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Genome distribution and validation of novel microsatellite markers of *Fusarium verticillioides* and their transferability to other *Fusarium* species

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ABSTRACT

Improved population studies in the fungus *Fusarium verticillioides* require the development of reliable microsatellite markers. Here we report a set of ten microsatellite loci that can be used for genetic diversity analyses in *F. verticillioides*, and are equally applicable to other fungi, especially those belonging to the *Gibberella fujikuroi* clade.

1. Introduction

Fusarium verticillioides, the most common maize pathogen in the world, is the cause of stalk, root and ear rot (Munkvold, 2003; Morales-Rodríguez et al., 2007). In addition to this disease and its negative impacts on grain yield and quality, *F. verticillioides* produces a diversity of mycotoxins, some of which are potentially carcinogenic. Among these are the fusarins, fumonisins, and 8-bostrycoidin (Leslie and Summerell, 2006). *Fusarium* species show high levels of intraspecific genetic diversity, which could explain such traits as pathogenicity variation, mycotoxin production and host selectivity between isolates of the same species (Manicom et al., 1990; Miedaner et al., 2001; Saharan and Naef, 2008). This genetic diversity has been investigated by molecular techniques including Random Amplification of Polymorphic DNA (RAPDs; Gherbawy et al., 2001; Pamphile and Azevedo, 2002), Polymerase Chain Reaction-Restriction Fragment Length Polymorphism (PCR-RFLPs; Patiño et al., 2006) and microsatellites (Saharan and Naef, 2008; Xu et al., 2012). Microsatellites, simple sequence repeats (SSR), or short tandem repeats (STR) are small motifs of DNA that are repeated in tandem, which are typically 1–6 nucleotides in length. Microsatellites have been successfully used to elucidate genetic diversity and to examine species differentiation and population structure in several *Fusarium* species, including *Fusarium oxysporum* (Bogale et al., 2005), *Fusarium culmorum* (Giraud et al., 2002; Bayraktar et al., 2008), *Fusarium circinatum* (Santana et al., 2009) and *Fusarium graminearum* (Karaoglu et al., 2005; Naef and Dégafo, 2006; Saharan and Naef, 2008; Vogelgsang et al., 2009). Genetic diversity in *F. verticillioides* has also been assessed by microsatellites designed for related species, e.g. *F. graminearum* (Saharan and Naef, 2008), although cross-species transferability often results in the amplification of either only a small set of microsatellites or no amplification at all (Saharan and Naef, 2008; Vogelgsang et al., 2009). With the recent sequencing of the *F. verticillioides* genome (Ma et al., 2010), significant advances have been made in the mining and generation of microsatellites in this species. For instance, Santana et al. (2009) identified genome microsatellites and Xu et al. (2012) reported di-, tri- and tetranucleotide microsatellites that are useful for genetic diversity studies in Chinese populations of *F. verticillioides*. To date, microsatellites used in population genetic studies of *Fusarium* species have been mainly di- and trinucleotides that range from seven to twenty-eight repetitions. Almost all of these are polymorphic, and have been shown to be useful for differentiating diverse *formae specialis* of *F. oxysporum* (Bogale et al., 2006), isolate identification (Giraud et al., 2002; Vogelgsang et al., 2009), and genetic variability assessment (Naef and Dégafo, 2006; Bayraktar et al., 2008; Saharan and Naef, 2008). Tetra-, penta- and hexanucleotides are rarely used, since it is presumed

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that they are less likely to be polymorphic, due to their lower repetition numbers. However, short microsatellites (arranged in five to seven tandem repeats) are reported to be polymorphic in other *Fusarium* species (Giraud et al., 2002; Bogale et al., 2006; Naef and Dégafo, 2006; Bayraktar et al., 2008; Saharan and Naef, 2008; Vogelgsang et al., 2009). Thus, polymorphism can also occur in microsatellites that are longer than hexanucleotides, as well as in compound microsatellites (Bogale et al., 2005).

A successful examination of the high genetic diversity of *F. verticillioides* will require a greater number of microsatellites than currently available. To respond to this need, we provide detailed information on the distribution and patterns of microsatellite regions over eleven of the twelve chromosomes and validate a set of ten microsatellite loci that include tetra-, penta- and hexanucleotides.

