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Structural organization of the mating-type locus in Clade 3 of *Fusarium solani* species complex and development of PCR protocol for identification of *MAT* idiomorphs

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Abstract

Mating compatibility in ascomycetes is regulated by the mating-type locus. In this study, whole-genome sequence data were used to characterize the structural organization of the *MAT* loci in two heterothallic and one homothallic species of Clade 3 from *Fusarium solani* species complex - FSSC. Based on sequence analysis, we identified *MAT1-1* idiomorph in *Fusarium paranaense* with three genes, *MAT1-2* idiomorph in *F. solani* f. sp. *piperis* with two genes, and both *MAT* idiomorphs in *F. striatum*, homothallic species. The structural organization of the *MAT1-1* locus in *F. paranaense*, like the *MAT1-2* locus in *F. striatum* and *F. solani* f. sp. *piperis* was different to those of other FSSC-Clade 2 and *Fusarium* species. Moreover, the mating-type genes sequences in FSSC-Clade 3 were dissimilar resulting in a distinct amino acid profile. Despite these differences in the studied species, the process of sexual reproduction is not affected. Besides, we developed primers and applied in a reliable PCR protocol to *MAT* idiomorph identification. This paper presents the first investigation of the structural organization of the *MAT* locus from FSSC-Clade 3. The availability of *MAT* genes sequences can give support to downstream functional studies of individual mating-type transcripts. The MAT primers will be useful to assess the reproductive mode in other strains of FSSC-Clade 3, and to facilitate the application of the biological concept through sexual crosses.

Key words – Heterothallic – Homothallic – MAT primers – *Neocosmospora* – Sexual reproduction – Whole-genome

Introduction

Sexual stage of *Fusarium solani* species complex (FSSC) is characterized by red or salmon-colored perithecia, which are frequently encountered in fallen rainforest fruits, recently dead trees and on litter in the tropics (Nalim et al. 2011). They are also present in diseased plants, such as in *Piper nigrum* with symptoms of black pepper fusariosis in Bahia, Brazil (Vaz et al. 2012). The *Fusarium solani* species complex (O'Donnell 2000) was included within genus *Neocosmospora*

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(Sandoval-Denis & Crous 2018, Sandoval-Denis et al. 2019); however, phylogenomic analysis supports the inclusion of the FSSC in *Fusarium* (Geiser et al. 2020). This species complex comprises more than 70 phylogenetic species, distributed in three main clades (Sandoval-Denis & Crous 2018, Sandoval-Denis et al. 2018, 2019, Geiser et al. 2020). Clade 1 represents a group of species exclusively from New Zealand, while Clade 2 includes saprobic species and pathogens of soybean and common bean, which occur in America and Asia. Aoki et al. (2003, 2005, 2011, 2012) described several species of this clade, namely: *Fusarium tucumaniae, F. virguliforme, F. brasiliense, F. cuneirostrum, F. crassistipitatum* and *F. azukiicola*. Most of the species belong to Clade 3, which includes plant, human and animal pathogens, as well as endophytic and saprobic species.

FSSC contains both heterothallic and homothallic species, and species that have unknown sexual stage. In ascomycetes, mating-type locus (*MAT*) contains two idiomorphs, *MAT1-1* and *MAT1-2*, encoding transcriptional regulators related to the expression of genes required for sexual development (Lopes et al. 2017, Wallen & Perlin 2018). Reproduction of heterothallic or self-sterile species occurs between two opposing mating type isolates, while homothallic species are considered self-fertile and complete their life cycle from a single spore, due to existence of both idiomorphs in the genome (Leslie & Summerell 2006). Initially in FSSC, seven mating populations (MP I to MP VII) were reported, based on mating experiments (Matuo & Snyder 1973). This was further extended through the description of mating populations *F. paranaense* (Costa et al. 2016) and *F. solani* f. sp. *piperis* (Albuquerque & Ferraz 1976, Vaz et al. 2012) in Clade 3, and *F. tucumaniae* (Covert et al. 2007) in Clade 2. Homothallic species in this complex are represented by *Neocosmospora vasinfecta* (Rossman et al. 1999), *Fusarium striatum* (Nirenberg & Brielmaier-Liebetanz 1996, Rossman et al. 1999), and others.

