Curr Genet (2008) 53:299–311
DOI 10.1007/s00294-008-0186-x

RESEARCH ARTICLE

Construction of a genetic linkage map of the fungal pathogen of banana *Mycosphaerella fijiensis*, causal agent of black leaf streak disease

Gilberto Manzo-Sánchez · Marie-Françoise Zapater · Francisco Luna-Martínez · Laura Conde-Ferráez · Jean Carlier · Andrew James-Kay · June Simpson

Received: 20 September 2007 / Revised: 29 February 2008 / Accepted: 4 March 2008 / Published online: 26 March 2008

Abstract A genetic linkage map of the fungal plant pathogen *Mycosphaerella fijiensis*, causal agent of black leaf streak disease of banana was developed. A cross between the isolates CIRAD86 (from Cameroon) and CIRAD139A (from Colombia) was analyzed using molecular markers and the *MAT* locus. The genetic linkage map consists of 298 AFLP and 16 SSR markers with 23 linkage groups, containing five or more markers, covering 1,879 cM. Markers are separated on average by around 5.9 cM. The *MAT* locus was shown to segregate in a 1:1 ratio but could not be successfully mapped. An estimate of the relation between physical size and genetic distance was approximately 39.0 kb/cM. The estimated total haploid genome size was calculated using the genetic mapping data at 4,298.2 cM. This is the first genetic linkage map reported for this important foliar pathogen of banana. The great utility of the map will be for anchoring contigs in the genome sequence, evolutionary studies in comparison with other fungi, to identify quantitative trait loci (QTLs) associated with aggressiveness or oxidative stress resistance and with the recently available genome sequence, for positional cloning.

Keywords Ascomycete · *Mycosphaerella fijiensis* · Black leaf streak disease of banana · Genetic mapping · AFLP markers · SSR markers

Communicated by J. Heitman.

G. Manzo-Sánchez · L. Conde-Ferráez · A. James-Kay (✉)
Unidad de Biotecnología, Centro de Investigación Científica de Yucatán, Calle 43 No. 130. Colonia Chuburná de Hidalgo, 97200 Mérida, Yucatán, Mexico
e-mail: andyj007@cicy.mx

M.-F. Zapater · J. Carlier
UMR BGPI, CIRAD, TA A 54/K, 34398 Montpellier CEDEX 05, France

F. Luna-Martínez · J. Simpson
Departamento de Ingeniería Genética de Plantas, Centro de Investigación y de Estudios Avanzados del Instituto Politécnico Nacional, Unidad Irapuato, Km 9.6 del Libt. Nt., Carr. Irapuato-León, PO box 629, 36821 Irapuato, Guanajuato, Mexico

Present Address:
G. Manzo-Sánchez
Laboratorio de Biotecnología, Facultad de Ciencias Biológicas y Agropecuarias, Universidad de Colima, km 40 Autopista Colima-Manzanillo, 36, km 40 Autopista Colima-Manzanillo, Tecomán 28100, Colima, Mexico

Introduction

The haploid, hemibiotrophic ascomycete fungus *Mycosphaerella fijiensis* Morelet [anamorph *Paracercospora fijiensis* (Morelet) Deighton] causes black leaf streak disease (BLSD) (commonly known as black Sigatoka), the most economically important disease of banana (*Musa* spp.) in tropical and subtropical areas (Marín et al. 2003). BLSD causes leaf necrosis and plant defoliation, resulting in yield losses and poor-quality fruit, which is prone to premature ripening. Fungicide control of the disease can contribute up to 40% of the direct costs of production, and these costs are beyond the means of many smallholders (Marín et al. 2003). *M fijiensis* is a heterothallic, filamentous fungus, which can be easily cultured on both liquid and solid media; in addition, sexual crosses can be made under laboratory conditions (Mourichon and Zapater 1990). Both anamorphs and teleomorphs are present on infected leaves. Ascospores, produced during the sexual stage, play an important epidemiological role through wind-borne dispersal.

The analysis of *M. fijiensis* population structure has been undertaken using different types of molecular markers (Carlier et al. 1996; Neu et al. 1999; Rivas et al. 2004). Genetic diversity in populations from other geographical regions outside South East Asia (South America, Africa and the Pacific Islands) have been considered to indicate founder events, since alleles in common with populations from Papua New Guinea and the Philippines were identified but lower levels of genetic diversity in general were also observed (Carlier et al. 1996). Analysis of gene flow and dispersal processes of *M. fijiensis* at the continental scale from population structure analysis in recently invaded regions was carried out by Rivas et al. (2004), who showed that founder effects accompanied the recent invasion of *M. fijiensis* in the African and Latin America-Caribbean regions, suggesting stochastic spread of the disease at the continental level.

In order to study the location and inheritance of important genes of interest such as those for avirulence, toxins, intersterility, vegetative compatibility, host specificity and the mating type, genetic linkage maps of fungal genomes have been constructed using restriction fragment length polymorphism (RFLP), random amplified polymorphic DNA (RAPD) amplified fragment length polymorphisms (AFLP), simple sequence repeat (SSR) markers and diversity array technology (DArT). Genetic linkage maps have been constructed in fungal Ascomycetes (Cozijnsen et al. 2000; Jurgenson et al. 2002; Zhong et al. 2002; Pedersen et al. 2002; Luo et al. 2005; Kubisiak and Milgroom 2006; Luna-Martínez et al. 2007), in fungal Basidiomycetes (Muraguchi et al. 2003; Lind et al. 2005; Forche et al. 2000; Marra et al. 2004) and in Oomycetes (May et al. 2002; Van der Lee et al. 1997; Sicard et al. 2003).

Knowledge of the genome organization of *M. fijiensis* should provide an insight into the high phenotypic variability observed among isolates, specifically, the variability in pathogenicity and aggressiveness (Fullerton and Olsen 1995). Electrophoretic karyotyping of 10 Mexican isolates resolved between 8 and 13 chromosomes giving a genome size ranging from 27 to 35 Mb (Rodríguez-García et al. 2006). At present the only genetic linkage map available within the Mycosphaerellaceae is from *Mycosphaerella graminicola* (Kema et al. 2002; Goodwin et al. 2007). Both *M. fijiensis* (http://genome.jgi-psf.org/Mycfi1/Mycfi1.info.html) and *M. graminicola* (http://jgi-psf.org/Mycgr1/Mycgr1.info.html) were selected for whole-genome sequencing within the DOE-JGI Community Sequencing Program. The sequence of *M. fijiensis* isolate CIRAD86 became available in August 2007 and is one of the parents of the mapping population reported in the present work. The genetic map of *M. fijiensis* will be useful for anchoring contigs for sequence assembly, for map-based cloning and for the identification of genomic regions of interest for comparative genomic studies with other species. The objectives of this study were (1) to perform crosses under in vitro conditions between isolates of *M. fijiensis* from different continents and from different cultivars, for the development of F_1 populations, (2) to analyze an F_1 population with AFLP, SSR markers and the *MAT* locus in order to generate a genetic linkage map of *M. fijiensis* and (3) to estimate the genome size of this organism based on the mapping data.

Materials and methods

Fungal isolates and growth conditions for crosses

Twelve isolates of *M. fijiensis* from different countries and cultivars of bananas were used as parentals (Table 1). Isolates from different continents and with differing levels of aggressiveness were used in order to maximize the genetic variation between parental isolates. Crosses were made following the method previously described by Mourichon and Zapater (1990). To constitute F1 populations, all ascospores were isolated from different pseudothecia and thus were from distinct meioses. The F_1 progeny population chosen for mapping was derived from isolates CIRAD86 (from Cameroon, isolated in 1988, *MAT1-1*; this same isolate has been used for genome sequencing) and CIRAD139A (from Colombia, isolated in 1990, *MAT1-2*). The F_1 progeny population consisting of 61 isolates was used for genetic map development. The mating type of both parents and the 61 F_1 isolates was determined by PCR (polymerase chain reaction) with specific primers based on the sequence of the mating type idiomorphs (Conde-Ferráez et al. 2007).

