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RESEARCH ARTICLE

MpSaci is a widespread *gypsy-Ty3* retrotransposon highly represented by non-autonomous copies in the *Moniliophthora perniciosa* genome

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Abstract Transposons are an important source of genetic variation. The phytopathogen *Moniliophthora perniciosa* shows high level of variability but little is known about the role of class I elements in shaping its genome. In this work, we aimed the characterization of a new g*ypsy/Ty3* retrotransposon species, named *MpSaci*, in the *M. perniciosa* genome. These elements are largely variable in size, ranging from 4 to 15 kb, and harbor direct long terminal repeats (LTRs) with varying degrees of similarity. Approximately, all of the copies are non-autonomous as shifts in the reading frame and stop codons were detected. Only two elements (*MpSaci6* and *MpSaci9*) code for GAG and POL proteins that possess functional domains. Conserved domains that are typically not found in retrotransposons were detected and could potentially impact the expression of neighbor genes. Solo LTRs and several LARDs (large retrotransposon derivative) were detected. Unusual elements containing small sequences with or without interruptions that are similar to *gag* or different *pol* domains and presenting LTRs with different levels of similarities were identified. Methylation was observed in *MpSaci* reverse transcriptase sequences. Distribution analysis indicates that *MpSaci* elements are present in high copy number in the genomes of C-, S- and L-biotypes of *M. perniciosa*. In addition, C-biotype isolates originating from the state of Bahia have fragments in common with isolates from the Amazon region and two hybridization profiles related to two chromosomal groups. RT-PCR analysis reveals that the *gag* gene is constitutively expressed and that the expression is increased at least three-fold with nutrient depravation even though no new insertion were observed. These findings point out that *MpSaci* collaborated and, even though is primarily represented by non-autonomous elements, still might contribute to the generation of genetic variability in the most important cacao pathogen in Brazil.

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Nucleotide sequence data reported are available in the DDBL/EMBL/GenBank databases under the accession numbers: KJ526182 (*MpSaci1*), KJ526183 (*MpSaci2*), KJ526184 (*MpSaci3*), KJ526185 (*MpSaci4*), KJ526186 (*MpSaci5*), KJ526187 (*MpSaci6*), KJ526188 (*MpSaci7*), KJ526189 (*MpSaci8*), KJ526190 (*MpSaci9*), KJ526191 (*MpSaci10*), KJ526192 (*MpSaci11*), KJ526193 (*MpSaci12*), KJ526194 (*MpSaci13*), KJ526195 (*MpSaci14*), KJ526196 (*MpSaci15*), KJ526197 (*MpSaci16*), KJ526198 (*MpSaci17*), KJ526199 (*MpSaci18*), KJ526200 (*MpSaci19*), KJ526201 (*MpSaci20*), KJ526202 (*MpSaci21*), KJ526203 (*MpSaci22*), KJ526204 (*MpSaci23*), KJ526205 (*MpSaci24*), KJ526206 (*MpSaci25*), KJ526207 (*MpSaci26*), KJ526208 (*MpSaci27*), KJ526209 (*MpSaci28*), KJ526210 (*MpSaci29*), and KJ526211 (*MpSaci30*).

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Introduction

In various organisms, transposable elements are an important source of genetic variability and significantly impact genome organization and evolution. Transposable elements can be directly involved in spontaneous mutations via base insertions and deletions, gene expression alterations, chromosomal rearrangements, and other changes (Daboussi and Capy 2003; Shnyreva 2003; Pereira et al. 2006; Wicker et al. 2007). The investigation of the diversity, distribution and expression of these elements can provide important information about molecular biology of an organism.

Transposons are divided into two major classes according to their mechanism of transposition: class I, whose elements are transposed by reverse transcription of an RNA intermediate, and class II, whose transposition acts by a cut-and-paste mechanism not requiring an RNA intermediate. The class I elements that are flanked by long terminal repeats (LTRs) and possess a *pol* region, encoding a protease, reverse transcriptase, RNase H and integrase in this particular order, in addition to the *gag* gene are termed *gypsy/Ty3*-like. In contrast, class I elements with an integrase located between the protease and reverse transcriptase are referred to as *copia/Ty1*-like (Daboussi and Capy 2003; Wicker et al. 2007). *Copia/Ty1* elements are classified into *Pseudoviridae* family while *gypsy/Ty3* elements belong to the *Metaviridae* family, which is separated into three genus, *Metavirus*, *Errantivirus* and *Semotivirus* (King et al. 2012). Retrotransposons from the *gypsy/Ty3* group (*Metaviridae*) are the major transposable elements found in phytopathogenic fungi (Daboussi and Capy 2003; Pereira et al. 2006). Given the fact that transposons encode for proteins necessary for transposition, transposons can be classified as follows: autonomous elements encode for all the domains; defective elements contain small mutations but still possess intact coding regions; and non-autonomous elements lack the complete domain(s) necessary for transposition (Wicker et al. 2007). Non-autonomous elements are capable of propagating using the proteins coded by other elements (Tanskanen et al. 2007). In addition, if these elements display sufficient sequence similarity to other copies, these elements can be used by the cell machinery for various types of rearrangements through recombination. Changes in transposon sequences can be performed by defense mechanisms like RIP (repeated-induced point mutation) where C:G to A:T transversions are generated in repetitive DNA frequently leading to epigenetic silencing through DNA methylation (Galagan and Selker 2004). In fact, those transversions were detected in reverse transcriptase sequences from *M. perniciosa* evidencing the presence of a putative RIP-like process (Pereira et al. 2007).

Moniliophthora perniciosa (*Agaricales, Tricholomataceae*) is the most important pathogen of cocoa in Brazil. After that phytopathogen arrived in the state of Bahia, the main cocoa-producing region in the country, Brazil shifted from one of the largest world producers to a major importer (Evans et al. 2013). Other plants in addition to cocoa are infected by *M. perniciosa,* and pathological data is used to divide this species into biotypes as the C-biotype, which infects species from the *Theobroma* and *Herrania* (*Sterculiaceae*) genera; the S-biotype, which infects species from the Solanaceae family; and the L-biotype, which colonizes a variety of plants (Meinhardt et al. 2008). While the L-biotype shows an out-crossing reproductive strategy (bifactorial heterothallism) and, consequently, higher levels of genetic diversity, C- and S-biotypes exhibit primary homothallism being the basidiospores produced when the disease cycle is completed (Griffith and Hedger 1994). Curiously, the C-biotype possesses A- and B-mating-type-like genes orthologous to the ones of heterothallic basidiomycetes (Kües and Navarro-González 2010).

Genetic diversity in *M. perniciosa* has been evaluated using several methods, and various levels of variability have been reported. In general, S- and L-biotype isolates display considerable diversity. C-biotype isolates from the Amazon region are more heterogeneous than isolates from Bahia, where two genotype groups have been identified. For example, restriction fragment length polymorphism targeting the mitochondrial DNA and ribosomal DNA ITS regions display no differences among isolates from various biotypes (Arruda et al. 2003a). In contrast, AFLP (Amplified Fragment Length Polymorphism) displays substantial variation among C-biotypes isolates from Bolivia, Brazil, Ecuador and Trinidad, but it shows similarity among isolates from the same geographic region (Ploetz et al. 2005). However, differences among C- and S-biotype isolates were detected using RAPD (Andebrhan et al. 1999), rDNA IGS region sequencing (Arruda et al. 2003a) and ERIC-PCR (Arruda et al. 2003b). IRAP and REMAP analyses revealed intraspecific variability among C-biotype isolates from different regions (Santana et al. 2012). Using pulsed field gel electrophoresis, distinct chromosomal patterns among S- and L- biotypes isolates were identified compared with C-biotype isolates (Rincones et al. 2003, 2006). In the Bahia state, C-biotype isolates typically possess eight chromosomes with different electrophoretic patterns; some isolates have eight bands, and others isolate display six bands with two overlapping bands. Microsatellites have also been isolated from *M. perniciosa* (Gramacho et al. 2007; Silva et al. 2008), indicating various polymorphisms

for each locus. Some of these microsatellites and the ITS and IGS regions of the rDNA were evaluated in Colombian *M. perniciosa* isolates, and clear genetic variability was demonstrated (Osorio-Solano et al. 2012). Diversity studies have also been performed using somatic compatibility and SDS- PAGE (Ferreira et al. 2012).

In a survey of *M. perniciosa* genome sequences, *gypsy/Ty3* elements were reported as the most abundant transposon type identified in this species (Mondego et al. 2008). A *gypsy/Ty3*-like reverse transcriptase, which is distributed across the *M. perniciosa* genome (Rincones et al. 2003), was characterized by our group (Pereira et al. 2007). Other types of elements, such as a transposase from the *TC1-mariner* superfamily (Ignacchiti et al. 2011) and an interesting element from the *PIF/harbinger* superfamily called *Boto* (Pereira et al. 2013), demonstrate that class II transposons are also distributed throughout the genome of this pathogen. Transposons were identified in the differentially expressed transcripts between biotrophic and saprophytic *M. perniciosa* mycelia (Rincones et al. 2008); however, *Boto* element expression has been detected in mycelium grown normally in culture medium (Pereira et al. 2013). It has been postulated that stresses can activate the transposition of these elements, thereby increasing the genetic variability necessary for adaptation to various environmental conditions (McClintock 1984). To date, it has been demonstrated that elements from various phytopathogenic fungi species are activated by some type of biotic or abiotic stress, such as heat shock, nutrient stress, toxic substances, or others (Anaya and Roncero 1996; Mes et al. 2000; Eto et al. 2001; Ikeda et al. 2001; Rep et al. 2005; Bouvet et al. 2008; Amyotte et al. 2012; Chadha and Sharma 2014).