Sexual cycle ensures recombinant offspring improving species fitness (Wallen & Perlin 2018) by promoting genetic variation. Moreover, perithecia formed in plant tissue produces ascospores that are forcibly ejected into the air column, facilitating their dispersal to susceptible hosts (Ma et al. 2013). Although homothallism does not increase genetic diversity within species like heterothallism, it contributes to removal of deleterious combinations of alleles (Wallen & Perlin 2018); furthermore, ascospores are more prone to survive in stressful environments (Dyer 2007). According to Ge et al. (2019) "mating-type genes are pivotal in the sexual development of fungi". Therefore, sequence analysis and identification of *MAT* loci represents the first and fundamental step to assess the reproductive mode of fungal species, and evolution of *MAT* loci (Martin et al. 2011, Wilken et al. 2017). Studies on *MAT* genes have been conducted mainly with species from the *Fusarium fujikuroi* species complex (FFSC), *Fusarium sambucinum* species complex (FSamSC) and Clade 2 from FSSC. Heterothallic species from the FFSC and FSSC-Clade 2 have three genes in the *MAT1-1* idiomorph (*MAT1-1-1*, *MAT1-1-2* and *MAT1-1-3*) and two genes in the *MAT1-2* idiomorph (*MAT1-2-1 MAT1-2-3*) (Martin et al. 2011, Hughes et al. 2014), while in the genome of the homothallic species of FSamSC five MAT genes are present (Martin et al. 2011). The *MAT1-2-3* gene was first described for several *Coccidioides* species, then a *MAT1-2-3* gene unrelated to that of *Coccidioides*, was identified from the *MAT1* locus in several Sordariomycetes, including several *Fusarium* species, *Cordyceps militaris*, and *Paecilomyces tenuipes* as well as *Clonostachys rosea*. Therefore, the *MAT1-2-3* locus from Sordariomycetes was renamed *MAT1-2-9* to avoid confusion with the homolog found in *Coccidioides* (Wilken et al. 2017).

Thus, in this study, we used genomic data to assess *MAT* locus differences in the structure and organization between homothallic and heterothallic species on FSSC-Clade 3, to compare amino acid profiles of FSSC-Clade 3 with those of FSSC-Clade 2 and FSamSC species, and to develop MAT primers for mating types identification of strains from FSSC-Clade 3. The sequence analysis and identification of the MAT loci and the understanding of the MAT loci evolution will give support to functional analyses of individual mating-type transcripts in FSSC species. Besides, the development of primers for mating-type diagnostic in PCR-based assays for strains from FSSC-Clade 3 species will help in one of the stages of the biological species identification process. Within the FSSC, the application of the concept of biological species contributes to the delimitation

of species, given that in this complex the separation of species through morphology is almost impossible.

Materials & Methods

Fungal strains

All strains used in this study were derived from single conidia and obtained from the Coleção Micológica de Lavras – CML, Universidade Federal de Lavras (http://www.dfp.ufla.br/cml). Fungi strains were maintained as spore suspensions in 15 % glycerol at -80ºC. The strains used in this study are listed in Table 1.

Self-fertility assay

Homothallism assays were established on 60 mm Petri dishes by two methods. First, the single-spore was grown on carrot agar (Klittich & Leslie 1988) at 25°C in complete darkness for one week. Next, 1.5 mL of 2.5% Tween 80 was spread on the surface and incubated at 20°C (Covert et al. 2007). Another approach consisted of transferring the strain to synthetic low nutrient agar (SNA), growing cultures for 12 h light/dark photoperiod in room temperature (Leslie & Summerell 2006). Plates were evaluated weekly for production of perithecia and exudation of ascospores. A cirrus of ascospores was collected from three perithecia of each fertile cross, suspended in sterile water, and spread on the surface of 2% water agar in a Petri dish. Plates were incubated overnight at 25°C in the dark and checked for ascospore germination (Lima et al. 2012). Sexual morph was characterized from cultures growing on SNA at 12 h light/dark photoperiod in room temperature. Dimensions of perithecia, asci, and ascospores were based on at least 20 independent measurements. The asexual stage produced by ascospores was characterized according to Aoki et al. (2003).

DNA extraction

Strains were cultivated in 100 mL of 2% malt extract broth medium (20 g of malt extract L^{-1}; Himedia Laboratories, Mumbai, India) and incubated for three days under agitation on a rotary shaker (100rpm). The biomass was harvested by filtration, macerated under liquid N_2, and total genomic DNA was extracted using the DNeasy Plant Mini Kit (Qiagen, Hilden, Germany) according to the manufacturer's protocol. DNA concentrations were estimated through visual inspection in agarose gel.

Analysis of MAT locus sequences

Paired-end libraries were prepared with the TruSeq Nano DNA LT Library Prep (Illumina) and sequenced on a HiSeq 2000 instrument at Macrogen (Macrogen Inc., Seoul, Rep. of Korea). The quality of sequencing reads (2 × 100 bp) was checked using FastQC 0.11.5 (https://www.bioinformatics.babraham.ac.uk/projects/fastqc/). Quality filtering was performed with Trimmomatic 0.35 (Bolger et al. 2014). Reads were assembled using SPAdes 3.10 (Bankevich et al. 2012). Genome assembly and annotation completeness was assessed with BUSCO 4.0.5 using Hypocreales as the reference dataset, which comprises 4494 single-copy orthologues (Simão et al. 2015). Gene prediction was performed using Augustus (Stanke et al. 2006). MAT genes were identified by blastp (Altschul et al. 1990) searches using predicted coding sequences of the assembled genomes to build a database, MAT query sequences were obtained from Genbank (Clark et al. 2016). E-value (1e-10) and identity (70%) thresholds were applied.