DNA extraction

The parental isolates (CIRAD139A and CIRAD 86) and F_1 progeny population were grown in PDA medium for 8–12 days at 25°C; the mycelia were harvested and dried for 1 day in an oven at 50°C. Total genomic DNA was extracted following a cetyltrimethyl ammonium bromide (CTAB)-based procedure (Carlier et al. 1996).

AFLP analysis

Restriction, ligation, and preamplification reactions were performed essentially as described in the original AFLP protocol of Vos et al. (1995). A total of 48 *EcoRI* + 2/*MseI* + 2 primer combinations (Table 2) were analyzed. The AFLP genotyping protocols using primer combinations *EcoR1* + AX/M + YX (where X = A, T, G, or C and Y = A, T or G) were carried out essentially as described by Vos et al. (1995). All *Mse1* + 2 selective primers were synthesized by

Table 1 Level of mating between 13 single ascospore isolates of *Mycosphaerella fijiensis*

Isolate	Mating type	Country of origin	Host	Aggressiveness	Successfully crossed with	Production of pseudothecia
CIRAD86	MAT 1-1	Cameroon	ND	High	CIRAD139a	A
					CIRAD1229	C
					CIRAD1231	B
CIRAD89	MAT 1-2	Cameroon	ND	High	CIRAD138	B
					CIRAD1233	C
CIRADG1	MAT 1-2	Philippines	ND	Low	CIRAD138	A
					CIRAD1233	A
CIRAD1240	MAT 1-1	Cameroon	Yangambi km 5	ND	CIRAD1229	C
					CIRAD1231	B
CIRAD139a	MAT 1-2	Colombia	French plantain	Medium	CIRAD86	A
CIRAD1229	MAT 1-2	Mexico	Grand Naine	ND	CIRAD86	C
CIRAD1231	MAT 1-2	Mexico	Grand Naine	ND	CIRAD86	B
					CIRAD1240	B
CIRAD138	MAT 1-1	Colombia	Figue Suc	Medium	CIRAD89	B
					CIRADG1	A
CIRAD1233	MAT 1-1	Mexico	Grand Naine	ND	CIRAD89	C
					CIRADG1	A

A high production of pseudothecia, *B* medium production of pseudothecia, *C* low production of pseudothecia, *ND* not determined

SIGMA GENOSYS (The Woodlands, TX) and IRDye™-700 and IRDye™-800 labeled *Eco R1* + 2 primers were purchased from LI-COR (Lincoln, NE). AFLP fragments were resolved on gels prepared with 6% KB Plus Polyacrylamide Gel Matrix (LI-COR, Lincoln, NE). Electrophoresis and detection of the AFLP fragments were performed on a 4200 LI-COR IR2 automated DNA sequencer and gel AFLP images were scored using SAGA^{MX} software (LI-COR, Lincoln, NE) and saved as TIF files for further analysis.

SSR markers

Twenty-seven SSR markers were used (Table 3). Seven and 18 were published by Neu et al. (1999) and Zapater et al. (2008), respectively. Two other markers *MfSSR058.3* and *MfSSR175.2* were developed in this study (GenBank Accession number EU200694 and EU200695, respectively). The corresponding sequences overlap the locus *MfSSR175* previously published by Neu et al. (1999). Characteristics of all the markers used are summarized in Table 3. Isolates were genotyped with all these SSR markers using an automated infrared fluorescence technology of a LI-COR IR2 sequencer (LI-COR, Lincoln, NE) as described in Zapater et al. (2008).

Linkage analysis and genetic map construction

The gel images (for AFLP and SSR markers) were scored using a binary scoring system that recorded the presence and absence of bands as 1 and 0, respectively. AFLP markers were denominated by the primer combination used for the amplification and the approximated length of the generated fragment. The length of each AFLP marker was estimated on the basis of a fluorescently labeled 700-bp ladder (LI-COR, Lincoln, NE) and data analysis was performed using SAGA^{MX} (LI-COR, Lincoln, NE) software. Monomorphic markers common to both parents were not scored. For the AFLP markers, the prefix A or B indicates the origin of each marker, the former corresponds to parent CIRAD86, while the latter prefix corresponds to parent CIRAD139A; this prefix is followed by the primer combination used and the molecular size (in base pairs). For example, BEAAMAG372 represents an AFLP marker originating from the isolate parent CIRAD139A and amplified by primer pairs *Eco R1* + AA and *Mse1* + AG, and showing a molecular size of 372 bp. The SSR markers are identified by *Mf*SSR followed by the locus number and finally by the letter a or b, corresponding to Zapater et al. (2008) or Neu et al. (1999), respectively. Segregation ratios of all markers were analyzed with JoinMap 3.0 software (Van Ooijen and Voorrips 2001). Map construction was carried out essentially as described in Luna-Martínez et al. (2007).

The population type was coded as HAP, i.e., a haploid population derived from a heterozygous diploid parent. Markers with segregation ratios significantly different from the expected 1:1 ratio ($\chi^2 \leq 7.7$; $P < 0.01$) were set aside because initial mapping analysis showed that they may cause erroneous grouping of unlinked markers. Linkage analysis was performed on the remaining markers with $P \geq 0.01$. Initial assignment to linkage groups (LG) was based on the logarithm of the odds (LOD) ratio for each possible marker pair. In this study we used LOD values of 3.0–9.0, but the final assembly of LG was determined by using a minimum LOD value of 3.0 with a maximum recombination fraction of 0.4 (LOD $\geq$ 0.8) and a threshold

Table 2 Segregation and mapping data for AFLP markers generated for the construction of the genetic linkage map of *Mycosphaerella fijiensis*

AFLP primers combination		Polymorphic bands obtained			Distorted markers[a]	Unlinked markers[b]	Mapped markers		
		CIRAD86	CIRAD139	Total			CIRAD86	CIRAD139	Total
1	E + AA/M + AA	7	8	15	0	10	2	3	5
2	E + AA/M + AC	8	7	15	2	9	3	1	4
3	E + AA/M + AG	16	7	23	3	12	6	2	8
4	E + AA/M + AT	4	2	6	1	3	2	0	2
5	E + AA/M + GA	5	3	8	1	3	2	2	4
6	E + AA/M + GC	13	6	19	0	7	8	4	12
7	E + AA/M + GG	10	6	16	1	4	7	4	11
8	E + AA/M + GT	8	6	14	3	5	4	2	6
9	E + AA/M + TA	6	6	12	3	2	4	3	7
10	E + AA/M + TC	8	9	17	1	4	5	7	12
11	E + AA/M + TG	1	15	16	2	6	1	7	8
12	E + AA/M + TT	12	6	18	1	3	9	5	14
13	E + AC/M + AA	3	6	9	0	7	0	2	2
14	E + AC/M + AC	4	6	10	0	4	2	4	6
15	E + AC/M + AG	4	2	6	0	5	1	0	1
16	E + AC/M + AT	2	3	5	0	5	0	0	0
17	E + AC/M + GA	6	3	9	0	3	4	2	6
18	E + AC/M + GC	2	11	13	1	4	0	8	8
19	E + AC/M + GG	12	6	18	1	5	8	4	12
20	E + AC/M + GT	6	9	15	1	8	2	4	6
21	E + AC/M + TA	12	6	18	1	4	9	4	13
22	E + AC/M + TC	10	7	17	0	7	7	3	10
23	E + AC/M + TG	7	7	14	0	3	6	5	11
24	E + AC/M + TT	9	6	15	1	6	5	3	8
25	E + AG/M + AA	5	8	13	2	7	1	3	4
26	E + AG/M + AC	7	5	12	0	5	4	3	7
27	E + AG/M + AG	4	6	10	0	3	3	4	7
28	E + AG/M + AT	2	4	6	0	4	1	1	2
29	E + AG/M + GA	6	4	10	2	3	3	2	5
30	E + AG/M + GC	10	7	17	2	11	3	1	4
31	E + AG/M + GG	8	7	15	2	7	2	4	6
32	E + AG/M + GT	7	4	11	1	2	5	3	8
33	E + AG/M + TA	7	9	16	1	8	3	4	7
34	E + AG/M + TC	10	8	18	1	6	4	7	11
35	E + AG/M + TG	5	6	11	1	6	1	3	4
36	E + AG/M + TT	7	9	16	0	4	5	7	12
37	E + AT/M + AA	5	9	14	1	5	3	5	8
38	E + AT/M + AC	4	10	14	1	1	4	8	12
39	E + AT/M + AG	3	4	7	0	1	3	3	6
40	E + AT/M + AT	8	5	13	0	3	5	5	10
41	E + AT/M + GA	11	4	15	1	9	3	2	5
42	E + AT/M + GC	9	9	18	0	9	3	6	9
43	E + AT/M + GG	12	9	21	0	3	9	9	18
44	E + AT/M + GT	4	3	7	0	3	3	1	4
45	E + AT/M + TA	15	6	21	3	6	9	3	12
46	E + AT/M + TC	12	15	27	4	11	4	8	12