Given that transposable elements are important in the evolution of new variants of plant pathogenic fungi, it is important to analyze the presence and activity of transposable elements to understand the biology of a species and the mechanisms that may be involved in the generation of genetic variability. In this article, we characterize several *gypsy/Ty3* transposable elements that belong to a new species, named *MpSaci*, in the *M. perniciosa* genome. The majority of the elements are non-autonomous as only two of the characterized copies produce GAG and POL proteins with intact domains. We also report the distribution of this species in various biotype isolates, phylogenetic analyses and element expression under nutrient deprivation. *MpSaci* elements are likely involved in the generation of genetic variability in *M. perniciosa* given the number of elements, their putative activity, the rearrangements observed among the unusual elements, the presence of solo LTR and conserved domains not typically found in transposable elements.

Materials and methods

MpSaci sequences

The *Moniliophthora perniciosa* genome was obtained from the Witches' Broom Genome Project database (www.lge.ibi.unicamp.br/vassoura). LTR transposons were detected using LTR-Finder software (Xu and Wang 2007). The resulting sequences were aligned with the reverse transcriptase sequence characterized by Pereira et al. (2007), leading to the identification of the *MpSaci1* element. This element was used to identify additional *MpSaci* elements in the sequences generated by the LTR-finder software, and similarities greater than 1^{e-10} were not considered.

M. perniciosa isolates

Moniliophthora perniciosa isolates examined in this study are listed in Table 1. Stock cultures were maintained at room temperature after growth on PDA (Potato Dextrose Agar) at 27 °C for 2 weeks.

Primers

To amplify a 394 bp DNA fragment corresponding to a region of the *MpSaci* reverse transcriptase coding region, we used the primers 5′ TGCCAAAGAAACAGGGACTT 3′ and 5′ GAATTTGCGAGACCGAAAAA 3′ described by Pereira et al. (2007). The primers SNF1-R (5′ GCTGTCCGTATGTCCAAGGT 3′) and SNF1-L (5′ GATGTACCACACGCCTGAGA 3′) that amplify part of the *M. perniciosa snf1* gene were used as RT-PCR experimental controls (Medina 2006). The primers ITS1 and ITS4 were used to amplify the rDNA ITS region as described by White et al. (1990). The primers GAG1 (5′ AACTTGACGAGGCGAATCAT 3′) and GAG2 (5′ TTTGACCGACTTCAGGAAGC 3′) were constructed based on the *MpSaci14* sequence using the Primer3 program (http://www-genome.wi.mit.edu/cgibin/primer/primer3.cgi) (Rozen and Skaletsky 2000). These primers were used to amplify a 400 bp DNA fragment corresponding to a portion of the *gag* coding region. For the IRAP and REMAP analyses (Kalendar et al. 1999), primers CPLTR1F (5′ CTCGCTTCTTCAGCTTGACC 3′), CPLTR1R (5′ GTGCAAGCGACACACAAACT 3′), CPLTR2F (5′ GCCGTACTTAGCGTACTTCTTT 3′), CPLTR2R (5′ GCAGTGTTTCTACAGCGAAG 3′), MS1 (5′ GGCGGCGGCGGCGGCGGCGGCT 3′), MS2 (5′ ATGATGATGATGATGATGATGG 3′), and MS4 (5′ CACACACACACACACACACAG 3′) developed by Santana et al. (2012) were used in different combinations (Fig. 7).

Table 1 Information on the *Moniliophthora perniciosa* isolates used in this study

Isolate number	Isolate identification	Biotype	Chromosomal group[a]	Location[b]	Host
1	FA551	C	–	Tabatinga/AM	*Theobroma* sp.
2	ESJOH1	C	–	Marituba/PA	*Theobroma cacao*
3	ESJOH2	C	–	Ouro Preto do Oeste/RO	*Theobroma cacao*
4	ESJOH3	C	–	Belém/PA	*Theobroma cacao*
5	CP02[c]	C	CG1	Itajaípe/BA	*Theobroma cacao*
6	Belmont	C	CG1	Belmonte/BA	*Theobroma* sp.
7	Ilhéus	C	CG1	Ilhéus/BA	*Theobroma* sp.
8	FA277	C	CG1-A	Itabuna/BA	*Theobroma cacao*
9	FA317	C	CG1-B	Ilhéus/BA	*Theobroma cacao*
10	FA563	C	CG1	Itabuna/BA	*Theobroma cacao*
11	Santo Amaro	C	CG2	Santo Amaro/BA	*Theobroma* sp.
12	FA42	C	CG2	Itabuna/BA	*Theobroma cacao*
13	FA276	C	CG2	Itabuna/BA	*Theobroma cacao*
14	FA281	C	CG2	Aiquara/BA	*Theobroma cacao*
15	FA293	C	–	Gandu/BA	*Theobroma cacao*
16	FA562	C	CG2	Itabuna/BA	*Theobroma cacao*
17	Lep1	L	–	Pichilingue/Equador	*Arrabidaea verrucosa*
18	SCFT	L	–	San Carlos/Equador	*Arrabidaea verrucosa*
19	SCL4	L	–	San Carlos/Equador	*Arrabidaea verrucosa*
20	FA607	S	–	Coimbra/MG	*Solanum lycocarpum*
21	FA609	S	–	Poços de Caldas/MG	*Solanum lycocarpum*
22	DOA-105	S	–	Jataí/GO	*Solanum lycocarpum*

[a] Chromosomal groups 1 and 2 for C-biotype isolates from Bahia determined according to Rincones et al. (2006)

[b] AM, PA, RO, BA, MG and GO indicate the Brazilian states of Amazonas, Pará, Rondônia, Bahia, Minas Gerais and Goiás, respectively

[c] The isolate CP02 was used in the Witches' Broom Genome Project

Sequence analysis

The *MpSaci* sequences were compared with sequences deposited in GenBank using the BLAST program (Basic Local Alignment Search Tool) (Altschul et al. 1997). Subsequent analyses were performed using ClustalW (Thompson et al. 1994) and CDD to search for conserved domains (Marchler-Bauer et al. 2011) with a maximum *E* value of 1^{e-5}. For the alignment analysis, the following GAG and POL sequences (with the respective accession numbers) were used: *grh* (M77661, M77661), MAGGY (AAA33419, AAA33420), *marY1* (BAA78624, BAD10925, BAA78625), *REAL* (BAA89271, BAA89272), and *skippy* (AAA88790, S60179). For the phylogenetic analysis of the *MpSaci* reverse transcriptase protein, representative sequences of the *Metaviridae* (genus *Metavirus*, *Errantivirus* and *Semotivirus*) and *Pseudoviridae* families were obtained using the following access numbers: *Bel* (U23420), *CfT-1* (Z11866), *copia* (D10880), *grh* (M77661), *gypsy* (X03734), MAGGY (AAA33420), *marY1* (BAA78625), *ninja* (D83207), *Pao* (L09635), *REAL* (BAA89272), *skippy* (S60179), *suzu* (AF537216), *Tf1-107* (M38526), *Tom* (Z24451), *Tv1* (AF056940), *Ty1* (Z48149), and *yoyo* (U60529). Phylogenetic analyses were performed with the ClustalW program (Thompson et al. 1994) via the neighbor-joining method (Saitou and Nei 1987) with bootstrap values using 1,000 replicates.

DNA extraction and PCR

Total DNA was extracted as described by Pereira et al. (2014). PCR was performed in a thermocycler (PTC-100—MJ Research) programmed for 40 cycles of 1 min at 94 °C; 1 min at 55 °C for the reverse transcriptase, 55 °C for *gag* or 52 °C for the ITS region; 1 min at 72 °C and a final extension at 72 °C for 10 min. The reactions were prepared in a final volume of 25 µl containing 1× thermophilic DNA poly Buffer (Promega), 2.5 mM $MgCl_2$ (Promega), 100 µM of each dNTP, 0.5 µM of each primer, 20 ng of DNA and one unit of Taq DNA polymerase (Promega). Negative controls (without DNA) were used for each set of experiments to assess non-specific amplification. The DNA fragments were analyzed by electrophoresis using a 1.5 % agarose gel.

Southern hybridization analysis

Total DNA (3.0 μg) was cleaved with *Bam*HI, which does not cleave the reverse transcriptase sequence used as a probe, *Hpa*II or *Msp*I, which recognize the same sequence (5′ CCGG 3′) but the cut is dependent of the cytosine methylation status (Bender 2004). The cleaved DNA was electrophoresed overnight in 0.7 % agarose gel and transferred to Duralon membrane-UVTM (Stratagene) according to standard protocols (Sambrook et al. 1989). Hybridization was performed at 65 °C using the 394 bp DNA fragment corresponding to part of the reverse transcriptase as the probe as well as the "Gene ImagesTM Random Primer Labeling Module" and "CDP-StarTM Detection Module" (Amersham) according to the manufacturer's instructions.