2. Materials and methods

2.1. Genome survey and distribution of microsatellites

The nucleotide sequences of eleven of the twelve chromosomes in the *F. verticillioides* (strain 7600) genome (previously obtained by the Broad Institute (Ma et al., 2010)) were downloaded from the National Center for Biotechnology Information (NCBI; http://www.ncbi.nlm.nih.gov/). The sequences of all chromosomes were scanned for microsatellite repeats with the Tandem Repeat Finder (TRF) software, version 4.04 (Benson, 1999). The following parameters, recommended by the software authors, were used: +2, −7, and −7 for match, mismatch and indels respectively; 0.80 and 0.10 for matching and indel probability respectively; a maximum period size of 500 for patterns and a minimum alignment score to report repeats of 50. Finally, only tandem repeats of exact copies of a particular motif (perfect repeats or perfect microsatellites) were selected for further analysis with the following selection criteria: seven or more repetitions for mononucleotides; five or more repetitions for di- and trinucleotides; and four or more repetitions for tetra-, penta- and hexanucleotides. The identified repeat motifs were grouped into classes that include all possible positions of nucleotides in the repeat and its complementary sequence. For example, $(AG)_n$ is equivalent to $(GA)_n$, $(CT)_n$, and $(TC)_n$. This yields two classes for mononucleotide repeats, four classes for dinucleotides, 10 classes for trinucleotides, 33 classes for tetranucleotides, 102 classes for pentanucleotides and 350 classes for hexanucleotides (Jurka and Pethiyagoda, 1994). To confirm the identified microsatellites using the TRF software, all chromosome sequences were also scanned by the SciRoKo 3.4 software (Kofler et al., 2007). This tool has been successfully used in previous studies to identify microsatellite repeats (Merkel and Gemmell, 2008; Kofler et al., 2008; Xiao et al., 2011). Both programs employ probabilistic methods, but use different approaches making SciRoKo a faster tool (Sharma et al., 2007; Grover et al., 2012). Aside from their differences in speed and approach, both programs are recommended for mining perfect, imperfect and compound microsatellite repeats (Sharma et al., 2007) and the characterization of genomic microsatellite distribution (Merkel and Gemmell, 2008; Grover et al., 2012). A compound microsatellite is an arrangement where two or more repeated motifs are next to each other without interruptions (e.g. $(CT)_{22}$-$(CA)_6$-), whereas an interrupted compound microsatellite is interrupted by a sequence (e.g. $(AC)_{14}$-AG-AA-$(AG)_{12}$-), and a complex microsatellite is an arrangement with three or more repeat units that may or may not be interrupted (e.g. $(TTTC)_{3-4}$-$(T)_6$-$(CT)_{0-1}$-$(CYKY)_n$-CTCC-$(TTCC)_{2-4}$) (Chambers and MacAvoy, 2000). The frequency of each repeat unit (mono-, di-, tri-, tetra-, penta- and hexanucleotide) in the genome was also determined, and the relative frequency was calculated by dividing the total amount of each type of microsatellite by the total number of microsatellites found in the *F. verticillioides* genome. Relative abundance (number of microsatellites of each type/Mb of analyzed sequence) and relative density (length of microsatellites/Mb of analyzed sequence) were calculated for each repeat unit. Normalizing the data per Mbp allows comparisons with similar studies.

Finally, microsatellites were mapped to their respective chromosomes with the software pDRAW32 version 1.0 (ACACLONE), using the chromosomal coordinates obtained by TRF.

2.2. Development of markers

Eleven microsatellite loci were selected on the basis that they showed a higher number of repetitions than average for each microsatellite repeat (mono-, di-, etc.). For instance, if the average number of repetitions observed for hexanucleotides was five, then a hexanucleotide with more than five repetitions would be selected for primer design. Subsequently, a set of eleven primer pairs was designed to target the surrounding sequence (500 bp) of the 11 microsatellites, using the Primer3 software (http://frodo.wi.mit.edu/cgi-bin/primer3/primer3.cgi). Primer design considerations included: an amplicon size from 100 to 300 bp; a Tm between 50 and 65 °C; GC content between 40 and 60 °C; and a primer size between 18 and 21 bp (Roux, 1995; Singh and Kumar, 2001). Four of the selected *loci* (Fv-98, Fv-114, Fv-120 and Fv-312) matched the microsatellites reported by Xu et al. (2012). However, new primer pairs were designed for each microsatellite, and therefore the Tm and expected size of the fragments were different (Table 3).