Table 2 continued

AFLP primers combination		Polymorphic bands obtained			Distorted markers[a]	Unlinked markers[b]	Mapped markers		
		CIRAD86	CIRAD139	Total			CIRAD86	CIRAD139	Total
47	E + AT/M + TG	5	7	12	2	1	4	5	9
48	E + AT/M + TT	5	6	11	1	2	3	5	8
Total		346	317	663	48	249	185	181	366
Percent		52.2	47.8	100			50.5	49.5	100
Average				13.8	1.0	5.2			7.6

[a] Number of markers from the primer combination with a segregation distortion deviating from the assumed 1:1 ratio ($\chi^2 \geq 7.7$, $P \geq 0.01$)

[b] Number of markers from the primer combination not linking to any other marker

of 5.0 for the removal of loci with respect to jumps in goodness-of-fit during the calculation of a linear order of loci within a linkage group. The Kosambi mapping function was used to compute recombination distance in centimorgans (cM). Genome length and coverage was determined essentially as described in Luna-Martínez et al. (2007).

Results

Mating between isolates

Among 38 pairs evaluated for crosses under in vitro conditions, only nine (Table 1) produced mature pseudothecia within 25–35 days following the confrontation of the potential parental strains. Each of the pairs produced different numbers of pseudothecia, indicating variable levels of fertility. Of the nine fertile pairs, the following three produced the greatest number of pseudothecia: CIRAD86 (from Cameroon) × CIRAD139A (from Colombia); CIRAD138 (from Colombia) × CIRADG1 (from Philippines); and CIRAD1233 (from Mexico) × CIRADG1 (from Philippines). Isolates CIRAD282, CIRAD 1241, CIRAD1239, CIRAD1231 and CIRAD1235 were completely sterile (produced no pseudothecia) when crossed with either mating type *MAT1-1* or *MAT1-2* (Table 1). The cross between isolates CIRAD139A × CIRAD86 was chosen to constitute a first F_1 population since isolate CIRAD86 was used recently for genome sequencing. In total, 61 progeny were obtained from this cross.

AFLP and SSR analysis of the mapping population

Forty-eight AFLP primer combinations produced in total 663 AFLP bands, which were polymorphic between the parents; 346 were present in the parent CIRAD86, and 317 fragments were present in the parent CIRAD139A (Table 2). The number of polymorphisms between the parents ranged from 5 (for primer pairs *Eco R1* + AC and *Mse1* + AT) to 27 (for primer pairs *Eco R1* + AT and *Mse1* + TC) with an average of 13.8 polymorphic markers per primer pair (Table 2). There was no evidence of a relationship between the composition of the selective nucleotides and the number of polymorphic fragments obtained. Of the 27 SSR markers that were analyzed, all were polymorphic between the parental isolates CIRAD86 and CIRAD139A, and were used for genotyping the progeny and linkage analysis.

Linkage analysis and map construction

A total of 691 loci were analyzed (663 AFLP markers, 27 SSR markers and the *MAT* locus) using the JoinMap 3.0 software (Van Ooijen and Voorrips 2001). Forty-eight AFLP markers (6.8%) showed significant distortion in segregation from the expected 1:1 ratio ($\chi^2 > 7.7$; $P < 0.01$) and they were excluded from the linkage analysis. Of these markers, 32 were identified in CIRAD86 and 16 in CIRAD139A, therefore 643 (93%) markers had $P \geq 0.01$ (615 AFLP, 27 SSR markers and the *MAT* locus) and were included in the linkage analysis. Of these 643 markers, 10 were significantly different from a 1:1 ratio at $P = 0.01$, 48 markers were significantly different from a 1:1 ratio at $P = 0.05$, and 585 markers were not significantly different from a 1:1 segregation ratio. Nevertheless, 258 (40%) markers with $P \geq 0.01$, including the *MAT* locus did not show linkage and only 385 markers (19 SSR and 366 AFLP) showed linkage with another marker (60%).

A total of 298 AFLP markers and 16 SSR markers were assigned to 23 LGs containing five or more markers with minimum LOD values of 3.0 (Tables 2, 3). The map covered a total length of 1,879 cM (Fig. 1) with an average distance between markers of 5.9 cM. The largest LG had 31 markers and was 192 cM in size (LG 1), and the smallest LG contained seven markers and was 35 cM in size (LG 23). Analysis of the genetic linkage map showed that 161

Table 3 Characteristics and segregation of the 27 SSR markers used for the construction of the genetic linkage map of *Mycosphaerella fijiensis*

Number of marker	Linkage group	Position in the linkage group	Locus name	A:B[a]	Value of P[b]	Forward primer	Reverse primer	T[c] (°C)	Expected size	Source
1	1	44	*Mf*SSR329a	33:28	0.522	GTCGATAGAGCCAGCGGTAG	GGGAAGGGGAATGGAATTTA	55	184	1
2	1	52	*Mf*SSR314a	29:32	0.700	CACTGTGCCAAAAGAAACCA	AGTGACTTGATGCGGATGTG	55	142	1
3	1	63	*Mf*SSR137b	30:31	0.898	GGCTCGAAGTGGACTAGCAC	CTGGTCGAGGGTCGGG	55	243	2
4	2	75	*Mf*SSR359a	33:28	0.522	ACGTAACCACATCGGCCA	GTCCAGTGCCAAGGCTTG	55	100	1
5	2	82	*Mf*SSR358a	31:29	0.796	GGAAGTGCCAAGAAAAGG	GACAGTGAGGAGATGAGC	55	154	1
6	2	93	*Mf*SSR175.2a	32:27	0.515	TGCCACCAAGATCTCACTTG	CAGGTCCATAGGCATGGTG	55	298	This study
7	2	111	*Mf*SSR354a	34:27	0.370	AGACCCATTCTTCATGACACG	GCAGAAACAACAGACCCACA	55	221	1
8	5	26	*Mf*SSR315a	30:28	0.792	GAGCGACCTTCCTCTTTCAA	CGAGAGCTTGGGAAAAAGTG	55	178	1
9	5	54	*Mf*SSR058.3a	30:29	0.896	CCGTGTCATTTCCAGTTTGA	GATGGAGGCACGAAAAGGTA	55	188	This study
10	6	18	*Mf*SSR340a	27:34	0.370	CCCACTCTTACTCCCCACAA	CACACGACAAAAACCCCTTT	57	270	1
11	6	18	*Mf*SSR328	27:34	0.370	ATCACTGACGGTCGGTAAGG	CTGGTCAGAGCGTCTTTTCC	55	150	2
12	6	67	*Mf*SSR244b	22:37	0.050	GGCCATTTCATTTGCAAGAC	ATGCCACAAAATCTCCATCC	55	215	2
13	8	36	*Mf*SSR392a	32:26	0.430	GCCATTGCTATTGATTTGTG	GCCAGCTTTTCTCCCACA	55	162	1
14	8	50	*Mf*SSR025b	31:27	0.599	CATGACTGACGTCCTCTTCTCA	ATATGGGAAGGGGAAAGGTG	55	176	2
15	9	23	*Mf*SSR333a	19:25	0.365	GCATGAGCAGGCGAGATA	GCAAGGACAAGGATTAGC	55	140	1
16	10	58	*Mf*SSR394a	26:35	0.249	GACGACGGACTTATTACCAGCTA	CGAACTCCTATCTAACGCTATTTC	55	99	1
17	33	0	*Mf*SSR061b	33:28	0.522	TGCAAACTCTGATGCTGGAC	TTCAGAGGCTCGTCTTTGGT	55	124	2
18	33	5	*Mf*SSR319a	32:29	0.700	CGTCATCGGGCTTTATGTCT	ATCATGGGTAGGGGGAGAAC	55	270	1
19	33	19	*Mf*SSR362a	30:31	0.898	TTCCCACTACATCCGGAAAG	CCCTCCTTTTCCTCAACACA	57	182	1
20	NG		*Mf*SSR355a	24:37	0.096	CGACCATGCCAAGCGTTTC	TGCCACTAGAGGAGACAGC	55	149	1
21	NG		*Mf*SSR353a	28:33	0.522	AATGGCAACCGCTTATGA	ACTCATAGATGTAAATACACAC	55	130	1
22	NG		*Mf*SSR324a	34:27	0.370	ACAGACGAACGACCGACGAC	ATTACGCCCAGAACACCTTT	55	115	1
23	NG		*Mf*SSR301a	33:27	0.438	CAGCCCAGCCTCCTTTTACT	TGTTCAGCCACCACCCTCTC	55	113	1
24	NG		*Mf*SSR194b	28:32	0.605	CATCTTTGAGGAGGCAAAGC	AGATTCCTTAGGCGGCATTT	55	294	2
25	NG		*Mf*SSR357a	34:26	0.301	GAATGGAAGCTTTGCAGGAG	GCCTAAGACACACACGCACA	55	223	1
26	NG		*Mf*SSR005b	28:30	0.792	TCCAAATTCCATCGTTGTCA	CGATGATTTGGGTGGTCAAGCTA	55	158	2
27	NG		*Mf*SSR203b	32:29	0.700	CTCTGTGGCGTAAGTGGGTG	GATTGCACAGCAGGAAGAG	55	227	2

