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Research Letter

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Molecular variability and genetic relationship among Brazilian strains of the sugarcane smut fungus

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One sentence summary: Genetic variation uncovered in Brazilian strains of the sugarcane smut fungi.

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ABSTRACT

Sporisorium scitamineum is the fungus that causes sugarcane smut disease. Despite of the importance of sugarcane for Brazilian agribusiness and the persistence of the pathogen in most cropping areas, genetic variation studies are still missing for Brazilian isolates. In this study, sets of isolates were analyzed using two molecular markers (AFLP and telRFLP) and ITS sequencing. Twenty-two whips were collected from symptomatic plants in cultivated sugarcane fields of Brazil. A total of 41 haploid strains of compatible mating types were selected from individual teliospores and used for molecular genetic analyses. telRFLP and ITS analyses were expanded to six Argentine isolates, where the sugarcane smut was first recorded in America. Genetic relationship among strains suggests the human-mediated dispersal of *S. scitamineum* within the Brazilian territory and between the two neighboring countries. Two genetically distinct groups were defined by the combined analysis of AFLP and telRFLP. The opposite mating-type strains derived from single teliospores were clustered together into these main groups, but had not always identical haplotypes. telRFLP markers analyzed over two generations of selfing and controlled outcrossing confirmed the potential for emergence of new variants and occurrence of recombination, which are relevant events for evolution of virulence and environmental adaptation.

Keywords: molecular markers; mating-type; telomere; *Sporisorium scitamineum*; Argentina; Brazil

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INTRODUCTION

Sporisorium scitamineum is the causal agent of sugarcane smut, a disease distributed worldwide (Comstock and Lentini 2005). The fungus presents three genetically and morphologically distinct phases during its life cycle: haploid yeast-like sporidia, dikaryotic hyphae and diploid teliospores. Infection culminates with the outgrowth of a whip-like structure from shoot primary meristem, characterizing the main symptom of the disease (Comstock 2000). Within this structure, teliospores develop as a result of karyogamy and hyphae fragmentation. Under appropriate environmental conditions, the teliospores germinate, undergo meiosis and produce haploid sporidia that grow saprophytically as yeast-like cells and *in vitro*. To infect the host, a combination of two haploid sporidia from opposite mating types is needed to form the dikaryotic infective hyphae (Alexander and Srinivasan 1966).

The complete genome sequence of *S. scitamineum* (strain SSC39B) confirmed the bipolar mating system by showing that the two sex-determining loci (*a* and *b*) are physically linked at a distance of 59 kbp apart on chromosome 2 (Taniguti *et al.* 2015). In general, the bipolar system favors a higher level of selfing compared with the tetrapolar system, leading to elevated homozygosity in the fungal population (Fraser and Heitman 2003), reducing the reassortment of genetic variability, and thus the potential of the pathogen to adapt to evolutionary changes of the host (Kaltz and Shykoff 1999). On the other hand, selfing could be selectively advantageous in cases of limited partner availability, ensuring reproductive success (Billiard *et al.* 2012). The advantages of inbreeding or outbreeding may depend on the ecological niche occupied by the fungus (Bakkeren, Kämper and Schirawski 2008) and their occurrence is also dependent on the number and frequency of alternate alleles at the mating-type *loci* (Giraud *et al.* 2008).

Many genetic studies have been conducted on global and local levels, using different strategies to assess *S. scitamineum* molecular variability and virulence degree (Braithwaite *et al.* 2004; Xu, Que and Chen, 2004, 2014; Singh, Somai and Pillay 2005; Raboin *et al.* 2007; Rago, Casagrande and Massola-Júnior 2009; Fattah *et al.* 2010; Luzaran *et al.* 2012; Que *et al.* 2012; Bhuiyan *et al.* 2015; Zhang *et al.* 2015). In an overall view, low levels of genetic variation were found in American and African isolates while high levels were found in Southeast Asian populations. Southeast Asia was predicted as the center of origin of *S. scitamineum* from where a single lineage was dispersed to other continents through exchange of infected plant material for breeding purposes (Braithwaite *et al.* 2004; Raboin *et al.* 2007). The complete homozygosity of 17 codominant markers for all fungal isolates analyzed by Raboin *et al.* (2007) indicates that selfing is the predominant reproductive mode and that the current population structure is markedly homogeneous.