RNA extraction, RT-PCR, IRAP and REMAP

To obtain the *M. perniciosa* mycelial mass for the nutritional stress experiments, 7-mm mycelial disks were incubated on plates containing PDA medium at 27 °C for 10 days. After growth, 30 small mycelium fragments were transferred to 125-ml Erlenmeyer flasks containing 50 ml of liquid minimal medium (6.8 g KH_2PO_4, 8.6 g K_2HPO_4, 6.0 g KNO_3, 0.52 g KCl, 0.01 g $FeSO_4$, 0.01 g $ZnSO_4$, 10 g glucose, and 1,000 ml distilled water) and incubated for 7 days at 27 °C/180 rpm. After 7 days, the mycelia were filtered, washed with distilled water, transferred to fresh minimal medium with and without a carbon or nitrogen source, and incubated for 10 h at 27 °C/180 rpm. The mycelia was used for total DNA extraction (as described above) and total RNA extraction as described by Watkinson et al. (2008). For the RT reactions, total RNA was treated with RNase-Free RQI DNase (Promega) and quantified at 260 nm. The first cDNA strand was generate using 5 μg of RNA, 1× RT buffer (Promega), 0.5 mM dNTPs, 500 ng oligo $(dT)_{15}$ (Promega), 20 U of ribonuclease inhibitor RNasin (Promega) and 10 U Avian Myeloblastosis Virus Reverse Transcriptase (Promega). The volume of each reaction was adjusted to 20 μl, and the reactions were incubated at 25 °C for 5 min and then at 42 °C for 60 min. PCR reactions were performed to detect the cycles corresponding to the logarithmic phase of DNA amplification. For these analyses, we used 1 μl cDNA and SNF1-L/R primers that amplify a 230 or 300 bp fragment of the *M. perniciosa snf1* gene depending on whether the template is cDNA or genomic DNA (Medina 2006). The amplification program was 1 min at 94 °C, 1 min at 57 °C and 1 min at 72 °C. Aliquots were removed at 20, 22, 24, 26 and 40 cycles. Based on the results from these analyses, all PCR reactions were performed using 25 cycles, and the entire content of each amplification reaction (25 μl) was separated on a 1.5 % agarose gel. The intensity of the amplified fragments was analyzed using ImageJ software (Abramoff et al. 2004) obtained from http://rsb.info.nih.gov/ij/. The expression of the *gag* gene was calculated in relation to *snf1* gene expression. The total DNA was analyzed by IRAP (inter-retrotransposon amplified polymorphism) and REMAP (retrotransposon-microsatellite amplified polymorphism) techniques with PCR amplification and thermocycling conditions as described by Santana et al. (2012). Amplifications were performed twice.

Results

Harvesting the *MpSaci* elements

A reverse transcriptase (RT) sequence previously characterized by our group (Pereira et al. 2007) was used to identify a complete LTR retrotransposon from the sequences in the *M. perniciosa* genome harvested by the LTR-Finder software. The first element identified, named *MpSaci1*, is 8,499 bp with 529 bp direct long terminal repeats (LTRs) displaying 99 % similarity. *MpSaci1* possesses two open read frames (ORFs) coding for the GAG and POL proteins; however, the first ORF is interrupted by two premature stop codons (Supplementary Fig. 1). The *MpSaci1* sequence was used to search for other elements from this species in the sequences harvested by the LTR-Finder software. We were able to obtain several sequences demonstrating various levels of similarity with the *MpSaci1* sequence. Fourteen of these sequences displayed an organization typically found in *gypsy-Ty3* elements; however, the majority of the copies did not code for intact GAG and POL proteins due to premature stop codons, frameshift mutations, or the lack of start codons and coding capacity (Fig. 1). The additional sequences demonstrated several rearrangements leading to truncated elements with low similarity to GAG or to some POL protein domains and LTRs in the same or opposite direction. These regions either flanked or did not flank sequences with similarities to GAG and/or POL (Fig. 2). Given that only two elements coded for all the domains necessary for transposition, non-autonomous elements primarily represent the *MpSaci* species in the *M. perniciosa* genome.

Characterization of the *MpSaci* species

The direct LTRs identified in the 14 elements with *gypsy/Ty3* organization display similarities ranging from 94 to 100 % and sizes ranging from 223 to 581 bp. Most of the LTRs are larger than the ones found in the majority of *gypsy/Ty3* LTR retroelements (200–450 bp); however, the *Rhizot* element has LTRs greater than 1 kb (Abe et al. 2009). Various characteristics have been reported for LTR

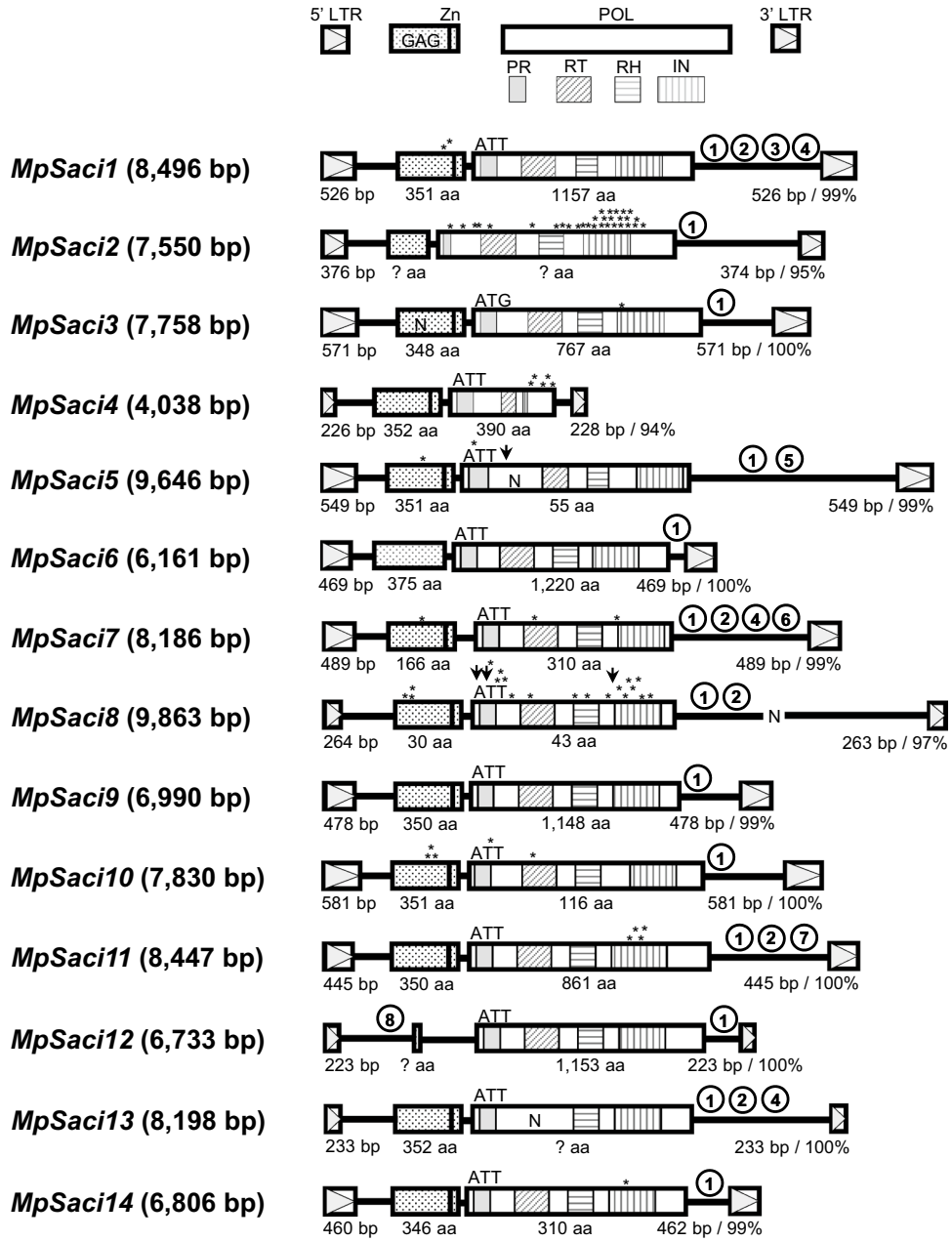


Fig. 1 Schematic representation of the fourteen *MpSaci* elements in *M. perniciosa* genome that display organization similar to the *gypsy/Ty3* group. For convenience, all elements are presented in the same orientation. The GAG protein contains a zinc finger domain (Zn) displayed in *black*. The POL protein contains protease (PR), reverse transcriptase (RT), RNase H (RH) and integrase (IN) domains. The *asterisks* correspond to premature stop codons, and the *arrows* indicate frameshift mutations. *N* indicates sequences with a stretch of undefined bases. *?* indicates protein sizes that could not be detected. The *bars* are proportional to the element size of the elements and putative proteins (not considering the stop codons when possible). The sizes of the GAG and POL proteins in *aa* (amino acids) are indicated considering stop codons. The LTR sizes and similarity between the LTR 5′ and 3′ are indicate below each element. The *numbers inside the circles* represent the presence of the following conserved domains: *1* chromatin organization modifier, *2* GAL4-like Zn2Cys6 binuclear cluster DNA-binding domain, *3* Tymovirus 45/70Kd protein, *4* C-terminal domain of homeodomain 1 for mating typing, *5* Atrophin-1, *6* AF-4 proto-oncoprotein, *7* Herpes-TAF50 super family, and *8* DUF3295 super family