1 Neu et al. 1999, *2* Zapater et al. 2008

[a] Ratio of parents CIRAD86 (A) to CIRAD139A (B) alleles

[b] Probability of χ^2 values. The null hypothesis of the χ^2 test is that the A:B segregation equals the 1:1 ratio ($\chi^2 \geq 7.7$, $P \geq 0.01$)

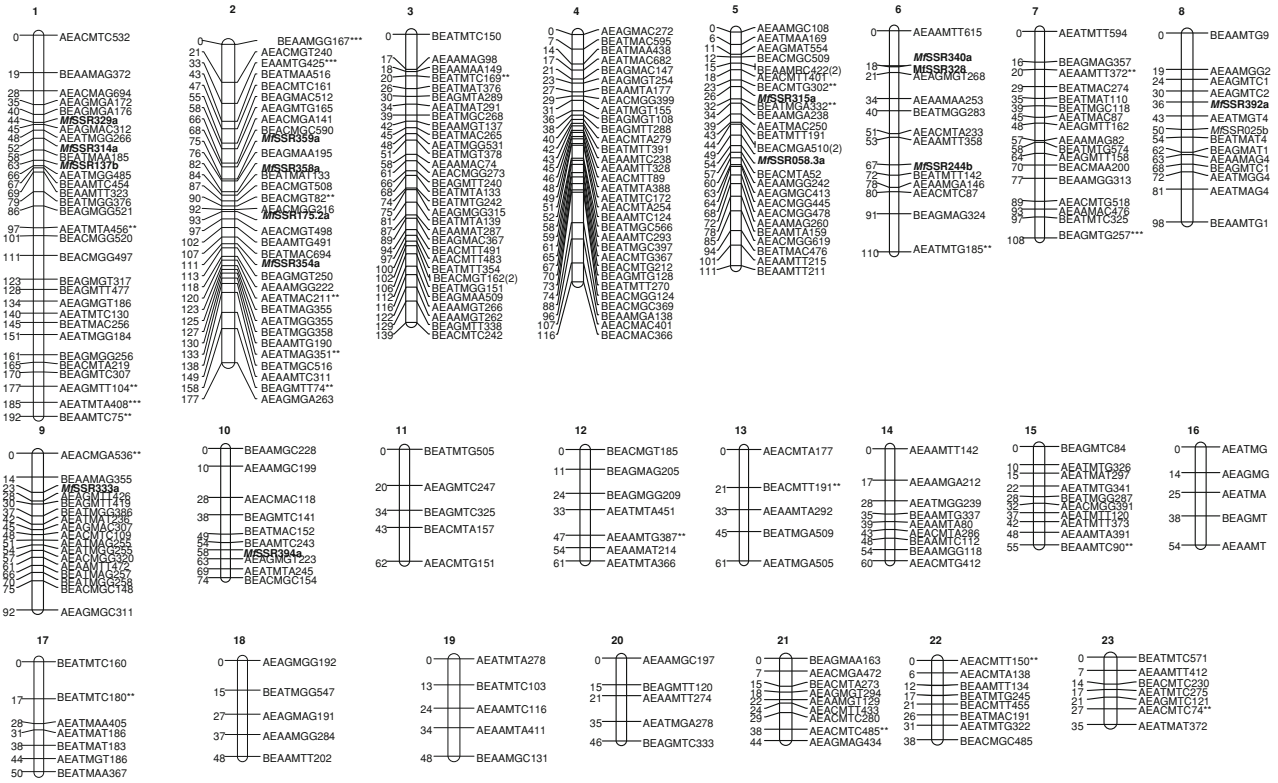

Fig. 1 The genetic linkage map of *Mycosphaerella fijiensis*. A total of 298 AFLP markers and 16 SSR markers are included in the 23 larger linkage groups analyzed with LOD threshold values minimum of 3.0. Marker distance derived using the Kosambi function is reported in cM and indicated on the left of each linkage group. AFLP and SSR markers were denominated as in "Materials and Methods". The numbers between parentheses indicate markers, which link at the same locus on the linkage groups *3* and *5*. Markers with distorted segregation ratios are identified by asterisks to the right of the marker's name are marked with "**" ($P = 0.05$) and "***"($P = 0.01$). The total length of the genetic linkage map was 1,879 cM

markers originated from the parental isolate CIRAD86 and 153 markers from CIRAD139A. The largest mapped distance between two markers was 21 cM (between AEACMTA177 and BEACMTT191) on LG 13. The general features of the map are summarized in Table 4.

A further 68 AFLP markers and three SSR markers were assigned to smaller LGs linked to just one other marker. These markers formed another 26 smaller LGs; 6 groups of 4 markers, 7 groups of 3 markers and 13 LGs containing just 2 markers, the proportion of mapped markers in both parents, CIRAD86 and CIRAD139A, were 34 and 37 markers, respectively.

The genome size of *M. fijiensis* in terms of map units calculated considering all LGs consisting of two markers or more was 2,581 cM in total (Table 4). Using specific PCR primers based on the sequence of the mating type idiomorphs (Conde-Ferráez et al. 2007), 25 isolates amplified MAT1-1 and 36 isolates amplified MAT1-2. Both idiomorphs were present in nearly equal frequencies ($\chi^2 = 2.0$; $P = 0.159$). The most tightly linked marker to the MAT locus was BEACMTG503 at LOD 2.0; this value is below the LOD of 3.0 threshold used to construct the map.

Segregation analysis reveals segregation distortion of mapped markers

The initial analysis showed 48 (at $P = 0.05$) and 10 markers (at $P = 0.01$) with segregation distortion from the expected 1:1 ratio. They were included in the analysis and some of them were successfully mapped. Of the 385 linked markers that showed the expected 1:1 Mendelian segregation ratios in the progeny mapping population, 57 (6.2%) markers included in 18 of 23 LG showed segregation distortion: 27 markers with a significance level of 95% and 10 markers with 99% (Fig. 1). The numbers of markers with distortion by LG varied between 1 (on LG 15, LG 17, LG 21, and LG 23) to 10 markers (on LG 2). The five LG with no distorted markers were LG 10, LG 11, LG 16, LG 19 and LG 20.