Despite the importance of Brazil as the largest producer of sugarcane in the world and the continuous maintenance of the fungus in cultivated areas, a detailed survey of the genetic variability of this pathogen in Brazilian fields is still missing. In order to better understand the molecular variation and the genetic relationship among Brazilian strains of *S. scitamineum* for further improve breeding strategies for smut resistance, we used a combination of molecular approaches. Each technique accesses different genomic regions: AFLPs (amplified fragment length polymorphisms) are randomly distributed throughout the genome (Mueller and Wolfenbarger 1999); telRFLPs (telomere-associated restriction fragment length polymorphisms) are located at chromosome termini (Wu *et al.* 2009); and ITS (internal transcribed spacers) are located at ribosomal DNA and is a popular region for variability studies (Nilsson *et al.* 2008). In addition, we also investigate (i) the genetic relationship between Brazilian and Argentine isolates, since Argentina is a Brazil's neighbor country where the sugarcane smut disease was first recorded in America in 1940 (Ferreira and Comstock 1989); (ii) the presence of polymorphisms between two sexual compatible haploid strains derived from a single teliospore; (iii) the usage of distinct restriction enzymes from those used by Braithwaite *et al.* (2004) in AFLP-based genotyping; (iv) the stability of telRFLP profiles over generations.

MATERIAL AND METHODS

Ethics statement

Smut whips were collected from 22 plants growing at sugarcane production areas in six states of Brazil (Table 1). Symptomatic canes were obtained from selection areas managed by 'Instituto Agronômico de Campinas (IAC) - Centro de Cana', located in Ribeirão Preto, Brazil. Six whips were obtained from three different substations of the 'Agro-industrial Experimental Station Obispo Colombres (EEAOC)' in Tucumán state (TUC), Argentina (Table 1). The reference SSC39A and SSC39B haploid strains were submitted to the culture collection of the 'Fundação Oswaldo Cruz – FIOCRUZ' under the accession numbers INCQS 40413 and INCQS 40412, respectively. The SSC39B strain, whose complete genome was sequenced (assembly accession: ASM101084v1), is herein referred to as BR_39B.

Sporisorium scitamineum strains isolation

Whips and teliospores were collected, and haploid strains with opposite mating-types were derived from each isolate (see Supplementary File1 for images of each lifecycle phase). Shortly, teliospores were scraped off from the whips and dried during 4 h at 37°C. A pinch of teliospores was dipped in 1 mL of saline solution (NaCl 0.85M) and treated with 0.5 mg L^{-1} of streptomycin sulphate (Sigma-Aldrich, St Louis, MO, USA) for 30 min. Serial dilutions were prepared, plated on YM medium (0.3% yeast extract, 0.3% malt extract, 0.5% soybean peptone, 1% D-glucose, 1.5% agar) and incubated in the dark for 24 h at 28°C. A single germinated spore was inoculated in YM liquid, incubated overnight at 28°C in orbital shaker and then plated on solid YM to obtain single haploid sporidial colonies (strains). The resulting yeast-like cells were stained with 1% orcein solution to confirm single nuclei state (Supplementary File1). Strains derived from each teliospore were randomly selected for the plate mating assay. Because the genomic context of the two alleles controlling mating types was determined for the *S. scitamineum* strains SSC39A (MAT2 allele) and SSC39B (MAT1 allele) (Taniguti *et al.* 2015), they were used as reference to assign strains to the A or B mating types. The strains were grown in liquid YM medium in orbital shaker for 24 h at 28°C. A droplet of each paired culture was mixed and placed on YM plates, and incubated overnight at 28°C. Successful matings were identified by the formation of white and fuzzy colonies typical of the filamentous growth (Supplementary File1). Total DNA was extracted from strains of both mating types, and *S. scitamineum* identity was confirmed by PCR amplification with modified primers bE4 and bE8 (Taniguti *et al.* 2015).

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Table 1. *Sporisorium scitamineum* isolates collected from symptomatic sugarcane plants and molecular strategies applied.