Diagnostic PCR for of the MAT locus in the Clade 3 from FSSC

Based on the alignment of *MAT1-1-1* sequences, the following oligonucleotide primers for *MAT1-1* locus amplification were designed: FsolMat1F (5'-CGCCCTCTGAATGCCTTTATG-3') and FsolMat1R (5'-GGAGTAGACCTTGGCAATGAGGGC-3'). Appropriate PCR conditions were also established. In addition, the oligonucleotide primers for *MAT1-2* locus determination,

described by Costa et al. (2016), were evaluated. PCRs were performed in 20 μL volumes containing about 20 ng of genomic DNA, 1x PCR buffer, 1.5 mM $MgCl^2$, 0.2 mM concentration of each deoxynucleoside triphosphate, 0.25 μM concentration of each primer, and 1 U of recombinant Taq DNA polymerase (Invitrogen Life Sciences, São Paulo, Brazil). For PCR optimization different annealing temperatures were used. Reactions were performed on a My Cycler thermocycler (Bio-Rad, Hercules, USA) using the following cycling conditions: 1 min at 94°C, followed by 35 cycles of 30 s at 94°C, 30 s at 55°C (*MAT1-1*) and 60°C (*MAT1-2*), and 90 s at 72°C, with a final extension step at 72°C for 1 min. PCR products were stained using Gel-Red stain (Biotium, Hayward, USA), separated by electrophoresis in 1% agarose gels, and visualized under UV light (312 nm).

Results

Self-fertility assay

In order to detect homothallism of FSSC-Clade 3 species, strains were subjected to a self-fertility *in vitro* assay. *Fusarium striatum* strain derived from single conidia and capable of producing perithecia on carrot agar or SNA was deemed as homothallic. Perithecia were first observed between 10-15 days after transfer of single conidia to media assessed. This sexual morph showed red perithecia solitary or aggregated in groups, superficial, nonstromatic on agar medium, globose to pyriform, and warty, 275–400 x 177–330 μm, non-papillate (Fig. 1A, B). Asci unitunicate, broadly cylindric clavate, 52–87.5 x 5–10 μm, containing eight ascopores often arranged obliquely (Fig. 1C). Ascospores hyaline, ellipsoidal, uniseptate and slightly constricted at the septum, 10–14 x 4.5–6 μm, exuded in cirrus (Fig. 1D). All ascospores germinated, forming hyphae in 24 to 32 h after transfer to water agar. Asexual stage produced by ascospores showed conidiogenous cells in long monophialides on SNA (27.5–167.5 x 2.5–5 μm) or sparsely branched, forming monophialides integrated in the apices (Fig. 1E, F). Sporodochia normally formed abundantly on PDA, but sparsely in some strains on SNA, showing conidial pustules pale yellow. Sporodochial conidiophores verticillate forming simple apical phialides, often with a conspicuous collarette at the tip (Fig. 1G, H). Sporodochial conidia typically falcate, with a rostrate apical cell and a protruding basal foot cell, mostly five-septate, occasionally three and four-septate, 3-septate (38–50 x 2.5–5 μm), 4-septate (30–43.5 x 3.5–5 μm) and 5-septate (38–62.5 x 4.5–6.5 μm) (Fig. 1I). Aerial conidia minute, oblong-ellipsoidal and reniform mostly 0-septate, occasionally 1-2 and three-septate: 0-septate (6–15 x 2.5–5 μm), 1-septate (12–24 x 3–6 μm), 2-septate (17.5–30 x 2.5–3.5 μm) and 3-septate (21–28 x 4–6 μm) (Fig. 1J). Chlamydospores formed abundantly in hyphae, mostly subglobose, and in chains, smooth to rough-walled (Fig. 1K). Colonies on PDA showing radial mycelial growth rates of 35 to 47 mm after four days at 25°C in the dark. Aerial mycelium generally abundant, loose to sometimes dense floccose, white, yellowish-white to pale yellow; conidial pustules yellowish-white, pale yellow or greyish-turquoise (Fig. 1L).