of fungal retrotransposons, including inverted repeats at the ends, tRNA primer binding sites, and transcriptional initiation and polyadenylation signals. Inverted terminal repeats are typically found in the majority of retroviral and retrotransposon LTRs (Bingham and Zachar 1989). Similar to the LTRs found in *skippy*, *Cf*T-1, *Boty*, *Cg*ret, *REAL* and ANiTa1 elements, *gypsy/Ty3* group elements typically end in 5′-TG…CA-3′ (McHale et al. 1992; Dobinson et al. 1993; Anaya and Roncero 1995; Diolez et al. 1995; Kaneko et al. 2000; Zhu and Oudemans 2000; Braumann et al. 2007). The LTRs of the majority of *MpSaci* elements also end in inverted repeats containing 5′-TG…CA-3′ (Fig. 3a). However, additional characteristics of the LTRs from other filamentous fungi were not found in the *MpSaci* elements, such as the primer binding site that is typically required for transcription initiation and is located immediately upstream of the 5′ LTR containing a conserved TGG in *Drosophila* (Bingham and Zachar 1989). For the *REAL* and *marY1* elements, the primer binding site was not found, but purine-rich sequences that correlate with the duplication process were identified (Kaneko et al. 2000; Murata and Yamada 2000). Three CT rich regions, one sequence similar to CCATT and two putative TATA boxes potentially linked to the transcription start site were identified in the *marY1* element. The majority of eukaryotic LTR retroelements are also flanked by small repeats related to the target

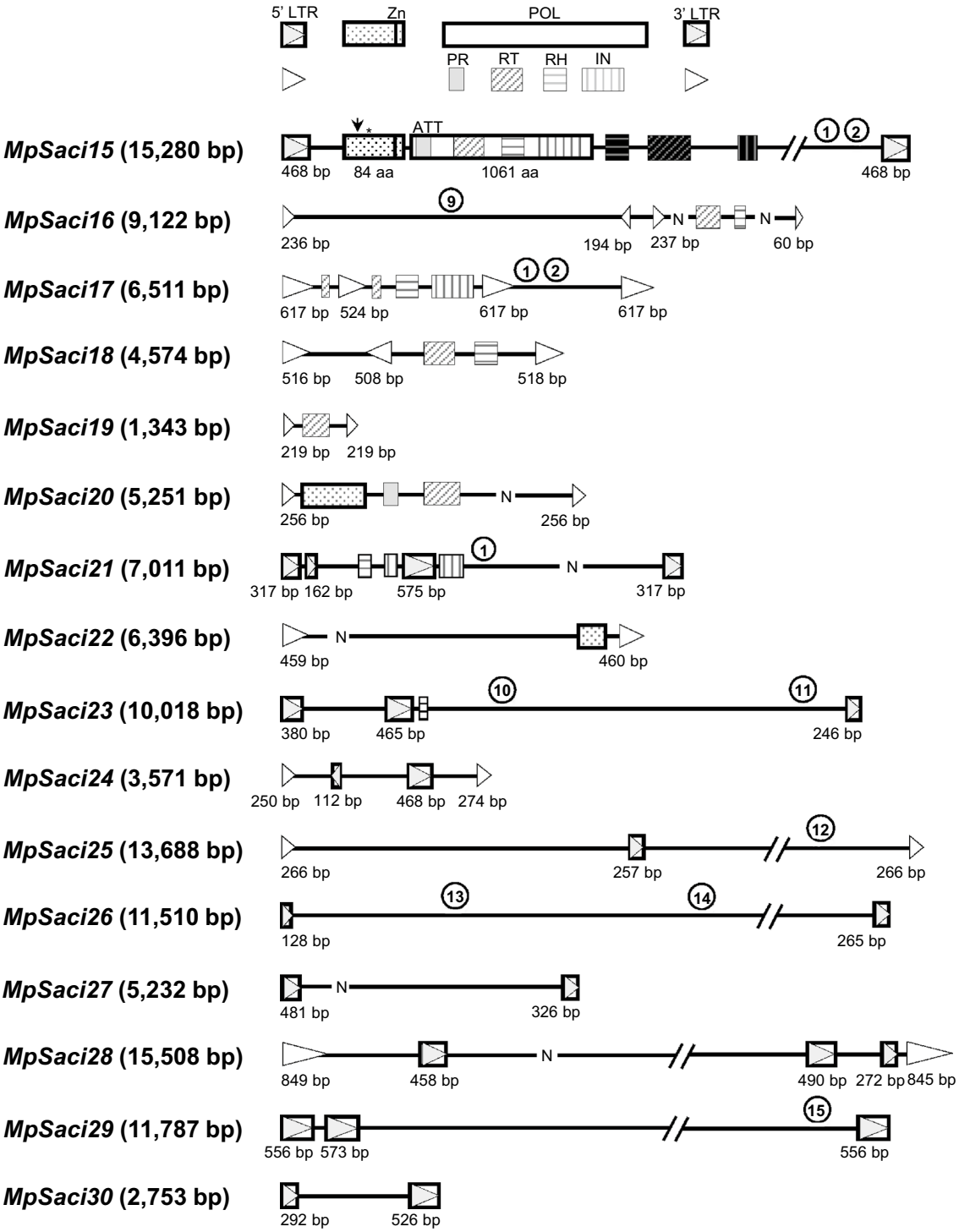
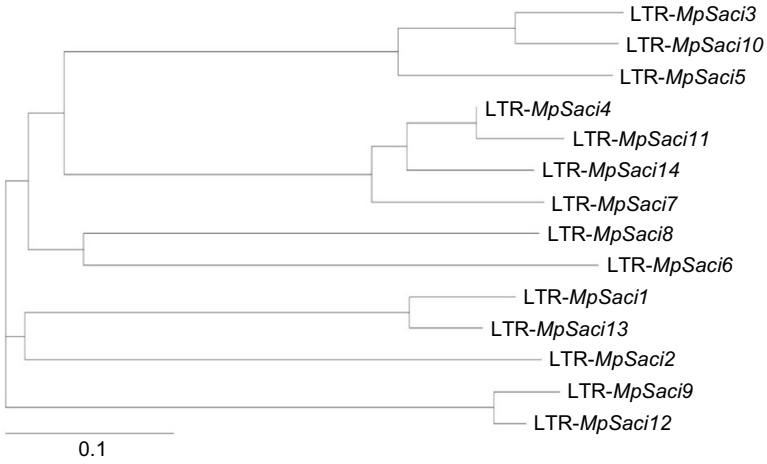


Fig. 2 Schematic representation of the unusual *MpSaci* sequences displaying various levels of homology with the *MpSaci1* element. For convenience, all elements are presented in the same orientation. *N* indicates sequences with a stretch of undefined bases. The *bars* are proportional to the sequence size. The LTRs inside the *boxes* (*gray horizontal arrowheads*) are similar to the LTRs from the *MpSaci1* to *Mpsaci14* elements. The LTRs indicated by *white horizontal arrowheads* are not similar to the *MpSaci* elements. The LTR sizes are indicated below each element. The *dark boxes* represent the domains of a *Ty1/copia*-like retrotransposon inside the *MpSaci15* element. The *numbers inside the circles* represent the presence of the following conserved domains: *1* chromatin organization modifier, *2* GAL4-like Zn2Cys6 binuclear cluster DNA-binding domain, *9* DnaQ-like-exo super family, *10* SDH-beta super family, *11 hAT* family dimerization domain, *12* Glyco-hydrolase-16 super family, *13 hAT* transposase, *14* DDE superfamily endonuclease, and *15* large tegument protein UL36

a

	5′LTR	3′LTR
• *MpSaci1*	TGTTA...TAACA	TGTAA...TAATA
MpSaci2	TGCTA...GACTT	TGCTA...GACTT
• *MpSaci3*	TGTTA...TAACA	TGTTA...TAACA
MpSaci4	TTACT...AGCAC	TTACT...AGCAC
• *MpSaci5*	TGTTA...TAACA	TGTTA...TAACA
MpSaci6	TGTTA...AAGCA	TGTTA...AAGCA
• *MpSaci7*	TGTTA...TAACA	TGTTA...TAACA
MpSaci8	TGTTA...ATGTA	TGTTA...GAGTA
• *MpSaci9*	TGTTA...TAACA	TGTTA...TAACA
• *MpSaci10*	TGTTA...TAACA	TGTTA...TAACA
• *MpSaci11*	TGTTA...TAACA	TGTTA...TAACA
MpSaci12	TGTTA...ATACG	TGTTA...ATACG
MpSaci13	ATTTT...AGATG	ATTTT...AGATG
• *MpSaci14*	TGTTA...TAACA	TGTTA...TAACA

