spm*-like transposon-transposase, that 72.5% contain a frame shift, a nonsense mutation or both within the sequence analyzed. Therefore, the majority of these cassava enzymes would be non-functional and defective.

Southern blot analysis did not reveal clear differences in the hybridization pattern between the cultivars tested (Figure 5, Panels a, b and c, and Figure 6). The observations made with cassava elements, *Meen*s, suggest that they have not been active during the recent history of cassava. The same restriction enzyme digest blots probed with cassava

Mutator-like transposable element (MULE) transposase cDNA show clear polymorphism among the same cassava cultivars (Gbadegesin et al., 2007). The hybridization pattern of the *Meens* in most cases, especially with the *Hpa*II or *Msp*I digestion of the genomic DNA, showed a strong smear over a range of low to high molecular weights (Figure 6). This indicates the presence of *Meen* 5 and homologues in many different genomic loci. These sequences were probably highly methylated as revealed by minor differences between the hybridization pattern of the *Hpa*II and *Msp*I digests. In contrast to the above, Southern analyses of the DNA digested by the isoschizomers, *Hpa*II and *Msp*I, revealed partial methylation sequences of cassava *Mutator*-like transposable elements for the same set of cultivars (Gbadegesin et al., 2007). These findings lend strong support to the association between decreases in DNA methylation and increased activity of transposable elements.

DNA methylation has tremendous effects on the heritability and activity of *En/Spm* (Banks et al., 1988). It is a key component of the mechanism that regulates transposition (Chandler and Walbot, 1986; Chomet et al., 1987; Fedoroff et al., 1995; Singer et al., 2001; Lisch et al., 2002). Transposons induce changes in host genes, some of which are detrimental (Zabala and Vodkin, 2007; Begin and Schoen, 2007; Beare et al., 2009). DNA methylation is especially important in plants, and mutants with reduced levels of cytosine methylation display highly abnormal developmental phenotypes (Finnegan et al., 1996; Kakutani et al., 1996; Ronemus et al., 1996). In conclusion, cassava *EnSpm*-like transposons are highly methylated, transcriptionally inactive and may not have been active during the recent history of cassava.

ACKNOWLEDGMENTS

M.A. Gbadegenin would like to acknowledge funding from the Commonwealth Scholarship Commission, UK. This publication is supported by University of Ibadan Senate Research Grant No. SRG/COM/2006/35A. It is also in part an output from a research project funded by the United Kingdom Department for International Development (DFID) for the benefit of developing countries: R8156 Crop Post-Harvest Programme. The views expressed are not necessarily those of DFID. This study has been carried out in compliance with the current laws governing genetic experimentation in the UK.

REFERENCES

Altschul SF, Gish W, Miller W, Myers EW, et al. (1990). Basic local alignment search tool. *J. Mol. Biol.* 215: 403-410.

Banks JA, Masson P and Fedoroff N (1988). Molecular mechanisms in the developmental regulation of the maize Suppressor-mutator transposable element. *Genes Dev.* 2: 1364-1380.

Beare PA, Unsworth N, Andoh M, Voth DE, et al. (2009). Comparative genomics reveal extensive transposon-mediated genomic plasticity and diversity among potential effector proteins within the genus *Coxiella*. *Infect. Immun.* 77: 642-656.

Begin M and Schoen DJ (2007). Transposable elements, mutational correlations, and population divergence in *Caenorhabditis elegans*. *Evolution* 61: 1062-1070.

Chandler VL and Walbot V (1986). DNA modification of a maize transposable element correlates with loss of activity. *Proc. Natl. Acad. Sci. U. S. A.* 83: 1767-1771.

Chang S, Puryear J and Cairney J (1993). A simple and efficient method for isolating RNA from pine trees. *Plant Mol. Biol. Rep.* 11: 113-116.

Chomet PS, Wessler S and Dellaporta SL (1987). Inactivation of the maize transposable element Activator (Ac) is associated with its DNA modification. *EMBO J.* 6: 295-302.

Dellaporta SL, Wood J and Hicks JB (1983). A plant DNA minipreparation: Version II. *Plant Mol. Biol. Rep.* 1: 19-21.

Fedoroff N, Schlappi M and Raina R (1995). Epigenetic regulation of the maize Spm transposon. *Bioessays* 17: 291-297.