Analysis of MAT locus sequences

Genomic information holds the key to uncover the relatedness of genes and organisms. Thus, we performed whole genome sequencing of three distinct FSSC-Clade 3 species (Table 1). Genome assemblies were deposited on Genbank under BioProject: PRJNA511482 (*Fusarium paranaense* CML1830), PRJNA511486 (*Fusarium solani* f. sp. *piperis* CML2186) and PRJNA511487 (*Fusarium striatum* CML2203). The predicted protein sequences were searched against BUSCO Hypocreales dataset for *F. paranaense* CML1830, *F. solani* f. sp. *piperis* CML2186 and *F. striatum* CML2203, which showed 98.8%, 98.6% and 98.8% of the single-copy orthologs. After assembly and gene prediction, we screened protein sequences for MAT related domains.

The *F. paranaense*, *MAT1-1* idiomorph contained the *MAT1-1-1*, *MAT1-1-2* and *MAT1-1-3* genes, while the *F. solani* f. sp. *piperis*, *MAT1-2* idiomorph contained the genes *MAT1-2-1* and *MAT1-2-9* (Fig. 2). The *F. striatum* strain contained both idiomorphs: *MAT1-1* and *MAT1-2*.

Introns were not equally present across *MAT* idiomorphs. The *MAT-1-2-1* and *MAT1-2-9* genes of homothallic species (*F. striatum*) showed less intronic positions (Table 2).

Protein alignments of *F. striatum* MAT sequences were performed using *F. paranaense*, *F. solani* f. sp. *piperis*, and other publicly available MAT sequences at GenBank. MAT1-1-2 and MAT1-1-3 protein sequences presented higher identity among species sequences, indicating higher conservation, while lower identity values were observed for MAT1-1-1 protein, MAT1-2-1, and MAT1-2-9 protein sequences. The *MAT* genes encoded within FSSC-Clade 3 species are more similar when compared to FSSC-Clade 2 and *F. graminearum* of FSamSC (Table 3).

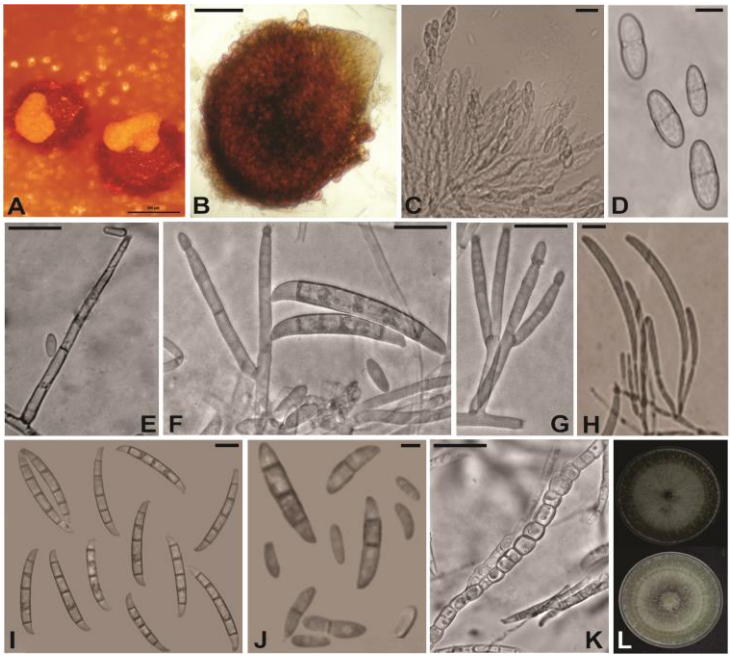

Fig. 1 – Morphological characters of sexual and asexual stage *Fusarium striatum*. A Perithecia on agar medium. B Perithecia. C Asci with ascospores. D Ascospores. E Aerial conidia formed on slender conidiophores arising from hyphae on the agar surface. F Branched conidiophores, forming monophialides integrated in the apices. G Sporodochium. H Sporodochium. I Sporodochial conidia. J Microconidia of aerial mycelium. K Chlamydospores. L Colony on PDA. Scale bars: B = 100 µm, C, J = 5 µm, D, H, I = 10 µm, E = 50 µm, F, G, K = 20 µm.