b



c

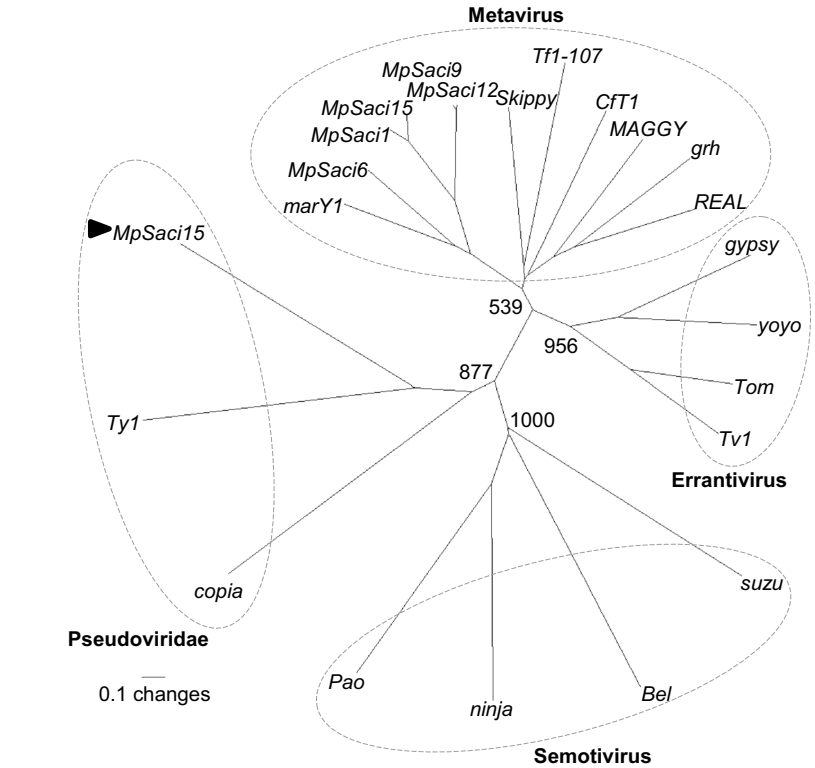


Fig. 3 Analyses of the LTRs and reverse transcriptases from the fourteen *MpSaci* elements displaying organization similar to the *gypsy/Ty3* group. **a** Sequence analysis of the first and last five bases in the 5′ and 3′ LTRs. *Black circles* before the name of the element represent elements possessing similar inverted repeats. **b** Relationship among the LTRs from the fourteen *MpSaci* elements. **c** Phylogenetic tree presenting the relationship among the reverse transcriptase domains from *MpSaci1*, *MpSaci6*, *MpSaci9, MpSaci12* and *MpSaci15* as well as other fungal retrotransposons and representatives of the *Metaviridae* (genus *Metavirus*, *Errantivirus* and *Semotivirus*) and *Pseudoviridae* families. The *arrowhead* indicates the second reverse transcriptase sequence found in *MpSaci15* that belongs to the *Ty1/copia* group of retroelements. The *numbers* in each branch indicate the *bootstrap* based on 1,000 replicates. The trees were constructed using the *neighbor-joining* method (Saitou and Nei 1987)

site duplication; however, these repeats could not be precisely identified for the *MpSaci* elements. Interestingly, the alignment of the LTRs from the 14 copies of *MpSaci* indicates minimal sequence similarity among some of the copies. In addition, these copies can be divided into different groups (Fig. 3b). The *gag*, *pol* and LTRs appear to be the target of several rearrangements because the *MpSaci15* to *MpSaci23* elements display an unusual pattern and solo LTRs were found (Fig. 2). Several sequences have LTRs but do not contain *gag* or *pol* coding regions, and these sequences can be classified as LARDs (large retrotransposon derivative) based on their sizes (*MpSaci24* to *MpSaci30*) (Havecker et al. 2004). Two of the unusual elements (*MpSaci15* and *MpSaci26*) display rearrangements leading to the presence of other transposable elements (*Ty1/copia* and *hAT*) between the LTRs of *MpSaci*. Transposable elements containing additional domains from other transposons inserted into the structure of a typical *Ty3* element were also observed by Muszewska et al. (2011). *MpSaci15* was considered part of the *MpSaci* species since most part of the sequence (around 80 %) that is flanked by LTRs is correlated to a *gypsy/Ty3* retroelement.

Intact ORFs coding for GAG (*MpSaci4, MpSaci6, MpSaci9, MpSaci11*, *MpSaci13* and *MpSaci14*) and POL (*MpSaci1*, *MpSaci6*, *MpSaci9, MpSaci12* and *MpSaci15*) proteins were detected. *MpSaci6* and *MpSaci9* are the only elements that code for both intact proteins. High sequence similarity among the GAG and POL proteins encoded by *MpSaci* elements and transposons from other filamentous fungi were detected. Conserved domains were identified in all of the elements. However, the region surrounding the GAG zinc finger in *MpSaci6* displayed reduced similarity. In addition, the glutamic acid from the integrase DDE motif in the POL protein from *MpSaci1* was changed to a lysine, likely resulting in a non-functional protein (Supplementary Fig. 2). Thus, *MpSaci6* and *MpSaci9* are the only elements that encode all of the domains typically required for transposition.

The sizes of the functional GAG proteins range from 346 to 375 aa with predicted molecular masses from 39.6 to 40.6 kDa. The mass is less than the masses reported for other retrotransposons with the exception of the *marY1* element, which has a 42 kDa GAG protein (Murata and Yamada 2000). However, a putative ribosomal frameshift site was identified in *marY1*, which could result in a 63.6 kDa GAG protein. This size is similar to the mass reported for other retrotransposons (60–80 kDa). Putative ribosomal frameshift sites were not detected in the *MpSaci* elements. A conserved zinc finger domain (Cys-X2-Cys-X4-His-X4-Cys), which is also present in other retrotransposon and retroviral GAGs, was detected in the GAGs from *MpSaci4, MpSaci6, MpSaci9, MpSaci11, MpSaci13* and *MpSaci14* (Supplementary Fig. 2A). The functional POL protein is formed by four different domains (protease, reverse transcriptase, RNase H and integrase) and ranges in size from 1,061 to 1,220 aa with a predicted molecular mass of 122.2–141.5 kDa. The POL protein displays high similarity to the *marY1* element from *Tricholoma matsutake* (Murata and Yamada 2000), *REAL* (Kaneko et al. 2000), MAGGY from *Magnaporthe grisea* (Farman et al. 1996), and *skippy* from *Fusarium oxysporum* (Anaya and Roncero 1995). The protease domain possesses the conserved motif DSG (Asp, Ser, Gly), which is the proposed active site (Katoh et al. 1987). The reverse transcriptase displays the seven domains described by Xiong and Eickbush (1990) and the motif YXDD (Tyr, Met, Asp, Asp), which is the proposed enzyme active site (Varmus and Brown 1989). The integrase possesses the motif DD(35)E (Asp, Asp, Glu), which is highly conserved among retrotransposon integrases (Supplementary Fig. 2E). Phylogenetic analyses using reverse transcriptase domains from various elements reveal that *MpSaci* is related to the *gypsy-Ty3* group. In addition, the other reverse transcriptase sequence found in *MpSaci15* is clearly related to a *Ty1/copia* element (Fig. 3c).

Another characteristic of the *MpSaci* elements is the long distance between the end of the *pol* coding region and the 3′ LTR; this region is greater than 2 kb in *MpSaci1, MpSaci2, MpSaci5, MpSaci7, MpSaci8, MpSaci11 and MpSaci13*. In different elements, this region possesses seven conserved domains that are not related to the domains typically found in retrotransposon sequences (Figs. 1, 2). The most common domains are the chromatin organization modifier (chromo) domain; a DNA-binding domain that is similar to the domain found in transcription regulators, such as GAL4; and the C-terminal domain of homeodomain 1, which is involved in fungi mating types. Some of the conserved domains detected in the *MpSaci* elements were previously reported to negatively or positively impact transcription (Marmorstein and Harrison 1994; Koonin et al. 1995).

Distribution of *MpSaci*

The *MpSaci* distribution was evaluated by PCR and Southern hybridization. Portions of the *gag* and *reverse transcriptase* coding region were amplified in all isolates (Fig. 4), indicating that the primers annealing sites were conserved among all isolates. Hybridization with the RT sequence indicates that *MpSaci* copies are present in the genomes of C-, S- and L-biotypes (Fig. 5). Although the resolution of the Southern analysis is not clear for most of the genotypes, the lowest *MpSaci* copy number, which was estimated to be seven, was detected for the FA609 isolate (L-biotype). Since the bioinformatics analysis resulted in 18 sequences with homology to the probe, we estimated the *MpSaci* copy number from seven to 18.