Isolate	Mating type	Location [city (state)]	Collection date	Sugarcane cultivar	AFLP	tel-RFLP	ITS	Approximate geographical coordinates
Brazilian isolates								
BR_04	A B	Barreiras (BA)	2009	IAC 91 5155	√	√	√	12° 09′ 10″ S 44° 59′ 24″ W
BR_05	A B	Frutal (MG)	2009	SP791011	√	√	√	20° 01′ 29″ S 48° 56′ 26″ W
BR_11	A B	Jaboticabal (SP)	2009	IAC911099	√	√		21° 15′ 17″ S 48° 19′ 20″ W
BR_17	A B	Macatuba (SP)	2009	CTC12	√	√		22° 30′ 08″ S 48° 42′ 41″ W
BR_18	A B	Paranaíba (MS)	2009	SP891115		√	√	19° 40′ 38″ S 51° 11′ 27″ W
BR_24	B	Paranaíba (MS)	2009	IACSP944004		√		19° 40′ 38″ S 51° 11′ 27″ W
BR_31	A B	Serra Azul (SP)	2009	IAC044084	√	√		21° 18′ 39″ S 47° 33′ 56″ W
BR_33	B	Vista Alegre do Alto (SP)	2009	RB855453		√		21° 10′ 14″ S 48° 37′ 45″ W
BR_35	A B	Limeira D´Oeste (MG)	2009	IACSP933046	√	√		19° 33′ 04″ S 50° 34′ 50″ W
BR_36	A B	Valparaíso (SP)	2009	RB72454	√	√		21° 13′ 40″ S 50° 52′ 06″ W
BR_38	A B	Guaíra (SP)	2009	SP903414	√	√		20° 19′ 06″ S 48° 18′ 38″ W
BR_39	A B	Ribeirão Preto (SP)	2009	IAC982053	√	√	√	21° 10′ 39″ S 47° 48′ 37″ W
BR_40	A B	Araçatuba (SP)	2009	nr*	√	√		21° 12′ 32″ S 50° 25′ 58″ W
BR_41	A B	Conchal (SP)	2009	nr*	√	√		22° 19′ 49″ S 47° 10′ 21″ W
BR_43	A	Paranaíba (MS)	2010	IACSP942094		√		19° 40′ 38″ S 51° 11′ 27″ W
BR_46	A B	Paranaíba (MS)	2010	CTC15		√		19° 40′ 38″ S 51° 11′ 27″ W
BR_58	A B	Goianésia (GO)	2009	IACSP044004		√	√	15° 19′ 03″ S 49° 07′ 03″ W
BR_65	A B	São José da Laje (AL)	2010	SP716849		√	√	09° 00′ 35″ S 36° 03′ 30″ W
BR_74	A B	São José da Laje (AL)	2010	“havaiana”		√		09° 00′ 35″ S 36° 03′ 30″ W
BR_85	A B	Paranaíba (MS)	2010	RB855002		√	√	19° 40′ 38″ S 51° 11′ 27″ W
BR_89	A B	São Simão (SP)	2011	nr*		√		21° 28′ 45″ S 47° 33′ 03″ W
BR_90	A B	São Simão (SP)	2011	nr*		√		21° 28′ 45″ S 47° 33′ 03″ W
Argentine isolates								
AR_01	A B	Cerco Represa (TUC)	2014	TUC 06–12		√	√	26°48′ 30″S 65°13′ 33″ W
AR_02	A B	Cerco Represa (TUC)	2014	TUC 06-25		√	√	26° 48′ 29″S 65° 13′ 35″W
AR_05	A B	Ingenio Santa Ana (TUC)	2014	TUC 06-04		√	√	27° 28′ 19″S 65° 40′ 53″W
AR_06	A B	Ingenio Santa Ana (TUC)	2014	TUC 05-10		√	√	27° 28′ 30″S 65° 40′ 55″W
AR_07	A B	Ranchillos (TUC)	2014	CP 65-357		√	√	26° 56′ 58″S 65° 03′ 00″W
AR_13	A B	Ingenio Santa Ana (TUC)	2014	TUC 06-25		√	√	27° 28′ 20″S 65° 40′50″W

*nr = not registered

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Molecular markers techniques

telRFLP

Total genomic DNA was extracted from haploid strains and completely digested with three restriction enzymes (*Eco*RI, *Hind*III and *Pst*I) in independent assays. The digested DNA was probed with the insert of pTEL13 recombinant plasmid, which corresponds to fungal telomeric region (Levis *et al.* 1997). The plasmid was digested and the insert gel purified with 'illustra GFX PCR DNA and Gel Band Purification kit' (GE Healthcare, Little Chalfont, UK). Labeling and detection of telRFLP fragments were performed using AlkaPhos Direct kit (GE Healthcare, Buckinghamshire, UK) following the manufacturer's instructions. Only reproducible and not ambiguous bands were scored (Supplementary File2). *In silico* predictions of endmost chromosome fragment size were performed based on the complete genome sequence of *S. scitamineum* (Taniguti *et al.* 2015).