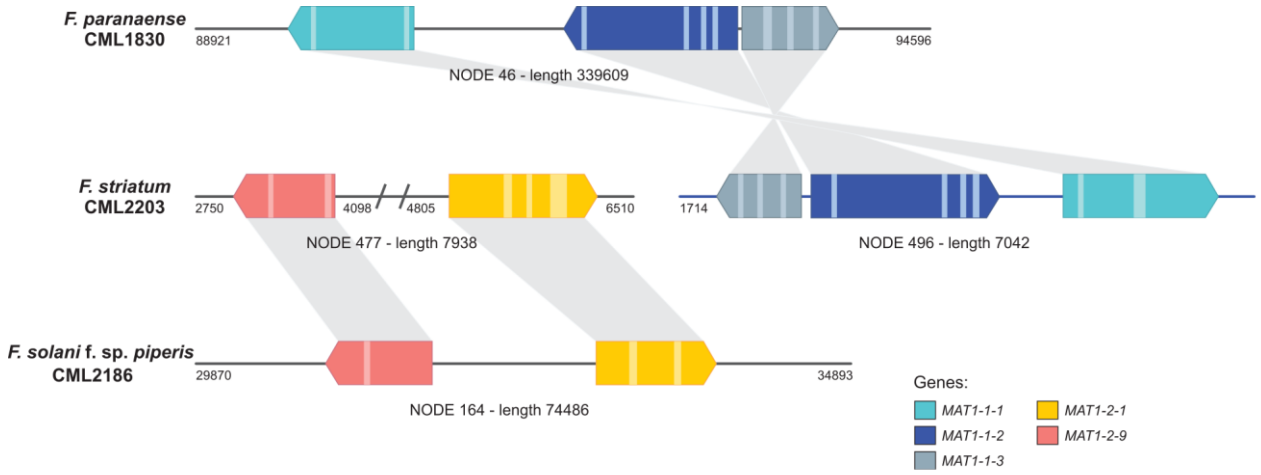

Fig. 2 – *MAT* genes annotated on FSSC sequenced genomes. *F. paranaense* CML1830 and *F. solani* f. sp. *piperis* CML2186 are heterothallic harboring *MAT1-1* and *MAT1-2* loci respectively. The homothallic *F. striatum* CML2203 bears both loci. *MAT-1-1* intron organization is highly conserved, which does not occur in *MAT-1-2*.

Table 1 Genome sequencing and gene prediction data of FSSC-Clade 3 species in this study

CML[a]	Species	Geographic origin[b]	Host	Genome size	No. of contigs	Largest contig	N50	No. of CDS	Reproductive style	Idiomorph
1830	*F. paranaense*	Cristalina, GO	*Glycine max*	53.367.726	523	2.263.987	480.828	16.536	Heterothallic	*MAT1-1*
2186	*F. solani* f. sp. *piperis*	Valença, BA	*Piper nigrum*	47.044.791	2118	1.041.978	238.575	13.831	Heterothallic	*MAT1-2*
2203	*F. striatum*	Ituberá, BA	*Piper nigrum*	60.495.063	1010	1.315.024	268.488	22.503	Homothallic	*MAT1-1/2*

[a] CML = Coleção Micológica de Lavras, Universidade Federal de Lavras, Lavras, MG, Brazil
[b] Brazilian state = BA: Bahia; GO: Goiás

Table 2 *MAT* gene sequences comparison of FSSC species

	Exons (bp)					Introns (bp)				
***Mat* genes**	***F. paranaense***	**f. sp. *piperis***	***F. striatum***	***F. tucumaniae* 34546[a]**	***F. tucumaniae* 31086[b]**	***F. paranaense***	**f. sp. *piperis***	***F. striatum***	***F. tucumaniae* 34546 [a]**	***F. tucumaniae* 31086 [b]**
MAT1-1-1	1,144	-	1,192	-	1068	47–46	-	44–91	-	47–56
MAT1-1-2	1,499	-	1,507	-	1023	48–47–45–45	-	45–45–47–53	-	45
MAT1-1-3	783	-	751	-	636	71–51–47	-	47–49–47	-	57–48–52
MAT1-2-1	-	841	1,142	690	-	-	55–50	64–44–129	47–51	-
MAT1-2-9	-	790	891	1,63	-	-	46	58–46	Not known	-

[a]Sequences of *F. tucumaniae* 34546: *MAT 1-2-1* (AIF79423) and *MAT 1-2-9* (AIF79423)
[b]Sequences of *F. tucumaniae* 31086: *MAT 1-1-1* (AIF79384), *MAT 1-1-2* (AIF79383) and *MAT 1-1-3* (AIF79382)

Table 3 MAT protein alignments using BLASTP. Qcov = Percentual of Query cover; ID = Percentual identity

F. striatum	***F. paranaense***		***F. tucumaniae* 31086**		***F. solani* f. sp. *pisi***		***F. graminearum* PH-1*a***		***F. solani* f. sp. *piperis***		***F. tucumaniae* 34546**	
MAT proteins	**Qcov**	**ID**	**Qcov**	**ID**	**Qcov**	**ID**	**Qcov**	**ID**	**Qcov**	**ID**	**Qcov**	**ID**
MAT 1-1	99	53,46	97	46,51	70	50,59	95	30,29				
MAT 1-2	100	92,68	75	57,58	100	89,47	88	32,2				
MAT 1-3	100	86,57	94	58,42	74	83,12	80	40,12				
MAT 2-1							68	51,13	72	68,35	94	64,5
MAT 2-9							37	47,56	85	60,36	*	*