Haplotype analysis

Distortion in the segregation ratios may be caused by skewing of markers, where the proportion of alleles from a single parental strain is much greater than expected. In order to visualize this skewing in the markers analyzed in this study, for each of the 61 F_1 progeny, the allelic frequency

Table 4 Length of linkage group, number of markers, average spacing between markers, largest genetic distance of the genetic linkage map of *Mycosphaerella fijiensis*

Linkage groups	Length (cM)	Number of markers	Average marker spacing (cM)	Largest distance (cM)
1	192	31	6.1	18.79
2	177	33	5.3	20.89
3	139	32	4.4	17.24
4	116	32	3.6	14.15
5	111	27	4.2	10.33
6	110	14	8.4	18.31
7	108	17	6.3	16.43
8	98	14	7.0	18.89
9	92	17	5.4	16.99
10	74	10	7.4	18.11
11	62	5	12.4	20.43
12	61	7	8.7	14.16
13	61	5	12.2	21.04
14	60	9	6.6	17.00
15	55	10	5.5	9.88
16	54	5	10.8	15.99
17	50	7	7.4	16.90
18	48	5	9.6	14.81
19	48	5	9.6	14.63
20	46	5	9.2	15.21
21	44	9	4.8	9.64
22	38	8	4.7	7.57
23	35	7	5.0	7.86
	1,879[a]	314[a]	5.97[a]	
24–49	702[b]	2, 3 and 4 (=71)[b]	9.89[b]	25.29
Total	2,581.5[c]	385[c]	6.68	

[a] Total of all major linkage groups

[b] Total of all minor linkage groups

[c] Total of all linkage groups

(AF) of each marker was calculated following the equation $AF = [a + (p/2)]/(a + b + p)$, where a = alleles from CIRAD86, b = alleles from CIRAD139A and p = missing alleles (Luna Martinez 2007), and plotted against the distal location (in cM) of the marker on the 23 linkage groups; an AF of 0.5 denoting independent segregation (Forche et al. 2000). An overall even distribution of parental alleles was observed, but some of the skewed markers are located at the ends of LGs. For the 61 F_1 progeny, at all 643 loci, there were 18,359 alleles from CIRAD86, 19,254 alleles from CIRAD139A and only 1,610 missing data points (4.1%) (data not shown). Nonetheless, some of the LGs show a slight, though insignificant bias toward one or the other parent. The LGs 3, 10, 12, 21 and 22 are skewed slightly toward parent CIRAD139A, while LG 2 is slightly skewed toward parent CIRAD86 as shown in Fig. 2.

Genome length and coverage

To estimate genome length, a combination of different methods based on mapping data and chromosome number was used (Hulbert et al. 1988; Remington et al. 1999; Chakravarti et al. 1991). A total of 4,373 linked marker pairs were detected using a LOD threshold of 2.0, letting n represent 643 markers with a level of significance less than 0.01 used in linkage analysis.

The maximum distance associated with a value of LOD of 2.0 was 49.19 cM. The results using our data suggest a genome length ($\hat{G}$) of 4,643.6 Kosambi cM employing Chakravati's method 3, whereas using the modified estimator reported by Remington et al. (1999), 4,437.7 and 4,298.2 Kosambi cM were estimated, considering 8 and 13 chromosomes, respectively, based on the data of Rodríguez-García et al. (2006). Based on an estimation of 2,581 cM for the genetic linkage map generated in this study (considering all the LGs), the map spans about 60% of the total length of the genome of *M. fijiensis* if this is considered to be 4,298.2 cM. The average map distance of 1 cM in kb of DNA is usually estimated by comparing the size of a physical map (in kb) to the length of its genetic map (in cM). To derive this calculation from the genome sequence we were able to find only three SSR markers

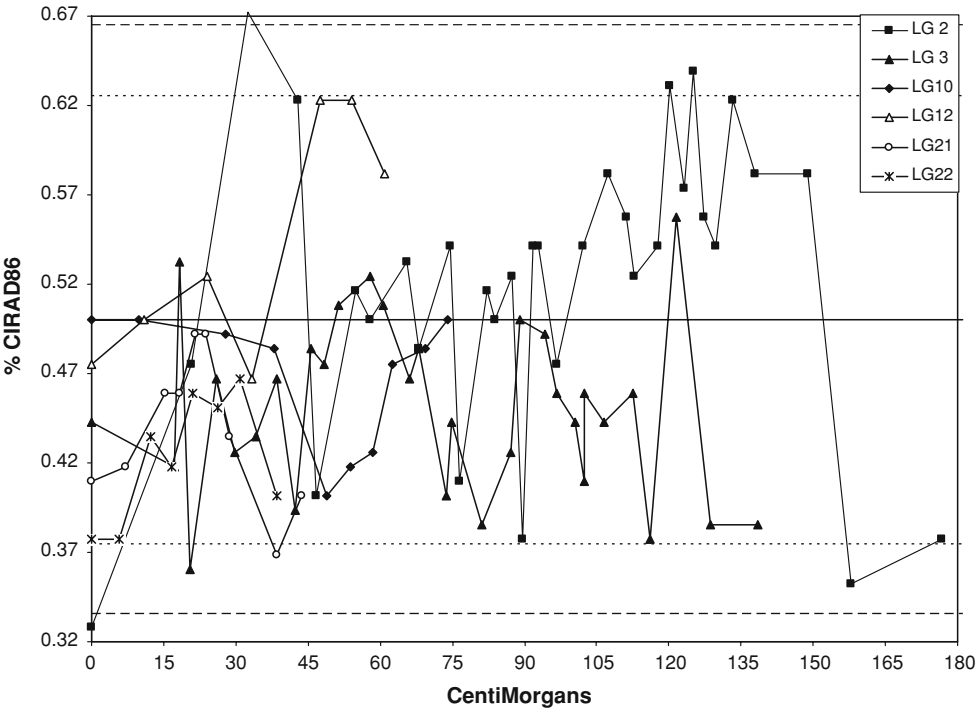

Fig. 2 Segregation ratios for LGs 2, 3, 10, 12, 21 and 22. The *y* axis describes the segregation skewed toward the CIRAD86 parent (0.5–1) or the CIRAD139A parent (0–0.5). Boundaries for the 95% (at 0.374 and 0.625) and 99% (0.335 and 0.664) confidence interval around the null hypothesis of Mendelian segregation (1:1) are indicated by *dotted lines*. The *x* axis describes the distance between the markers of each linkage group and is in cM

which occurred on the same linkage group (small LG33) and also the same supercontig and therefore could be used for checking physical and genetic distances. We found values of 42.96, 52.58 and 45.57 kb/cM. The physical order of the markers was congruent with the genetic map order.

Because the karyotype analysis estimated for *M. fijiensis* genome was approximately 35 Mb in size (Rodríguez-García et al. 2006), but the sequencing now indicates a genome size of 73.4 Mb (http://genome.jgi-psf.org/Mycfi1/Mycfi1.info.html) then this estimation of the genetic map length, considering only the large 23 LGs, gives an average of 39.0 kb/cM which is similar to values found in LG 33. In comparison, the genome size of *M. graminicola* is 41.2 Mb (http://genome.jgi-psf.org/Mycfi1/Mycfi1.info.html), or 1,322 cM, which includes the smallest linkage groups (Goodwin et al. 2007) where the physical to genetic distance is 31.2 kb/cM.

We evaluated the degree of map coverage using the formula $c = e^{-2dn/G}$ (Lange and Boehnke 1982) and using the lowest estimate of genome size $G = 4{,}298.2$ Kosambi cM, an estimated 70.4% of the genome is within 6.8 cM of one of the 385 linked markers. To obtain an average interval of 10.0 cM for coverage of 95% of the genome of *M. fijiensis*, all 644 markers are required to be on the map.