AFLP

Templates were prepared by digesting the total DNA with *Mse*I and either *Pst*I or *Eco*RI, followed by ligation of adaptors and selective PCR amplifications, according to Palhares *et al.* (2012) (Supplementary File2). AFLP bands were resolved in denaturing polyacrylamide gels using the electrophoresis apparatus Sequi-Gen GT (Bio-Rad Lab., Hercules, CA, USA), and visualized by silver staining. All samples were analyzed in duplicates and only reproducible bands were scored. The 125 bp and 10 bp ladders (Invitrogen Life Technologies, Carlsbad, CA, USA) were used in order to estimate the size of AFLP fragments. Additionally, polymorphic AFLP bands were excised directly from the gels, reamplified, size fragmented in agarose gel, purified with 'illustra GFX PCR DNA and Gel Band Purification kit' (GE Healthcare, Little Chalfont, UK) and sequenced in an ABI PRISM 3730 DNA Analyzer (Applied Biosystems).

ITS-nrDNA sequencing

The primers used to amplify the ITS region were designed by White *et al.* (1990) and modified by Stoll *et al.* (2003). The amplifications were performed with the high fidelity enzyme 'KAPA HiFi HotStart DNA Polymerase' (Kapa Biosystems) in a 'Veriti® 96-Well' thermocycler (Applied Biosystems). PCR products were purified with '™ illustra GFX PCR DNA and Gel Band Purification kit' (GE Healthcare) and sequenced in a ABI PRISM 3730 DNA Analyzer (Applied Biosystems, Foster City, CA, USA).

Stability of telRFLP markers across generations

Simulations of selfing (allowing natural crossing between teliospores-derived cells of BR_39 isolate) and outcrossing (using controlled crossing between BR_18A and BR_39B strains) were made across two consecutive generations in greenhouse (Supplementary File3). Single-budded sugarcane setts were inoculated with *S. scitamineum* by punctuation. For the selfing experiment, BR_39 teliospores were inoculated to produce the first inbreeding generation of progeny BR_39-F1. The resulting teliospores were separated into opposite mating-type strains and used to produce the next inbreeding generation (BR_39-F2).

For the outcrossing simulation, the strains BR_18A (from whip BR_18) and BR_39B (from whip BR_39) were chosen because they presented contrasting telRFLP profiles and came from different locations (Table 1). A mixture of the two strains was inoculated to produce the progeny BR_18×39-F1. The following progenies BR_18×39-F2 were produced by inoculating two sexually compatible sporidial colonies isolated from the whip of previous disease cycle.

At each generation, five strains of opposite mating types had their telRFLP profiles obtained with the restriction enzyme *Eco*RI.

Data analyses

Binary matrixes obtained from scoring telRFLP and AFLP polymorphic fragments as absent (0) or present (1) were used to estimate the genetic distance among *S. scitamineum* haploid strains using the Jaccard coefficient. When a marker was not scored for a particular strain, it was assigned as missing data. The distance matrix was used to construct a dendrogram based on the 'unweighted pair cluster method with arithmetic mean' (UPGMA) with 10 000 replications using the Pvclust package implemented in R (Suzuki and Shimodaira 2006). The optimal number of genetic clusters (K) was estimated using the STRUCTURE v2.3 software (Pritchard, Stephens and Donnelly 2000), working with 125 000 burn-in steps before 500 000 MCMC repeats, for K ranging from 1 to 7 with 10 repeat runs for each K, under a model assuming admixture. The web-based program STRUCTURE HARVESTER was used to summarize the output data and to execute the 'Evanno' method (Evanno, Regnaut and Goudet 2005), providing the K number that best fitted the data (Earl and vonHoldt 2012). The principal component analysis (PCA) for grouping the strains in the first two principal components (PCs) was conducted with the bioconductor package pcaMethods (Stacklies *et al.* 2007).

Mantel tests were performed with the 'APE' package in R (Paradis, Claude and Strimmer 2004) to verify the correlation between pairs of distance matrices (genetic, geographic, temporal and breeding program). To generate a distribution of Z (Mantel's test statistic Z) under the null hypothesis of no association between matrices, 10 000 Monte Carlo random permutations were done. A two-tailed test was used by default for computing the statistical significance.