[a]*MAT 1-1-1* and *1-2-1* sequences of *F. graminearum* CBS138561 (KT855196) and *MAT 1-2-9* of PH1 (XP011319817)
* *F. tucumaniae* strain NRRL 34546 *MAT 1-2-9* sequence not available

MAT loci architecture across FSSC and *Fusarium* species

The order and orientation of the *MAT* locus (*MAT1-1-1, MAT1-1-2, MAT1-1-3*) in *F. paranaense* was inverted in comparison to other *Fusarium* species and species from FSSC-Clade 2 (*MAT1-1-3, MAT1-1-2, MAT1-1-1*) (Fig. 3). Also *F. striatum* and *F. solani* f. sp. *piperis* exhibited different order and orientation (*MAT1-2-9, MAT1-2-1*) in comparison to *F. tucumaniae, F. brasiliense, F. crassistipitatum, F. verticillioides* and *F. graminearum* (*MAT1-2-1, MAT1-2-9*) (Fig. 3).

PCR-based mating type identification in FSSC-Clade 3

The primers developed herein along with the ones developed by Costa et al. (2016) were efficient for the determination of mating idiomorphs in all strains evaluated. The amplicon sizes of *MAT1-1* and *MAT1-2* were 200 and 212 bp, respectively (Fig. 4). The amplicons were sequenced to confirm the specificity of the *MAT* idiomorphs amplification.

Discussion

This work presents information on the structural organization of the mating type (*MAT*) locus in the FSSC-Clade 3 species. Using genomic data, we verified that the *MAT* locus in *F. paranaense* (*MAT1-1*), *F. solani* f. sp. *piperis* (*MAT1-2*) and *F. striatum* (*MAT1-1*/*MAT1-2*) shares conserved features with other *Fusarium* species. However, Clade 3 strains showed differential order and orientation of *MAT* locus, compared to other *Fusarium* species and FSSC-Clade 2, suggesting a segmental inversion.

F. striatum is a homothallic species, which showed five *MAT* genes, being the *MAT1-1* locus adjacent and downstream to the *MAT1-2*; the same pattern can be observed in *F. graminearum*. The *MAT1-1* locus exhibits a conserved orientation across *Fusarium* species, *MAT1-1-3*, *MAT1-1-2* and *MAT1-1-1* genes. Even though the same genes can be found in *F. paranaense*, their order is inverted with respect to other species (Fig. 2). Locus inversion is the product of the mediation of loop formation and crossing over caused by recombination between MAT flanking motifs (Hurles & Lupski 2006, Flores et al. 2007, Chitrampalam et al. 2013). Similar scenario has been described in other ascomycetes including *Cochliobolus kusanoi* (Yun et al. 1999), *Stemphylium* spp. (Inderbitzin et al. 2005), *Peyronellaea* spp. (Woudenberg et al. 2012). According to Chitrampalam et al. (2013), in *Sclerotinia sclerotiorum* inverted MAT loci occur in every meiotic generation, and these recurrent inversions result in mixed cell populations. Sexual reproduction in *F. paranaense* can lead to process homologue recombination, resulting in some individuals of progeny having inverted *MAT* genes. *MAT1-1-1* gene of *F. paranaense* encodes a 339 amino acid (aa) protein, *F. striatum* 351 aa, *F. tucumaniae* 355 aa (AIF79384.1), *F. solani* f. sp. *pisi* 225 aa (XP_003052790.1). Despite those discrepancies, the lack of these codons in genes did not affect the process of sexual reproduction in *F. paranaense* (Costa et al. 2016) and *F. solani* f. sp. *pisi* (Matuo & Snyder 1973). Based on MAT deletion in *Didymella zeae-maydis*, the homothallic strains showing *MAT1-1-1*; *MAT1-2-1*, were self-sterile, showing that MAT1-2-1 protein is essential to sexual reproduction. However, when upon *MAT1-1-1* knockout; *MAT1-2-1* strains produced some ascospores. This result suggested that the function of the *MAT1-1-1* HMG domain (alpha box domain) might be redundant with one or more of the other HMG proteins (Yun et al. 2013). The *MAT1-1-1* and *MAT1-2-1* have a close relationship and could have originated from a single ancestral HMG gene (Ge et al. 2019). Moreover, *MAT* gene deletion experiments in a self-fertile *Sordaria macrospora* revealed that *Smt A-1* (comparable to *MAT1-1-1*) was dispensable for fruiting body formation and it did not affect sexual reproduction (Klix et al. 2010).