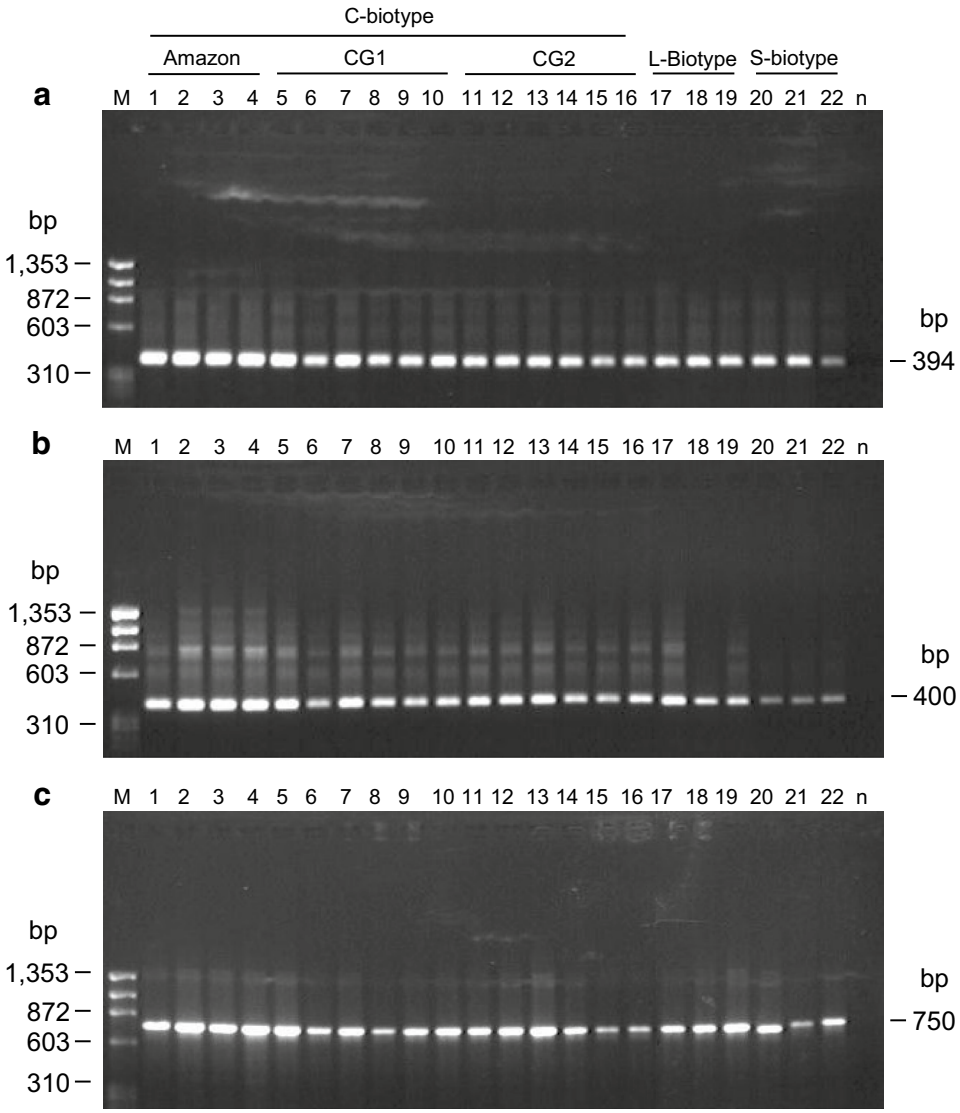


Fig. 4 PCR amplification of various *M. perniciosa* isolates with primers specific for the *MpSaci* elements. **a** Amplification of a 394 bp DNA fragment corresponding to part of the reverse transcriptase coding region; **b** amplification of a 400 bp DNA fragment corresponding to part of the *gag* coding region; and **c** amplification of the rDNA-ITS region as a control for DNA quality. *M* indicates DNA size marker (ϕX174 *Hae* III), and *n* indicates the negative control (reaction without total DNA). The isolate identification corresponds to the data presented in Table 1

However, considering the unusual elements, that number could increase to 30 sequences as detected for the CP02 genome (Figs. 1, 2). The C-biotype isolates originated from the Amazon region and the Bahia state isolates have different hybridization profiles; these two distinct profiles correlate with the two chromosomal groups, CG1 and CG2, described by Rincones et al. (2006). Although DNA fragments greater than 3 kb are not separated and hence more difficult to analyze, some DNA fragments from the Amazon isolates correlate with Bahia state isolates (Fig. 5). One common 1,630 bp fragment is present in isolate 3 (originated from Rondônia) and the CG1 isolates (from Bahia). This fragment is absent in isolates 2 and 4 (from the Pará state) as well as the CG2 isolates (Bahia). This finding creates a link among the different chromosomal groups from the Bahia isolates and C-biotypes from different locations in the Amazon region. L- and S-biotype isolates also possess an increased *MpSaci* element copy number; however, none of the copies could be correlated with a specific genotype or location (Fig. 5).

MpSaci reverse transcriptase methylation status

Hybridization of the *MpSaci* reverse transcriptase revealed a different profile depending if the total DNA was cleaved with *Hpa*II or *Msp*I (Fig. 5). The cleavage by *HpaII* was inhibited generating larger bands while *MspI* was able to cut the transcriptase sequences more often. The different hybridization profile is evidence that *MpSaci* reverse transcriptase sequence is methylated.

Expression analysis

RT-PCR was performed to evaluate the effect of nutritional stress (lack of nutrients) on *gag* gene expression (Fig. 6). After determining that the logarithmic phase

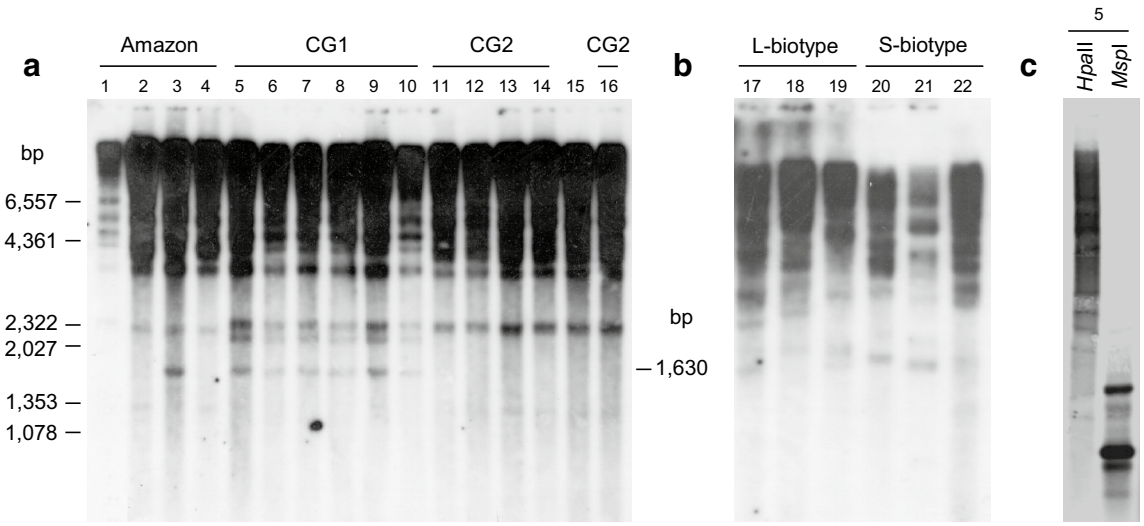


Fig. 5 Hybridization of the 394 bp DNA fragment containing part of the *MpSaci* reverse transcriptase coding region with total DNA from the *M. perniciosa* isolates. *Bam*HI cutted DNA of **a** C-biotypes and **b** L- and S-biotypes isolates. **c** DNA of CP02 isolate cleaved with *Hpa*II or *Msp*I. The isolate identification is presented in Table 1. "CG1" and "CG2" refer to the two chromosomal groups of the C-biotype from Bahia. The position of some fragments is presented in the *left*; the size and position of the fragment discussed in the text are displayed

of DNA amplification occurs between cycles 24 and 26 (Fig. 6c), the PCR reaction was ended at the 25th cycle. An intron was detected in the amplification of the control gene (*snf1*) (Fig. 6e). Amplification of the 400 bp DNA fragment that corresponds to a region of the *MpSaci gag* gene revealed that this element is constitutively expressed in *M. perniciosa* grown in minimal medium. In addition, nutritional stress activates element expression (Fig. 6f). A minimum of three-fold increase in DNA intensity was detected in stress conditions (Fig. 6i). No introns were detected in the 400 bp DNA fragment from the *MpSaci gag* gene. Using GenBank (http://www.ncbi.nlm.nih.gov/nucleotide/), we also detected several *MpSaci gag* and *pol* sequences generated from expression studies, indicating that *MpSaci* expression was also detected in other experiments (Supplementary Table 1).

IRAP and REMAP

To search for new insertions, DNA extracted from nutritionally stressed mycelia was used to amplify regions between LTRs (IRAP) or LTR insertions near to microsatellite markers (REMAP). Different primers combinations were used being the amplification profile highly similar among the stressed and non-stressed mycelia (Fig. 7). Differences in the amplification profile were observed in the stressed mycelia when the microsatellite primer MS2 was used in different combinations. New insertions were not detected.

Discussion

This report is the first one to characterize all members of a species, belonging to the class I of retrotransposons, in the most important pathogen of cocoa in Brazil. Since transposable elements are associated with increased adaptability and the phenotypic variation demonstrated by phytopathogenic fungi (Daboussi and Capy 2003; Shnyreva 2003; Pereira et al. 2006), the characterization of the retrotransposon *MpSaci* is an important step forward in understanding the genomic organization and adaptability of *M. perniciosa*. Almost entirely represented by non-autonomous elements, the *MpSaci* species contains elements whose gene organization are similar to *gypsy/Ty3* elements but also unusual elements and solo LTRs. This species is distributed across different biotypes and it is activated under nutritional stress although the lack of some structural characteristics might prevent an efficient transposition.