Discussion

In this study, we used AFLP and SSR markers and the *MAT* locus to construct the first genetic linkage map of *M. fijiensis*. This genetic linkage map was constructed using 314 markers; it comprises 23 large LGs and covers a length of 1,879 cM with an average interval of 5.9 cM between markers. In comparison, the *M. graminicola* genetic linkage map comprises 23 LGs containing a total of 280 markers, the *MAT* locus and the avirulence locus, giving a total length of 1,216 cM with an average of 5.3 cM between markers (Kema et al. 2002). An updated map of *M. graminicola* by Goodwin et al. (2007) gives information indicating that the total map length, including the smallest LGs, is 1,322 cM. The number of LGs in the *M. fijiensis* genetic linkage map was close to those found in *Leptosphaeria maculans* with 21 LGs containing 160 loci (Cozijnsen et al. 2000), *Ascochyta rabiei* with 28 LGs containing 126 loci (Phan et al. 2003), *Cochliobolus sativus* containing 27 LGs with 134 loci (Zhong et al. 2002) and *Cryphonectria parasitica* with 24 LGs and 144 loci (Kubisiak and Milgroom 2006).

The number of LG was considerably higher than the estimated number of 8–13 chromosomes reported for Mexican strains of *M. fijiensis* (Rodríguez-García et al. 2006). However the exact size and number of the largest chromosomes in this study was not certain; probably because their large size was beyond the resolving power of PFGE and there are no molecular markers available for PFGE above 5.7 Mb. Undoubtedly, when more markers are placed on the map, several linkage groups will be expected to merge, especially in the case of the smaller groups. This could be due to an underestimation of the number of chromosomes of the parents and may be resolved when additional markers are added to the map. The discrepancy between chromosome numbers and LGs could also be accounted for by the

possibility of co-migration of similar sized chromosomes (Lind et al. 2005). Indeed, the differential banding intensity of the autoradiograph for small chromosomes probably indicated that co-migration was likely, and therefore total genome size of the small chromosomes was probably underestimated. To associate LG and DNA markers with chromosomes, at least one AFLP or SSR marker from each LG could be used as a probe to hybridize with CHEF blots. In this regard, the genome sequence of *M. fijiensis* is available now at (http://genome.jgi-psf.org/Mycfi1/Mycfi1.info.html) and indicates that the genome size is 73.4 Mb which is double the estimated size obtained by electrophoretic karyotyping of chromosomal DNA.

Seventy-one markers (68 AFLP and 3 SSR) were found on small LGs suggesting that some parts of the genome still have a low marker density. When all the small LGs are taken into account, the genome size expanded to a total length of 2,581.5 cM, which is similar to the genetic linkage map of *Heterobasidion annosum* with 2,252 cM, which contains 39 LGs (Lind et al. 2005) and *Blumeria graminis* with 2,114 cM containing 34 LGs (Pedersen et al. 2002).

The genome length of *M. fijiensis* was estimated using the method of Hulbert et al. (1988) taking into account the modifications in method 3 of Chakravarti et al. (1991) and a modified estimator proposed by Remington et al. (1999). When the first method was applied, a genome size of 4,643.6 cM was obtained, while the second method gave a genome size between 4,298.2 and 4,437.7 cM. The genome sizes of most filamentous Ascomycetes lie within 1,500–2,000 cM (Beadle et al. 2003; Kupfer et al. 1997). However, larger estimates have been obtained for *A. nidulans* (4,000 cM; Swart 1996), *C. lindemuthianum* (3,470 cM; Luna Martinez et al. 2007) and *C. parasitica* (3,356 cM; Kubisiak and Milgroom 2006). The estimated genome size obtained for *M. fijiensis* is therefore exceptionally large but this is in agreement with a recent estimation of the physical size of the *M. fijiensis* genome (73.4 Mb), which is significantly larger than the estimates of the closely related *M. graminicola* of 41.2 Mb and 1,322 cM (Goodwin et al. 2007).

In our study 1–5 markers with distorted segregation were observed on 15 different LG; these markers were found on different regions of individual LG, indicating that no particular LG or large segments of a particular LG showed distortion (Fig. 1). No clustering of skewed segregating markers could be found in our analysis. The level of distortion accepted in this study was determined at $P > 0.01$, this value compares well with those taken for the construction of other fungal genetic linkage maps. In other studies some markers with a significantly distorted segregation were included on the LG forming the genetic linkage map. In *H. annosum* this included all 19 large LG of (Lind et al. 2005), on 7 of the 11 LG in *P. ostreatus* (Larraya et al. 2000), and in *B. lactucae* on 7 of the 12 LG (Sicard et al. 2003).

Several genomic processes could be responsible for this phenomenon; the expression of linked lethal genes, the development of the progeny from parents with different genome sizes and chromosome complements leading to non-disjunction during meiosis (Bert et al. 1999), and an accidentally biased selection of the ascospores used for the development of the segregating population (Kerrigan et al. 1992; Larraya et al. 2000).

The physical size to genetic distance of the large 23 LGs was estimated to be approximately 39.0 kb/cM and is similar to 31.2 kb/cM of *M. graminicola* (Goodwin et al. 2007). Linking the genome sequence information and the genetic map position of three SSRs we obtained a mean of 47.00 kb/cM, which is close to 39.0 kb/cM. So, we obtained similar results with both methods. These results are similar to those of maps developed for other ascomycetes, for example 32 kb/cm in *G. fujikuroi* (Xu and Leslie 1996), 50 kb/cM in *L. maculans* (Cozijnsen et al. 2000), 48.5 kb/cM in *A. bisporus* (Kerrigan et al. 1992), 27.9 kb/cM in *C. cinereus* (Muraguchi et al. 2003), 27 kb/cM in *L. edodes* (Kwan and Xu 2002) and 36.3 kb/cM in *P. ostreatus* (Park et al. 2006). If an average of 70 kb/cM is favorable for map-based cloning (Sicard et al. 2003), then the map generated in this study is well suited for the mapping of genes associated with aggressiveness or oxidative stress resistance.

The segregation ratio for *MAT1-1*:*MAT1-2* (25:36; $\chi^2 = 2.0$; $P = 0.159$) among the progeny of *M. fijiensis* used for the development of the genetic linkage map is similar (35:26; $\chi^2 = 2.20$; $P = 0.5–0.3$) to that reported for the *M. graminicola* map (Kema et al. 2002). However on the *M. fijiensis* map the *MAT* locus could not be located; similar results have occurred in other studies, for example, in *H. annosum* (Lind et al. 2005) and *B. lactucae* (Hulbert et al. 1988). This is probably due to the large genome size, small segregating population analyzed or that the *MAT* locus is localized in a difficult to map region of the genome and should be solved by using markers generated by the analysis of expressed sequence tags (EST) and the genome sequence of *M. fijiensis*.

The size of the progeny population is an important factor in the development of a genetic linkage map. The 61 progeny used in this study is a similar number to that of the 61 progeny used to generate the genetic linkage maps of *M. graminicola* (Kema et al. 2002); the 61 progeny used in *M. grisea* (Nitta et al. 1997) and the 60 progeny in *Magnaporthe oryzae* (Luo et al. 2005) and is larger than the mapping populations used to create the maps for *Agaricus bisporus* with 52 haploid offspring (Kerrigan et al. 1992), the 58 single spore progeny used for *L. maculans*, (Cozijnsen et al. 2000), the 40 progeny of *Coprinus cinereus*

(Muraguchi et al. 2003) and for *Fusarium oxysporum* f. sp. *lycopersici* 32 parasexual fusion products were analyzed (Teunissen et al. 2003).

A relatively relaxed minimum LOD value of 3.0 was applied. However many of the LG determined were based on LOD's between 3.0 and 9.0. The LOD values and the maximum recombination frequency of 0.4 used in this study are similar to values reported in previous studies in fungi (Cozijnsen et al. 2000; Pedersen et al. 2002; Phan et al. 2003; Luo et al. 2005) and Oomycetes (May et al. 2002).

AFLP markers have been found to cluster due to the methods of likelihood used to detect SNP's, which often occur in repetitive DNA regions (Qi et al. 1998). Only three pairs of AFLP markers were found to map to exactly the same position, one pair on linkage group 3 indicated as BEACMGT162(2) and two pairs on linkage group 3 indicated as BEAAMGC422(2) and BEACMGA510(2).