The *MAT1-1-2* gene of *F. paranaense* and *F. striatum* encode a 436 aa and 437 aa proteins respectively. In *F. solani* f. sp. *pisi*, this gene product also has 436 aa length (XP_003052789.1). MAT1-1-2 protein sequences from related species exhibits the same overall sequence size, namely: *F. gramineraum* has 463 aa (XP_011319816.1), *F. sporotrichioides* 460 aa (RGP65486.1), *F. subglutinans* 438 aa (AEP03806.1), while in *F. tucumaniae* this protein has only 355 aa (AIF79383.1) indicating that deletion events may have occurred in this locus, but it did not affect

the process of sexual reproduction of this species. *MAT1-1-2* in homothallics, *F. graminearum* and *S. macrospora*, and in heterothallic *Podospora anserina* has a crucial role in controlling the development and maturation of the fruiting body (Klix et al. 2010, Kim et al. 2012). This *MAT* locus interacts with all other MAT transcription factors, suggesting that they may form a protein complex during sexual reproduction (Zheng et al. 2013).

The *MAT1-1-3* gene encodes proteins of 203 aa in *F. paranaense*, 201 aa in *F. striatum*, 211 aa in *F. tucumaniae* (AIF79382.1) and 150 aa in *F. solani* f. sp. *pisi* (XP_003053292.1). In *F. graminearum*, *MAT1-1-3* mutant retained the capacity to produce barren perithecia, this showed that its sexual development was blocked at perithecia maturation stage. These results indicate the essential role of this gene to sexual development (Kim et al. 2012). However, the mating-type protein SMTA-3 (comparable to MAT1-1-3) of *S. macrospora* had no function in its sexual reproduction process (Klix et al. 2010).

F. striatum (homothallic) and *F. solani* f. sp. *piperis* (heterothallic) contain the *MAT1-2* genes, in different orientation of other *Fusarium* species and species from FSSC-Clade 2 (Fig. 3). *MAT1-2-1* gene encodes a 300 aa protein in *F. striatum*, while in *F. solani* f. sp. *piperis* 244 aa, *F. tucumaniae* 229 aa (AIF79417.1) and *F. graminearum* 245 aa (ABE98344.1). Despite differences in *MAT-1-2* sequence length among species, this did not prevent sexual reproduction. Single conidia of *F. striatum* strain produced perithecia on carrot agar and SNA with exudation of ascospores in a cirrus and ascospores germinated and formed hyphae that produced conidia (Fig. 1). *F. solani* f. sp. *piperis* strains are also able to outcross, as reported by Vaz et al. (2012), meaning that additional codons do not hinder the process of sexual reproduction.

The *MAT1-2-9* was found in the genome of *F. striatum* and *F. solani* f. sp. *piperis*. This locus was also reported in: the homothallic *S. macrospora* (Klix et al. 2010), the homothallic *F. graminearum*, strains Z3643 and Z3639 (Kim et al. 2012), heterothallic *F. verticillioides* (Martin et al. 2011) and in FSSC-Clade 2 species (Hughes et al. 2014). However, gene deletion analysis showed that *MAT1-2-9* was not essential for sexual reproduction in *F. graminearum* (Kim et al. 2012). Moreover, *F. graminearum* PH-1, wild type strain did not have this *MAT* gene (Zheng et al. 2013).

Study of *MAT* loci structure provides understanding of the evolutionary plasticity originating from multiple mutations, duplications, insertion and deletion events, along with rearrangements, which modulate speciation (Amselem et al. 2011, Chitrampalam et al. 2013, Yun et al. 2013, Hughes et al. 2014). Although MAT proteins are considered regulators of sexual development, their functions are not conserved among filamentous ascomycetes (Kim et al. 2012). To date, there are no functional analyses of individual mating-type transcripts in FSSC species, therefore, functional studies are needed.

Additionality in this study, primers for mating type determination for FSSC species were designed. These primers are important to differentiate among strains that can be used in sexual crosses in the laboratory for species identification. The biological concept can be applied to aid in the identification of morphologically similar species, as is the case of FSSC. The PCR method based on degenerate oligonucleotide primers was developed for the mating type assessment of different *Fusarium* species, including *F. solani sensu lato* (Kerényi et al. 2004), and multiplex-PCR was used for MAT detected both idiomorphs in FSSC-Clade 2 (Hughes et al. 2014). The primers developed herein will be useful in the determination of the *MAT1-1* as well as primers of the *MAT1-2* (Costa et al. 2016) for idiomorphs determination of strains from FSSC-Clade 3 species. Thus, allowing sexual crosses to be performed only between opposing strains, *MAT1-1* and *MAT1-2* idiomorphs; reducing time, culture media and area usage. Moreover, mating type diagnostic in PCR-based can avoid the laborious work of making numerous crosses, testing an array of conditions of temperature, luminosity, nutrition and receptivity of the mycelium (Leslie & Summerell 2006).

The findings reported in this work represent “the tip of the iceberg” in the comprehension of structure and evolution of *mating type* locus in the FSSC. Although FSSC-Clade 3 species have exhibited dissimilar *MAT* locus organization and amino acid profile from other *Fusarium* species

and FSSC-Clade 2, these differences did not affect sexual reproduction. Moreover, the availability of PCR protocol for *MAT* idiomorphs identification will facilitate the recognition of potentially sexually compatible strains from FSSC-Clade 3.