High copy numbers of the *MpSaci* elements exist in C-, S- and L-biotypes of *M. perniciosa* that originate from various geographic regions (Fig. 5). Based on a transposition mechanism wherein a new copy is inserted in a new target site, high copy numbers of retrotransposons are found in the genomes of phytopathogenic fungi. In general, transposable elements constitute less than 20 % of fungal genomes (Daboussi and Capy 2003; Wicker et al. 2007) although they can represent around 85 % of *Blumeria graminis* f.sp. *tritici* genome (Parlange et al. 2011). Class I elements are more abundant than class II elements in *M. perniciosa* genome (Mondego et al. 2008); however, the percentage

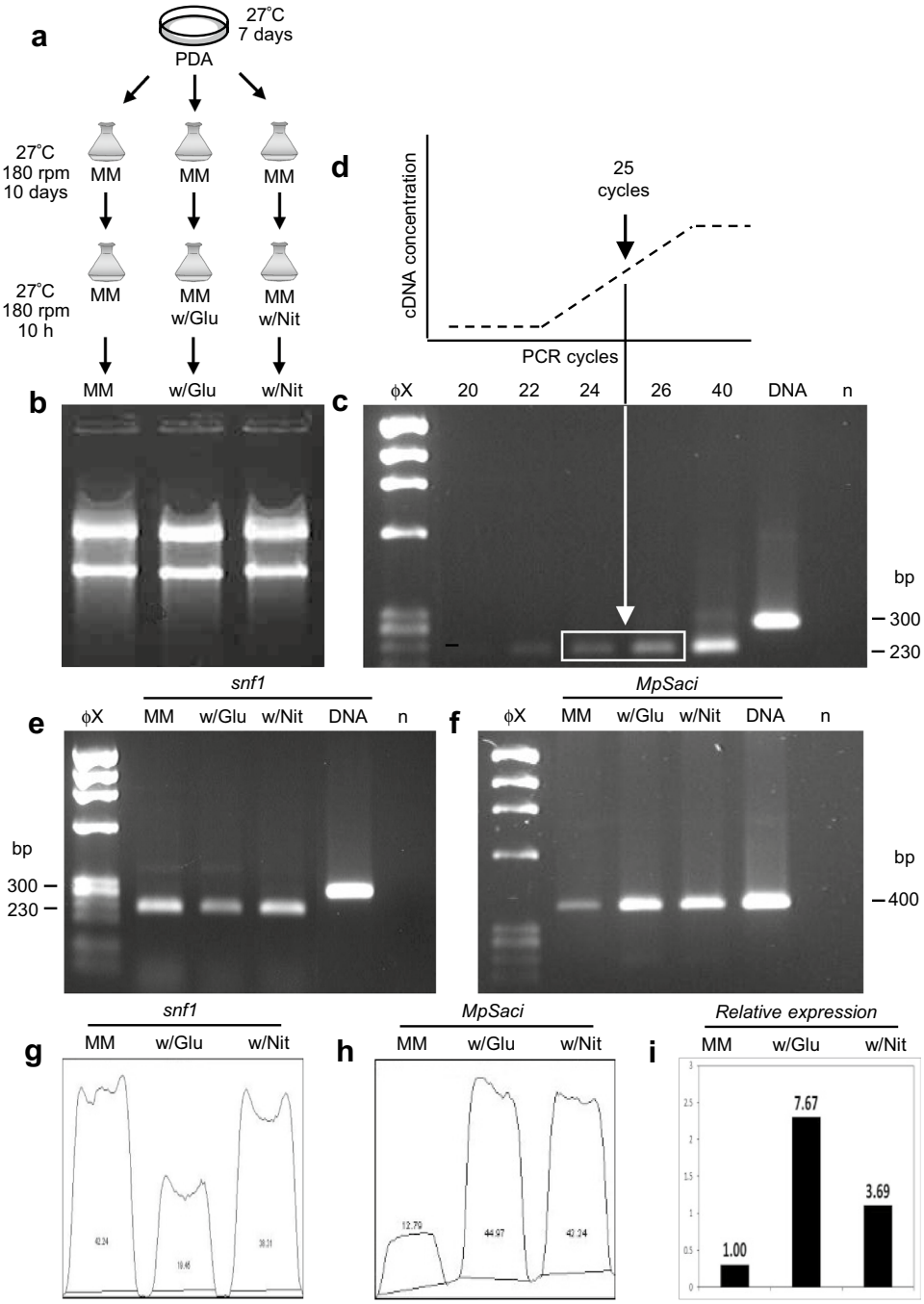


Fig. 6 RT-PCR analysis of *gag* expression from *MpSaci*. **a** Representation of the experiment. **b** Quality of the RNA extracted. **c**, **d** Evaluation of the amplification curve using the *snf1* gene to detect the logarithm phase of DNA amplification. Amplifications of the *snf1* control (**e**) and part of the *MpSaci gag* region (**f**) were performed until the 25th cycle. **g**, **h** Densitometric analysis of the amplified products. **i** Relative *gag* gene expression from the *MpSaci* elements in relation to the *snf1* gene. *ϕX* indicates the DNA size marker (ϕX174 *Hae* III); "MM" indicates minimum medium; *w/glu* indicates minimum medium without glucose; *w/nit* indicates minimum medium without nitrogen source; *DNA* indicates the total DNA from the CP02 isolate; and *n* indicates the negative control (amplification without adding cDNA or DNA). The DNA fragment sizes amplified are presented in **c**, **e** and **f**

of the genome that is comprised of these elements remains unknown. Phylogenetic analysis of the reverse transcriptase protein, the most conserved protein among retrotransposons (Havecker et al. 2004), and the organization of the *pol* region indicate that *MpSaci* is another species of the *Metaviridae* family (*gypsy/Ty3*) belonging to the genus *Metavirus* (Fig. 3c). The *gypsy/Ty3* group is the most abundant retrotransposon in the *M. perniciosa* genome (Mondego et al. 2008), and this group is typically the most abundant retrotransposon in other filamentous fungi (Muszewska et al. 2011). According to Daboussi (1996), *gypsy/Ty3*-like retrotransposons are ubiquitous because one common ancestor contained a copy of these elements and the elements were transferred vertically along with the other components of the genome during speciation events. The vertical transmission is also based on phylogenetic analyses of the reverse transcriptase. The majority of the LTR-retrotransposons are clustered, thereby creating a monophyletic group of fungal transposons; this finding is consistent with the vertical heritage (Daboussi 1996).

The high copy number of transposable elements in fungi can potentially lead to chromosome-length polymorphism

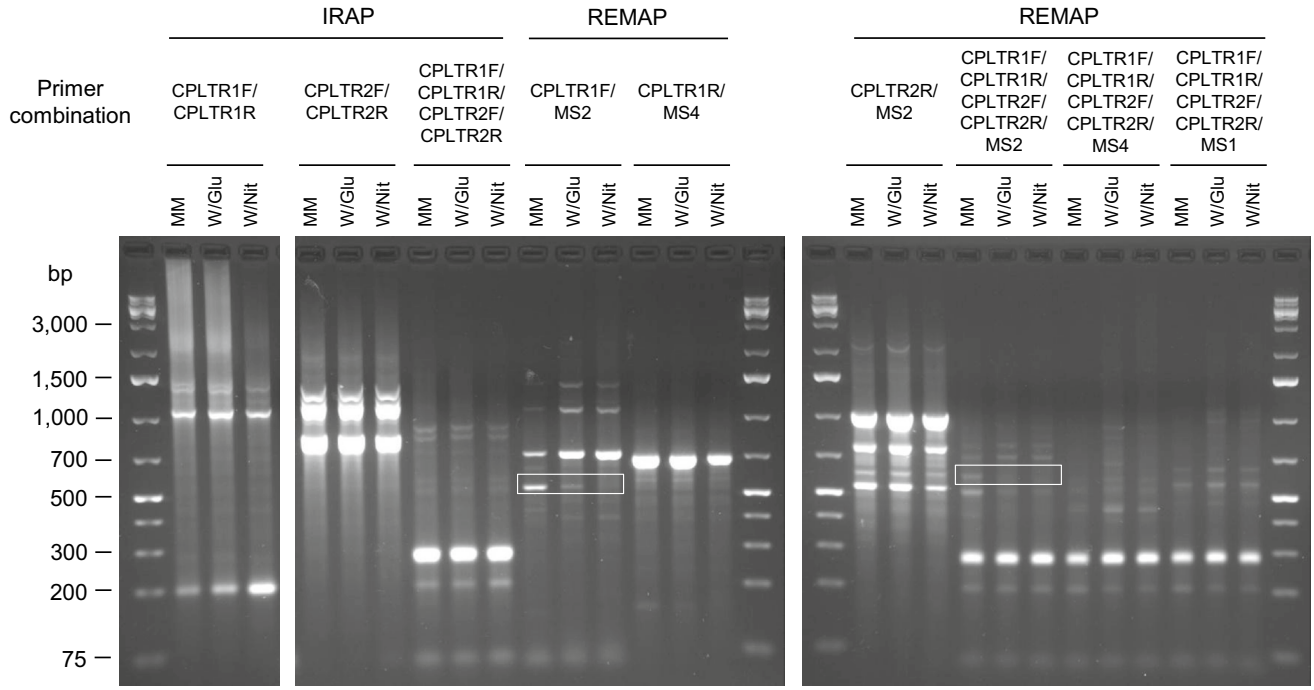


Fig. 7 IRAP and REMAP analyses (2 % agarose gel) in DNA extracted from the nutritionally stressed mycelia. Some fragments of the molecular size marker are presented in the *left*. *MM* indicates minimum medium; *w/glu* indicates minimum medium without glucose; *w/nit* indicates minimum medium without nitrogen source. *White rectangle* indicates differences in the amplification profile