Sequencing data were trimmed and multiple aligned using the genome sequence of SSC39B strain as reference (Taniguti *et al.* 2015) in the CLC Genomics Workbench v.8.0. As a single haplotype was obtained for ITS region in Brazilian and Argentine strains, the sequence can be recovered of the SSC39B genome sequence (accession assembly: ASM101084v1; Supplementary File4).

RESULTS AND DISCUSSION

Molecular variability of *Sporisorium scitamineum* unraveled by AFLP and telRFLP

In order to draw a panoramic picture of the genetic variation of Brazilian isolates, we chose a combination of molecular approaches (telRFLP, AFLP and ITS sequencing) to screen various regions of the genome and to compare markers used in previous studies.

Amplification with 19 AFLP combinations of restriction enzymes (*Eco*RI, *Pst*I and *Mse*I) and selective primers produced 36 polymorphic bands out of 1311 that were scored. The number of polymorphic bands was in general low per gel (up to 3) and the highest number obtained was 6 with [P+AGA, M+CTC] and [P+AGA, M+CAA] combinations. This approach increased the number of polymorphic loci compared to the previous data for American isolates (Braithwaite *et al.* 2004), but still reveal limited variation among Brazilian strains. The ITS sequencing data was even more inefficient, producing identical sequences for all

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Table 2. Variability detected by telRFLP and AFLP markers among sets of *S. scitamineum* strains.

Molecular marker	Marker ID	Polymorphic bands	Strains	Haplotypes
telRFLP	*EcoR1*, *HindIII*, *PstI*	41	53	45
	EcoRI	18	52	27
	HindIII	14	52	27
	PstI	9	33	17
AFLP	*EcoRI*, *PstI*, *MseI*	36	24	18

strains, including those from Argentina. The ITS data produced by other authors revealed some variability in isolates from different countries (Singh, Somai and Pillay 2005; Zhang *et al.* 2015). After aligning our data with other 30 ITS sequences available at *genbank*, the Brazilian and Argentine haplotypes were equal to the most common haplotype worldwide (Supplementary File4).

Lastly, the telomeric hybridization patterns of genomic DNA digested with *EcoRI*, *HindIII* and *PstI* resulted in the scoring of 18, 9 and 14 unambiguous polymorphic bands, respectively. Through telRFLP technique, almost a unique fingerprint was detected for each strain, unraveling a variation yet unknown for Brazilian isolates (Table 2).

We tried to assess the genomic region uncovered by polymorphic loci from the AFLP and telRFLP markers. From the 36 polymorphic AFLP fragments, only six could be recovered after reamplification and gel excision. The sequences were aligned by blastN against the SSC39B genome and the lowest e-values were scored in locations on chromosomes 02, 05, 08, 20, 22 and mtDNA (Supplementary File5), which seems to be a random scanning of the genome.

Examining the *in silico* prediction of telRFLP endmost cutting sites in the genome of SSC39B (Taniguti *et al.* 2015), a list of target putative genes were detected (Supplementary File5). Most of them were predicted members of the telomere-linked helicase (TLH) family that acts in telomere maintenance via recombination. Highly polymorphic TLHs are a common feature among filamentous fungi and yeast (Sánchez-Alonso and Guzmán 1998; Yamada *et al.* 1998; Gao *et al.* 2002). Rehmeyer *et al.* (2009) have hypothesized that repeat-induced point (RIP) mutation could be the mutational process that generates the variation among multicopies of TLHs. Besides RIP mutations, a general source of telRFLP polymorphisms can be subtelomeric rearrangements during mitosis or meiosis that lead to chromosome end variability by duplication, deletion or (non)homologous recombination events (Louis and Haber 1990; Zolan 1995; Winzeler *et al.* 2003). The source of telRFLP variation in *S. scitamineum* may be generated by a combination of all these processes.

Relationship among Brazilian and Argentine strains

The combined data analysis of the two markers was conducted totaling 77 polymorphic loci. Two main groups were identified by the UPGMA dendrogram (Fig. 1A). A and B strains isolated from the same diseased plant were clustered into the same main group, except for BR_41 isolate (Fig. 1A). The prevalence of selfing explains the clustering pattern of A and B strains (Raboin *et al.* 2007). However, these strains did not have identical markers profile, evidencing the presence of variation even between strains derived from the same teliospore.