In this study the markers are relatively evenly spread over the LG, spaced at an average of 7.0 cM between markers. The non-clustering of AFLP markers is similar to that found in a similar work (with an average distance between markers of 8.9–15.1 cM), for example, *C. neoformans* (Forche et al. 2000), *H. annosum* (Lind et al. 2005), *A. rabiei* (Phan et al. 2003) and *L. maculans* (Cozijnsen et al. 2000). Whereas in *M. graminicola* (Kema et al. 2002), *G. zeae* (Jurgenson et al. 2002) in *B. lactucae* (Sicard et al. 2003) such clustering has been seen (the maps showed an average distance between markers of 2.8–5.3 cM), the clustering phenomenon of AFLP markers seems to be associated with high density maps, hence addition of more AFLP markers might reveal clustering in *M. fijiensis* as well. Clustering has also been found to be associated with the use of the six-base cutter *Eco R1*, as used in this work; in future AFLP mapping this may be avoided by replacing *Eco R1* with *Pst1*. For example, Powell et al. (1997) reported that methylation sensitive *Pst1*, when used with *Mse1* in AFLP analysis provided markers in the recombinationally active, distal regions of barley chromosomes, while the methylation insensitive *EcoR1*/*Mse1* restriction endonuclease combination provided a high frequency of markers clustered near the barley centromere.

In our study 27 SSR markers were used to analyze the segregation among the 61 F_1 progeny; some SSR markers from *M. graminicola* and *M. grisea* were analyzed using the parental isolates (CIRAD86 and CIRAD139A), however, these were not informative (data not shown). The genomic sequencing of *M. fijiensis*, including 40,000 ESTs is now complete and manual annotation is in progress (http://genome.jgi-psf.org/Mycfi1/Mycfi1.home.html). This will provide a valuable resource of non-anonymous DNA markers to increase the density of markers on the genetic linkage map and to assemble contiguous clones (contigs).

The use of SSR markers for the construction of genetic linkage maps in fungi includes *M. graminicola*, where 23 SSR markers were integrated into an established genetic linkage map (Goodwin et al. 2007); *Magnaporthe grisea*, where 23 SSR markers were integrated (Kaye et al. 2003); *A. rabiei*, where 10 SSR markers were added to the map (Phan et al. 2003); *C. neoformans* where 63 SSR markers were included in the map (Marra et al. 2004) and recently Ma et al. (2006) developed a total of 121 SSR markers, which are evenly distributed over the whole genome of *M. grisea*. *M. fijiensis* is a major foliar pathogen of banana and the understanding of the molecular and genetic basis of aggressiveness and pathogenicity in the banana–*M. fijiensis* pathosystem is an important research objective.

A genetic linkage map is essential to investigate the genetics underlying complex or quantitative trait loci (QTL). For example, in *H. annosum*, two of the three QTL for mycelial growth rate were shown to be tightly linked to the intersterility genes S and P (Olson 2006); in *P. ostreatus*, QTLs controlling growth rate and some important industrial production traits have been found (Larraya et al. 2003). In *Gibberella zeae* Cumagun et al. (2004), identified a QTL associated with pathogenicity and aggressiveness on wheat. The genetic linkage map of the *M. fijiensis* genome may be useful to identify QTL associated with aggressiveness, for example, the parents of the cross used in the present work differ in levels of aggressivity (Table 1). Furthermore, this genetic linkage map provides a framework for the recently initiated *Mycosphaerella* genome sequencing project. It will help to assign sequence contigs to chromosomes and guide their ordering and orientation. For future mapping the DArT technology has proven its usefulness in the recent creation of two high-density genetic maps of *M. graminicola* (Wittenberg 2006); 1,793 new polymorphic markers were developed which were easily sequenced. Together with new SSR markers, DArT markers will enable high-density future maps to be constructed with more precision including gaining knowledge on the fate of dispensable chromosomes.

Acknowledgments G. Manzo-Sánchez acknowledges fellowship support from the Consejo Nacional de Ciencia y Tecnología, Mexico (CONACyT) (No. 128982). This project was financially supported by CONACYT (Project No. 37602-B) and the International Network for the Improvement of Banana and Plantain (INIBAP). We thank Emigdia Alfaro for technical support during the AFLP marker analyses.

References

Beadle J, Wright M, McNeely L, Bennet JW (2003) Electrophoretic karyotype analysis in fungi. Adv Appl Microbiol 53:243–270

Bert PF, Gharmet G, Sourdille P, Hayward MD, Balfourer F (1999) A high-density molecular map for the rye grass (*Lolium perenne*) using AFLP markers. Theor Appl Genet 99:445–452

Carlier J, Lebrun MH, Zapater M, Dubois C, Mourichon X (1996) Genetic structure of the global population of banana black leaf streak fungus, *Mycosphaerella fijiensis*. Mol Ecol 5:499–510

Chakravarti A, Lasher LK, Reefer JE (1991) A maximum likelihood method for estimating genome length using genetic linkage data. Genetics 128:380–390

Conde-Ferráez L, Waalwijk C, Canto-Canché B, Kema GHJ, Crous P, James A, Abeln E (2007) Isolation and characterization of the *mating type* locus of *Mycosphaerella fijiensis*, the causal agent of black leaf streak disease of banana. Mol Plant Pathol 8:111–120

Cozijnsen AJ, Popa KM, Rolls BD, Purwantara A, Howlett BJ (2000) Genome analysis of the plant pathogenic fungus *Leptosphaeria maculans*: mapping mating type and host specificity loci. Mol Plant Pathol 1:293–302

Cumagun CJR, Borden R, Jurgenson J, Leslie J, Miedaner T (2004) Genetic mapping of pathogenicity and aggressiveness of *Gibberella zeae* (*Fusarium graminearum*) toward wheat. Phytopathology 94:520–526

Forche A, Xu J, Vilgalys R, Mitchell TG (2000) Development and characterization of a genetic linkage map of *Cryptococcus neoformans* var. *neoformans* using amplified fragment length polymorphisms and other markers. Fungal Genet Biol 31:189–203

Fullerton RA, Olsen TL (1995) Pathogenic variability in *Mycosphaerella fijiensis* Morelet, cause of black sigatoka in banana and plantain. N Z J Crop Hortic Sci 23:39–48

Goodwin SB, van der Lee TAJ, Cavaletto JR, Hekkert BL, Crane CF, Kema GHJ (2007) Identification and genetic mapping of highly polymorphic microsatellite loci from an EST database of the septoria tritici blotch pathogen *Mycosphaerella graminicola*. Fungal Genet Biol 44:398–414

Hulbert SH, Ilott TW, Legg EJ, Lincoln SE, Lander ES, Michelmore RW (1988) Genetic analysis of the fungus, *Bremia lactucae*, using restriction fragment length polymorphisms. Genetics 120:947–958

Jurgenson JE, Bowden RL, Zeller KA, Leslie JF, Alexandra NJ, Plattner RD (2002) A genetic map of *Gibberella zea* (*Fusarium graminearum*). Genetics 160:1451–1460

Kaye C, Milazzo J, Rozenfeld S, Lebrun M-H, Tharreau D (2003) The development of simple sequence repeat markers for *Magnaporthe grisea* and their integration into an established genetic linkage map. Fungal Genet Biol 40:207–214

Kema GHJ, Goodwin SB, Hamza S, Verstappen ECP, Cavaletto JR, Van der Lee TAJ, de Weerdt M, Bonants PJM, Waalwijk C (2002) A combined amplified fragment length polymorphism and randomly amplified polymorphism DNA genetic linkage map of *Mycosphaerella graminicola*, the *Septoria tritici* leaf blotch pathogen of wheat. Genetics 161:1497–1505

Kerrigan RW, Royer JC, Baller LM, Kohli Y, Horgen PA, Anderson JB (1992) Meiotic behavior and linkage relationships in the secondarily homothallic fungus *Agaricus bisporus*. Genetics 133:225–236

Kubisiak TL, Milgroom MG (2006) Markers linked to vegetative incompatibility (*vic*) genes and a region of high heterogeneity and reduced recombination near the mating type locus (*MAT*) in *Cryphonectria parasitica*. Fungal Genet Biol 43:519–527