(Zolan 1995). Not only the transposition can cause chromosomal breaks but also the homologous recombination between copies spread throughout the genome can promote chromosomal rearrangements (Mieczkowski et al. 2006). With regard to phytopathogenic fungi, a relationship between karyotype instability and transposable elements has been reported in *F. oxysporum* and *M. grisea* (Shull and Hamer 1996a, b; Nitta et al. 1997; Davière et al. 2001; Thon et al. 2006; Starnes et al. 2012). In *M. perniciosa*, two different types of chromosomal patterns were reported for C-biotypes originating from the Bahia state (Rincones et al. 2003, 2006), and these chromosomal patterns display two unique hybridization patterns when analyzed using a *MpSaci* reverse transcriptase sequence (Fig. 5). Similarly, the *Boto* element from the *PIF/Harbinger* superfamily (Pereira et al. 2013) and a portion of an element belonging to the *TC1-Mariner* superfamily (Ignacchiti et al. 2011) are also distributed in *M. perniciosa* genome based on the chromosomal groups. *MpSaci* is the third species of transposable elements analyzed by our group in the *M. perniciosa* genome. Given that all of these elements are distributed according to the Bahia state C-biotype chromosomal groups, we hypothesize that the chromosomal polymorphism causes the different transposons profiles or, alternatively, the transposable elements are potentially linked to the origin of the two C-biotype chromosomal groups. Clearly, more concrete evidence should be provided to support these hypotheses. In addition to the link between *MpSaci* distribution and the chromosomal groups, two distinct *MpSaci* hybridization profiles were detected in the C-biotype isolates from the Bahia state. This finding indicates the presence of two genotypic groups, and each group displays similarities with different genotypes from the Amazon region. The presence of two distinct C-biotype groups in the Bahia state has been reported previously by Andebrhan et al. (1999) through RAPD analysis; this study concluded that witches' broom was introduced in Bahia via two independent points. In addition, a relationship between the Amazon and Bahia isolates was also reported by other authors (Andebrhan et al. 1999; Arruda et al. 2003a, b; Ploetz et al. 2005; Ignacchiti et al. 2011).

Interestingly, transposons can contribute to the evolution of phytopathogenic fungi by promoting phenotype alterations. Various alterations originate through inactivation via insertional mutagenesis; this mechanism is directly linked to transposition promoted by autonomous elements. For example, the insertion of *Pot3* in *AVR-Pita* promotes virulence of *M. grisea* in a cultivar containing the *Pi-Ta* resistance gene (Kang et al. 2001). This element was also identified in the *AVR-Pita* gene of other isolates (Zhou et al. 2007; Singh et al. 2014). Moreover, transposon insertions promoted gain of virulence in *C. fulvum* and increased drug resistance in *B. cinerea* (Luderer et al. 2002; Kretschmer et al. 2009). In addition, changes generated by

recombination are also reported, thereby providing evidence that non-autonomous transposable elements can also contribute to the evolution of plant pathogens. For example, retrotransposons near the avirulence gene *AvrLm1* in *L. maculans* could have increased the frequency of recombination events in that region, creating deletions that are linked to virulence in some isolates (Fudal et al. 2007). Transposons in another avirulence gene, *AVR-Pita*, potentially contribute to the increased proportion of translocation in the genomes of various *M. oryzae* isolates (Chuma et al. 2011). In addition, the super-expression of CYP51, which confers resistance to fungicide, is positively correlated with a truncated LINE-like element in the CYP51 coding region in *Blumeriella jaapii* (Ma et al. 2006). In *Neurospora* spp., retrotransposons play an important role in the transition between homothallic and heterothallic systems (Gioti et al. 2012). In *P. tritici-repentis*, alterations induced by transposons are likely to be involved in the emergence of pathogenicity (Manning et al. 2013). Thus, although the *MpSaci* species is primarily represented by non-autonomous elements, these elements still can contribute to genetic variability in *M. perniciosa*. It is also important to consider that some of the unusual domains in the *MpSaci* sequences could contribute to alterations in the expression of neighboring genes because these domains could potentially impact transcription (Marmorstein and Harrison 1994; Koonin et al. 1995).

The following characteristics demonstrate that the majority of *MpSaci* elements sequenced in the wild type isolate are likely non-autonomous: the absence of structures typically found in LTRs, the presence of premature stop codons and frameshift mutations, the complete absence of sequences resembling the coding region and the reduced similarity/lack of conserved domains (Figs. 1, 2). The reduced similarity among LTRs from various elements of the same species potentially suggests that the *MpSaci* is an old resident of the *M. perniciosa* genome. This finding might indicate that the host genome suppressed the majority of the deleterious effects of the transposable elements. Non-autonomous retrotransposons are typically found in various fungal species and can represent the majority of element copies in the genome (Muszewska et al. 2011). For instance, no autonomous elements were identified in the *N. crassa* genome (Galagan et al. 2003; Schulman 2012). These elements can be inactivated through genetic or epigenetic mechanisms by the host genome (Kazazian 2004). Two evidences indicate that a silencing mechanism related to RIP (repeat-induced point mutation) could be acting on *M. perniciosa* genome: methylated reverse transcriptase sequences as observed in Southern analysis (Fig. 5) and numerous G:C to A:T transitions at the *MpSaci* reverse transcriptase previously reported by Pereira et al. (2007). RIP is the mechanism of action implicated as a primary reason for the origin of degenerate transposable elements reported in other phytopathogenic fungi (Nakayashiki et al. 1999; Attard et al. 2005). Although RIP is reported in numerous fungal species (Clutterbuck 2011), this defense mechanism has not been shown to exist in *M. perniciosa*. In addition to RIP, other mechanisms act on *MpSaci* elements, leading to truncated copies, LTRs with reduced similarity to elements (Fig. 3b), solo LTR and several LARDs (Fig. 2). Solo LTRs have been detected in the *Phanerochaete chrysosporium* and *B. graminis* f.sp. *tritici* genomes (Martinez et al. 2004; Parlange et al. 2011) as well as in the *Ty1,* ANiTa1 and *yeti* retrotransposons of *Saccharomyces paradoxus, Aspergillus niger* and *Podospora anserina*. LTR–LTR recombination, which is based on homologous recombination between LTRs, is thought to be involved in the generation of solo LTRs (Kim et al. 1998; Hamann et al. 2000; Moore et al. 2004; Braumann et al. 2008). Primers based on the *MpSaci* LTRs were used for inter-retrotransposon amplified polymorphism (IRAP) to generate polymorphic loci in the different isolates, and the solo LTR and LARDs sequences may have also contributed to the polymorphism (Santana et al. 2012). It is also important to note that we analyzed the genome of one *M. perniciosa* isolate, and different isolates can potentially retain autonomous copies of *MpSaci*.

Gene expression analysis of transposable elements (Okuda et al. 1998; Kaneko et al. 2000; Kito et al. 2003; Rep et al. 2005; Bouvet et al. 2008; Amyotte et al. 2012; Pereira et al. 2013) is used to demonstrate transposon activity as well as gene inactivation (Daboussi et al. 1992; Langin et al. 1995; Maurer et al. 1997; Gómez-Gómez et al. 1999) and the appearance of new copies in the genome (Anaya and Roncero 1996; Mes et al. 2000; Pereira et al. 2013; Chadha and Sharma 2014). Stress-induced transposition is described in other fungi. For example, nutritional stress activates the transposition of the *hAT*-like *Drifter* element in *F. oxysporum* and various elements in *Verticillium dahliae* (Rep et al. 2005; Amyotte et al. 2012). Nutritional stress results in at least 3-fold increase in *gag* gene expression from *MpSaci* (Fig. 6). Given that we detected several *gag* and *pol* sequences from cDNA libraries (Supplementary Table 1), these mRNAs could produce active GAG and POL proteins. Although only two *MpSaci* elements produce intact GAG and POL proteins, *in trans* activation of the transposition cannot be ruled out. However, the LTRs, the primer-binding site and a polypyrine tract are minimally required for transposon replication (Havecker et al. 2004). Given that some of these characteristics were not detected in the *MpSaci* elements, *in trans* mobilization appears to be unlikely in the analyzed *M. perniciosa* genome. In fact, new fragments were not detected by IRAP and REMAP techniques in the mycelia obtained after nutritional stress (Fig. 7).

In conclusion, our results highlight that *MpSaci* species is highly represented by non-autonomous elements. Even the elements that code for intact proteins lack structures necessary for efficient transposition. This could explain why no new insertions were detected although nutritional stress can activate the expression of *gag* and reverse transcriptase genes. Nevertheless, these elements could be involved in the generation of polymorphisms through homologous recombination between two copies and also could impact the transcription of neighboring genes since conserved domains were detected inside all copies. The copy number, expression and distribution of the *MpSaci* elements throughout the genomes of various isolates added valuable information about the molecular biology of *M. perniciosa*. In addition, further studies can address the questions raised here about the possible link between transposable elements and the appearance of two chromosomal groups in C-biotype isolates and the presence of defense mechanisms that promote the degeneration of most of the copies in a transposon species.

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