A model-based clustering algorithm, performed with STRUCTURE software, identified that the most likely number of clusters is three (K = 3) and provided the quantification of membership to each cluster for each strain (Fig. 1B). Most individuals (42) have very high membership coefficients to a single cluster (> = 0.9), but an admixture composition was noticed for some strains, whose genetic makeup is drawn from more than one of the *K* clusters. The STRUCTURE results arranged by line according to the dendrogram were not corroborative. When the dendrogram was partitioned into three groups (which is also well supported), the STRUCTURE red cluster fully covered one dendrogram group and partially another. The PCA was used as a third method to examine the clustering pattern of *S. scitamineum* strains, producing a 2D graphic summarizing the maximal variance of the multilocus data (Fig. 1C). The two groups set up by the dendrogram can be viewed within ellipses. These two main groups were consistently organized by the three methods used.

The whip-like structure harboring the teliospores emerges 2 months after inoculation in highly susceptible varieties (Legaz *et al.* 2011). Spores are carried by the wind over a distance of 100 m from the source whip and by splash of rainfall water in the soil (Sreeramulu and Vittal 1972; Hoy, Grisham and Chao 1991). Despite the potential scattering over long distances, we found no association between genetic and geographical distances among isolates (Fig. 1D and E), which was confirmed by the Mantel correlation test (Supplementary File6). The genetic distance matrix among *S. scitamineum* strains was not significantly correlated with any variable tested (spatial, temporal and breeding program origin), whereas these other variables were correlated between them. The dispersion within the Brazilian territory and among countries was probably due to human activity by exchanging asymptomatic plant material, as already proposed on global scale by Braithwaite *et al.* (2004) and Raboin *et al.* (2007).

telRFLP marker over generations

Sporisorium scitamineum is a dimorphic fungus that requires sexual reproduction to switch from a saprophytic budding yeast phase to an infectious dikaryotic hyphal growth. This transition is essential for the completion of its life cycle, since sporulation is host dependent (Bakkeren, Kämper and Schirawski 2008). As an obligate sexual fungus that requires different mating types in the haploid strains for successful sexual reaction, one would expect high genotypic diversity in its population. The admix of pre-existing genetic variation due to sexual recombination can be observed in our outcrossing simulation experiment between *S. scitamineum* strains (BR_18A x BR39_B) in which occurs the reassortment of parental telRFLP polymorphisms into the progenies. However, *S. scitamineum* has a bipolar mating system and selfing seems to be the predominant sexual reproduction mode in natural populations (Raboin *et al.* 2007).

The parental strains derived from the BR_39 whip had identical telRFLP *EcoRI* profiles, whereas the parental strains BR_18A and BR_39B differed at four scored bands. Interestingly, a different hybridization pattern emerged in F1 progenies derived from selfing, whereas in the outcrossing simulation no new pattern was detected (Supplementary File3).