Kupfer DM, Reece CA, Clifton SW, Roe BA, Prade RA (1997) Multicellular ascomycetous fungal genomes contain more than 8,000 genes. Fungal Genet Biol 21:364–372

Kwan HS, Xu HL (2002) Construction of a genetic linkage map of shiitake mushroom *Lentinula edodes* strain L-54. J Biochem Mol Biol 35:465–471

Lange K, Boehnke M (1982) How many polymorphic genes will it take to span the human genome? Am J Hum Genet 24:842–845

Larraya LM, Perez G, Ritter E, Pisabarro AG, Ramirez L (2000) Genetic linkage map of the edible basidiomycete *Pleurotus ostreatus*. Appl Environ Microbiol 66:5290–5300

Larraya LM, Alfonso M, Pisabarro AG, Ramırez L (2003) Mapping of genomic regions (Quantitative Trait Loci) controlling production and quality in industrial cultures of the edible Basidiomycete *Pleurotus ostreatus*. Appl Environ Microbiol 68:3617–3625

Lind M, Olson A, Stenlid J (2005) An AFLP-marker based genetic linkage map of *Heterobasidion annosum* locating intersterility genes. Fungal Genet Biol 42:519–527

Luna-Martinez F (2007) Segregacion de genes de avirulencia y de apareamiento sexual y construccion del mapa de ligamiento de *Colletotrichum lindemuthianum*, agente causal de la anthracnosis de frijol. Dissertation, 2007, CINVESTAV, Unidad Irapuato, Guanajuato, Mexico

Luna-Martínez F, Rodríguez-Guerra R, Victoria-Campos M, Simpson J (2007) Development of a molecular genetic linkage map for *Colletotrichum lindemuthianum* and segregation analysis of two avirulence genes. Curr Genetics 51:109–121

Luo C-X, Yin LF, Koyanagi S, Farman ML, Kusaba M, Yaegashi H (2005) Genetic mapping and chrosmosomal assignment of *Magnaporthe oryzae* avirulence genes *AvrPik*, *AvrPiz*, and *AvrPiz-t* controlling cultivar specificity on rice. Phytopathology 95:640–647

Ma J-H, Wang L, Fena S-J, Lin F, Xiao Y, Pan Q-H (2006) Identification and fine mapping of *AvrPi15*, a novel avirulence gene of *Magnaporthe grisea*. Theor Appl Genet 113:875–883

Marín DH, Romero RA, Guzmán M, Sutton TB (2003) Black sigatoka: an increasing threat to banana cultivation. Plant Dis 87:208–222

Marra RE, Huang JC, Fung E, Nielsen K, Heitman J, Vilgalys R, Mitchell TG (2004) A genetic linkage map of *Cryptococcus neoformans* variety *neoformans* serotype D (*Filobasidiella neoformans*). Genetics 167:619–631

May KJ, Whisson SC, Zwart RS, Searle IR, Irwin JAG, Maclean DJ, Carroll BJ, Drenth A (2002) Inheritance and mapping of 11 avirulence genes in *Phytophthora sojae*. Fungal Genet Biol 37:1–12

Mourichon X, Zapater MF (1990) Obtention in vitro du stade *Mycosphaerella fijiensis* forme parfaite de *Cercospora fijiensis*. Fruits 45:553–557

Muraguchi H, Ito Y, Kamada T, Yanagi SO (2003) A linkage map of the basidiomycete *Coprinus cinereus* based on random amplified polymorphic DNA and restriction fragment length polymorphisms. Fungal Genet Biol 40:93–102

Neu C, Kaemmer D, Kahl G, Fisches D, Weising K (1999) Polymorphic microsatellite markers for the banana pathogen *Mycosphaerella fijiensis*. Mol Ecol 8:513–525

Nitta N, Farman ML, Leong SA (1997) Genome organization of *Magnaporthe grisea*: integration of genetics maps, clustering of transposable elements and identification of genome duplications and rearrangements. Theor Appl Genet 95:20–32

Olson A (2006) Genetic linkage between growth rate and the intersterility genes S and P in the basidiomycete *Heterobasidion annosum s. lat*. Mycol Res 110:979–984

Park SK, Peñas MM, Ramírez L, Pisabarro AG (2006) Genetic linkage map and expression analysis of genes expressed in the lamellae of the edible basidiomycete *Pleurotus ostreatus*. Fungal Genet Biol 43:376–87

Pedersen C, Rasmussen SW, Giese H (2002) A genetic map of *Blumeria graminis* based on functional genes, avirulence genes, and molecular markers. Fungal Genet Biol 35:235–246

Phan HTT, Ford R, Taylor PWJ (2003) Mapping the mating type locus of *Ascochyta rabiei*, the causal agent of ascochyta of chickpea. Mol Plant Pathol 4:373–781

Powell L, Thomas WTB, Baird E, Lawrence P, Booth A, Harrower B, McNicol JW, Waugh R (1997) Analysis of quantitative traits in barley by the use of Amplified Fragment Length Polymorphisms. Heredity 79:48–59

Qi X, Stam P, Lindhout P (1998) Use of locus-specific AFLP markers to construct a high density molecular map in barley. Theor Appl Genet 96:376–384

Remington DL, Whetten RW, Liu B-H, O´Malley DM (1999) Construction of an AFLP genetic linkage map with near complete genome coverage in *Pinus taeda*. Theor Appl Genet 98:1279–1292

Rivas G-G, Zapater M-F, Abadie C, Carlier J (2004) Founder effects and stochastic dispersal at the continental scale of the fungal pathogen of bananas *Mycosphaerella fijiensis*. Mol Ecol 13:471–482

Rodríguez-García CM, Raigosa-Flores N, Conde-Ferráez L, Peraza-Echeverría L, Canto-Canché B, James-Kay A (2006) Variation in electrophoretic karyotype among Mexican isolates of *Mycosphaerella fijiensis*. Can J Plant Pathol 28:236–241

Sicard D, Legg E, Brown S, Babu NK, Ochoa O, Sudarshana P, Michelmore RW (2003) A genetic map of the lettuce downy mildew pathogen, *Bremia lactucae*, constructed from molecular markers and avirulence genes. Fungal Genet Biol 39:16–30

Swart K (1996) Mapping and breeding strategies. In: Bos CJ (ed) Fungal genetics principles and practice. Marcel Dekker, New York, pp 295–315

Teunissen HAS, Rep M, Houterman PM, Cornelissen BJC, Haring MA (2003) Construction of a mitotic linkage map of *Fusarium oxysporum* based on *Foxy*-AFLP. Mol Genet Genomics 269:215–226

Van der Lee T, De Witte I, Drenth A, Alfonso C, Govers F (1997) AFLP linkage map of the Oomycete *Phytophthora infestans*. Fungal Genet Biol 21:278–291

Van Ooijen JW, Voorrips RE (2001) JoinMap® 3.0, software for the calculation of genetic linkage maps. Plant Research International, Wageningen

Vos P, Hogers R, Bleeker M, Reijans M, Van der Lee T, Hornes M, Frijtens A, Pot J, Peleman J, Kiper M, Zabeau M (1995) AFLP, a new technique for DNA fingerprinting. Nucleic Acids Res 23:4407–4414

Wittenberg AHJ (2006) Genetic mapping using the diversity arrays technology (DArT) :application and validation using the whole-genome sequences of *Arabidopsis thaliana* and the fungal wheat pathogen *Mycosphaerella graminicola.* Dissertation, no. 4153, Wageningen University

Xu JR, Leslie JE (1996) A genetic map of *Gibberella fujikuroi* mating population A (*Fusarium moniliforme*). Genetics 143(1):175–189

Zapater MF, Duchemin M, Dussart JF, Coste D, Brottier P, Carlier J (2008) Microsatellite markers for the fungal banana pathogens *Mycosphaerella fijiensis*, *Mycosphaerella musicola* and *Mycosphaerella eumusae*. Mol Ecol Notes (in press)

Zhong S, Steffenson BJ, Martinez P, Ciuffetti LM (2002) A molecular genetic map and electrophoretic karyotype of the plant pathogenic fungus *Cochliobolus sativus*. Mol Plant Microbe Interact 15:481–992