Finnegan DJ (1992). Transposable elements. *Curr. Opin. Genet. Dev.* 2: 861-867.
Finnegan EJ, Peacock WJ and Dennis ES (1996). Reduced DNA methylation in *Arabidopsis thaliana* results in abnormal plant development. *Proc. Natl. Acad. Sci. U. S. A.* 93: 8449-8454.
Gbadegesin MA, Gómez-Vásquez R, Reilly K and Beeching JR (2007). Transcriptionally active mutator-like transposable elements in the genome of Cassava (*Manihot esculenta* Crantz). *Asian J. Plant Sci.* 6: 129-136.
Grant SR, Gierl A and Saedler H (1990). En/Spm encoded tnpA protein requires a specific target sequence for suppression. *EMBO J.* 9: 2029-2035.
Grant SR, Hardenack S, Trentmann S and Saedler H (1993). Functional cis-element sequence requirements for suppression of gene expression by the TNPA protein of the *Zea mays* transposon En/Spm. *Mol. Gen. Genet.* 241: 153-160.
Han Y, Gómez-Vásquez R, Reilly K, Li H, et al. (2001). Hydroxyproline-rich glycoproteins expressed during stress responses in cassava. *Euphytica* 120: 59-70.
Itoh Y, Hasebe M, Davies E, Takeda J, et al. (2003). Survival of Tdc transposable elements of the En/Spm superfamily in the carrot genome. *Mol. Genet. Genomics* 269: 49-59.
Kakutani T, Jeddeloh JA, Flowers SK, Munakata K, et al. (1996). Developmental abnormalities and epimutations associated with DNA hypomethylation mutations. *Proc. Natl. Acad. Sci. U. S. A.* 93: 12406-12411.
Lander ES, Linton LM, Birren B, Nusbaum C, et al. (2001). Initial sequencing and analysis of the human genome. *Nature* 409: 860-921.
Lisch D, Carey CC, Dorweiler JE and Chandler VL (2002). A mutation that prevents paramutation in maize also reverses Mutator transposon methylation and silencing. *Proc. Natl. Acad. Sci. U. S. A.* 99: 6130-6135.
Luo D, Coen ES, Doyle S and Carpenter R (1991). Pigmentation mutants produced by transposon mutagenesis in *Antirrhinum majus*. *Plant J.* 1: 59-69.
McClintock B (1954). Mutations in maize and chromosomal aberrations in *Neurospora*. *Carnegie Inst. Washington Yearbook* 53: 254-261.
Peterson PA (1953). A mutable pale green locus in maize. *Genetics* 38: 682-683.
Ronemus MJ, Galbiati M, Ticknor C, Chen J, et al. (1996). Demethylation-induced developmental pleiotropy in *Arabidopsis*. *Science* 273: 654-657.
Sambrook J, Fritsch E and Maniatis T (1989). Molecular Cloning: A Laboratory Manual. Cold Spring Harbor Laboratory Press, Cold Spring Harbor.
SanMiguel P, Tikhonov A, Jin YK, Motchoulskaia N, et al. (1996). Nested retrotransposons in the intergenic regions of the maize genome. *Science* 274: 765-768.
Singer T, Yordan C and Martienssen RA (2001). Robertson's Mutator transposons in *A. thaliana* are regulated by the chromatin-remodeling gene Decrease in DNA Methylation (DDM1). *Genes Dev.* 15: 591-602.
Speulman E, Metz PL, van ArKel G, te Lintel HB, et al. (1999). A two-component enhancer-inhibitor transposon mutagenesis system for functional analysis of the *Arabidopsis* genome. *Plant Cell* 11: 1853-1866.
Staginnus C, Huettel B, Desel C, Schmidt T, et al. (2001). A PCR-based assay to detect En/Spm-like transposon sequences in plants. *Chromosome Res.* 9: 591-605.
Takeda S, Sugimoto K, Otsuki H and Hirochika H (1998). Transcriptional activation of the tobacco retrotransposon Tto1 by wounding and methyl jasmonate. *Plant Mol. Biol.* 36: 365-376.
Thompson JD, Higgins DG and Gibson TJ (1994). CLUSTAL W: improving the sensitivity of progressive multiple sequence alignment through sequence weighting, position-specific gap penalties and weight matrix choice. *Nucleic Acids Res.* 22: 4673-4680.
Tissier AF, Marillonnet S, Klimyuk V, Patel K, et al. (1999). Multiple independent defective suppressor-mutator transposon insertions in *Arabidopsis*: a tool for functional genomics. *Plant Cell* 11: 1841-1852.
Wisman E, Cardon GH, Fransz P and Saedler H (1998). The behaviour of the autonomous maize transposable element En/Spm in *Arabidopsis thaliana* allows efficient mutagenesis. *Plant Mol. Biol.* 37: 989-999.
Zabala G and Vodkin L (2007). Novel exon combinations generated by alternative splicing of gene fragments mobilized by a CACTA transposon in *Glycine max*. *BMC Plant Biol.* 7: 38.