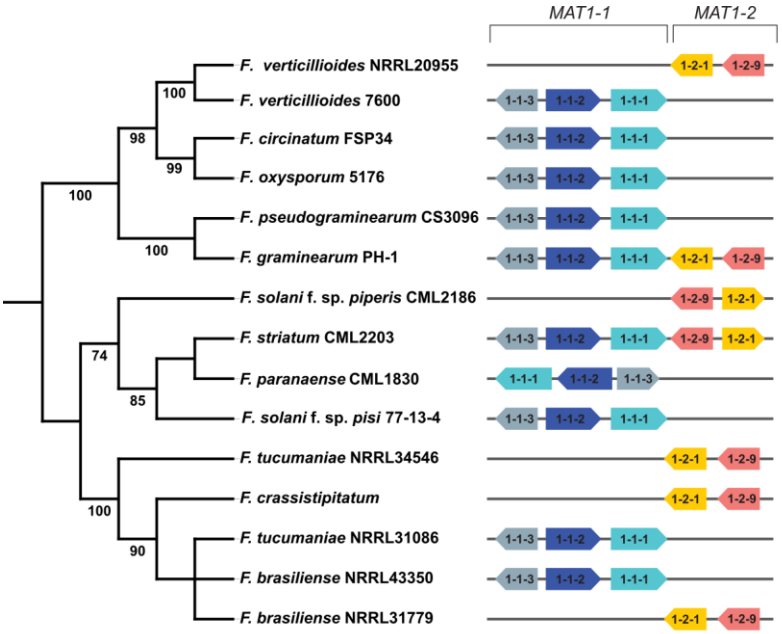

Fig. 3 – Comparison of the *MAT* loci in *Fusarium* genomes. The cladogram on the left shows the phylogenetic relationships based on *RPB2* sequence inferred by maximum likelihood. *MAT* genes and orientation are shown accordingly.

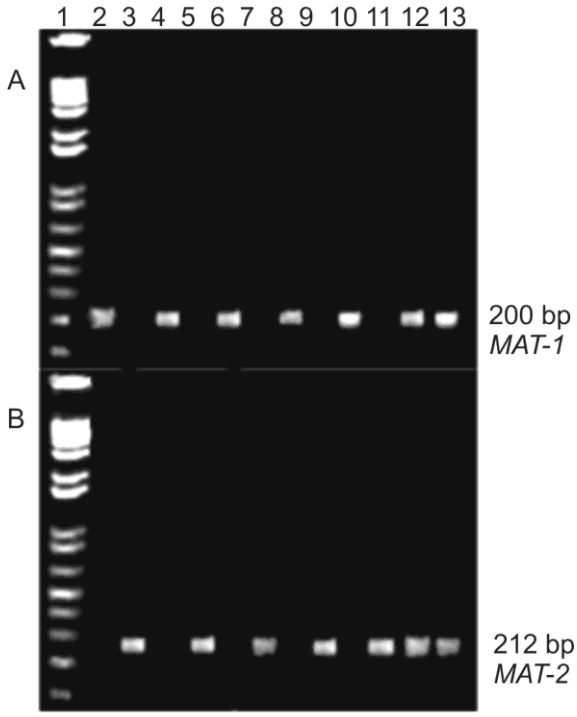

Fig. 4 – PCR amplification of mating type-specific boxes from species in the Clade 3 from FSSC. A Amplification of *MAT1-1*-specific by using the FsolMat1F and FsolMat1R primers. B Amplification of *MAT1-2*-specific by using the FsolMat2F and FsolMat2R primers. Lane 1, 100-bp DNA ladder; Lanes 2-11 Heterothallic strains. Lanes 2-3: *F. paranaense* CML1830 (*MAT1-1*), CML860 (*MAT1-2*); Lanes 4-5: *F. solani* f. sp. *piperis*, CML2187 (*MAT1-1*), CML 2186 (*MAT1-*

2); Lanes 6-7: *F. solani* f. sp. *batatas*, CML2350 (*MAT1-1*), CML 1894 = NRRL22400 (*MAT1-2*); Lanes 8-9: *F. solani* f. sp. *xanthoxyli*, CML 2230 = NRRL22277 (*MAT1-1*), CML1884 = NRRL22163 (*MAT1-2*); Lanes 10-11: *F. petroliphilum*, CML1895 = NRRL22141 (*MAT1-1*), CML1883 = NRRL 22142 (*MAT1-2*); Lanes 12-13 Homothallic strains. Lane 12: *Fusarium* sp. CML576, Lane 13: *F. striatum* CML2183.

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