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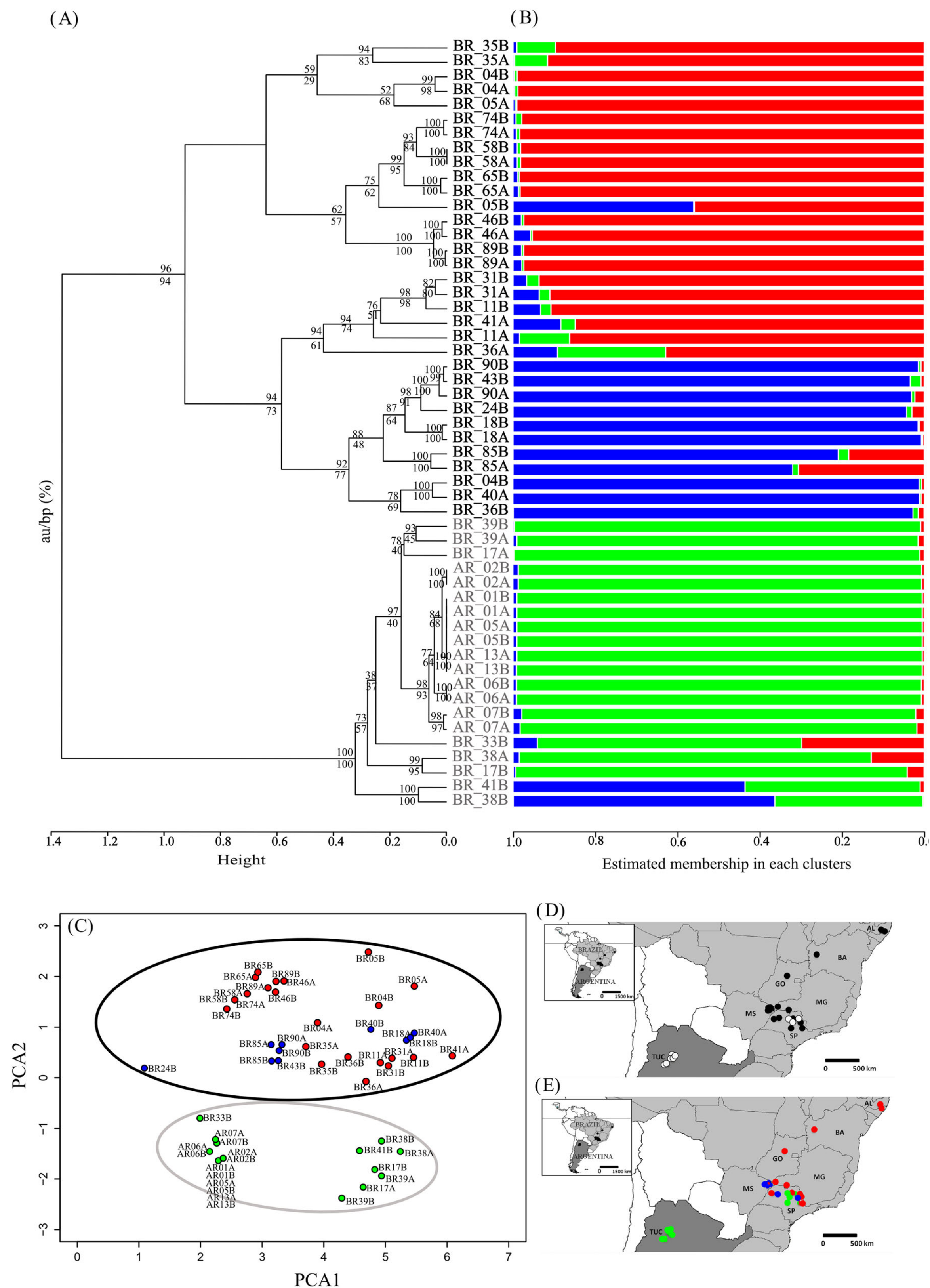

Figure 1. Relationship among S. *scitamineum* strains using telRFLP and AFLP markers. **(A)** UPGMA dendrogram of genetic distance using Pvclust R package. Black and gray colors were attributed to labels according to the two groups defined. Values at branches are AU p-values (above) and BP values (below). **(B)** Data structure obtained by STRUCTURE software for the best estimated value of K clusters (K = 3). Each strain is represented by a horizontal bar, ordered according to the dendrogram. Each bar is partitioned into three colored segments (red, blue and green) that represent the estimated membership in each cluster. **(C)** Graphic representation of the first two PCAs. Each point represents a strain. The color of the points corresponds to the primary color assigned by STRUCTURE analysis. The two ellipses indicate the groups generated by UPGMA dendrogram. **(D)** and **(E)** Approximate geographical distribution of *S. scitamineum* isolates (colored dots) included in this study. Dot colors indicate the grouping pattern generated by the dendrogram (A) and STRUCTURE (B) analysis, respectively.

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CONCLUSION

In summary, our study provides new clues about informative regions of the *Sporisorium scitamineum* genome, revealing genetic variability among Brazilian strains that so far were not described. No polymorphism was found by using ITS sequences, but taken together AFLP and telRFLP markers generate almost a unique fingerprint for each strain. A total of 53 strains were clustered into two genetically distinct major groups that do not reflect their geographical origins. We suggest that a mixture of isolates from these two major genetic backgrounds should be used in breeding programs to cover different adaptive potentials of Brazilian isolates. Our results do not allow us to infer about the emergence of new races, but showed that there is variability among strains and that new genetic variants may appear in every generation. It is worth mentioning that population genomics studies of this pathogen can also bring new knowledge to light regarding other polymorphic genomic regions and for the understanding of the disease dispersion history.

SUPPLEMENTARY DATA

Supplementary data are available at FEMSLE online.

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Conflict of interest. None declared.

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