2.3. Microsatellite validation

Sixty-two mono-conidial strains of *F. verticillioides* isolated from seed and root from different locations in Northern Mexico were used for this study (Table S1). Cross-transferability was examined using two mono-conidial isolates each of *Fusarium thapsinum* (isolates F33 and F65), *Fusarium nygamai* (isolates P01 and DA31), *Fusarium andiyazi* (isolates F116 and F133), and one isolate of *F. oxysporum* (Isolate Fol 11). *Fusarium* strains were previously identified using partial DNA sequences of the calmodulin (Mulé et al., 2004) and elongation factor 1α (O'Donnell et al., 1998) genes, which were deposited in the GenBank database (accession numbers are reported in Table S1). Isolates were grown in 125 ml Erlenmeyer flasks containing 50 ml of PD broth (BD Difco) on a shaker (180 rpm) at 25 °C. After four days, mycelia were harvested by centrifugation (15,000 *g* for 20 min), dried with sterile filter paper (Faga-lab No. 1), frozen, and ground in liquid nitrogen with a mortar and pestle. Genomic DNA was extracted from 10 mg of frozen mycelium using the DNeasy Blood and Tissue Kit (Qiagen, Hilden, Germany) according to the manufacturer's protocol adapted for yeast. Quality and purity of genomic DNA were estimated by measuring the 260/280 nm ratio in a NanoDrop 2000c spectrophotometer (Thermo Scientific, Wilmington, DE) and by electrophoresis in a 1% agarose gel. DNA extracted from all samples had a 260/280 ratio ranging from 1.7 to 1.9, indicating high quality. DNA was quantified using the Quant-iT ™ dsDNA HS kit (Invitrogen). PCR was performed in a total volume of 25 μl containing 1 ng template DNA, 1.5 mM $MgCl_2$, 0.5 mM of each dNTP, 0.4 μM of each primer (forward and reverse), and 0.05 U of Taq DNA polymerase (Invitrogen). The following amplification program was used: 5 min initial denaturation at 94 °C, followed by 35 cycles of 45 s denaturation at 94 °C, 30 s annealing at the optimal Tm for each primer pair (Table 3), and 30 s extension at 72 °C. PCR was completed with a 5 min final extension at 72 °C. The same PCR conditions were used for each primer pair to amplify microsatellite loci in all tested species. PCR yield was first measured in a NanoDrop 2000c spectrophotometer prior to QIAxcel analysis. Allele amplification and visualization were then determined by capillary electrophoresis in a QIAxcel system using the QIAxcel DNA High resolution kit (Qiagen). The DNA concentration used for all samples was between 100 and 135 ng/μL, and the OH800 method was therefore selected for microsatellite analysis in the QIAxcel system. This method provides a resolution of 3 bp for amplicons between 100 and 500 bp in size. Alleles obtained from the

Table 1
Frequency, relative frequency, relative abundance and relative density of each repeat unit in 11 chromosomes of the *F. verticillioides* genome.

Chromosome	Repeat unit	F[a]	RF[b]	RA[c]	RD[d]
I	Mono-	7	0.100	1.126	26.691
	Di-	6	0.086	0.965	38.268
	Tri-	11	0.157	1.769	57.403
	Tetra-	10	0.143	1.608	41.163
	Penta-	14	0.200	2.251	117.378
	Hexa-	19	0.271	3.055	94.546
	Other repeats	1	0.014	0.161	27.495
	Compound repeats	2	0.029	0.322	36.017
Total		70		11.255	438.962
II	Mono-	8	0.131	1.706	60.340
	Di-	2	0.033	0.426	11.940
	Tri-	9	0.148	1.919	85.073
	Tetra-	9	0.148	1.919	90.403
	Penta-	10	0.164	2.132	58.634
	Hexa-	19	0.311	4.051	108.740
	Other repeats	4	0.066	0.853	24.733
	Compound repeats	–	–	–	–
Total		61		13.006	439.864
III	Mono-	5	0.106	1.077	34.887
	Di-	3	0.064	0.646	24.550
	Tri-	5	0.106	1.077	34.241
	Tetra-	9	0.191	1.938	55.992
	Penta-	6	0.128	1.292	39.840
	Hexa-	15	0.319	3.230	85.280
	Other repeats	2	0.042	0.431	14.429
	Compound repeats	2	0.043	0.431	26.488
Total		47		10.122	315.708
IV	Mono-	3	0.073	0.708	32.103
	Di-	3	0.073	0.708	30.687
	Tri-	1	0.024	0.236	7.790
	Tetra-	7	0.171	1.652	45.322
	Penta-	4	0.098	0.944	25.966
	Hexa-	13	0.317	3.069	82.146
	Other repeats	4	0.098	0.944	44.614
	Compound repeats	6	0.146	1.416	73.176
Total		41		9.678	341.804
V	Mono-	12	0.154	2.826	101.265
	Di-	7	0.090	1.649	64.763
	Tri-	12	0.154	2.826	93.258
	Tetra-	11	0.141	2.591	85.722
	Penta-	9	0.115	2.120	65.940
	Hexa-	14	0.179	3.297	84.780
	Other repeats	4	0.051	0.942	60.288
	Compound repeats	9	0.115	2.120	93.494
Total		78		18.369	649.512
VI	Mono-	7	0.125	1.794	60.999
	Di-	4	0.071	1.025	29.218
	Tri-	4	0.071	1.025	26.142
	Tetra-	13	0.232	3.332	110.720
	Penta-	9	0.161	2.307	80.734
	Hexa-	13	0.232	3.332	83.040
	Other repeats	3	0.054	0.769	28.449
	Compound repeats	3	0.054	0.769	35.113
Total		56		14.353	454.425
VII	Mono-	5	0.114	1.540	47.130
	Di-	8	0.182	2.464	80.090
	Tri-	5	0.114	1.540	41.585
	Tetra-	1	0.023	0.308	7.393
	Penta-	8	0.182	2.464	66.229
	Hexa-	10	0.227	3.080	153.404
	Other repeats	3	0.068	0.924	83.787
	Compound repeats	4	0.091	1.232	63.456
Total		44		13.554	543.074
VIII	Mono-	3	0.120	1.049	29.719
	Di-	–	–	–	–
	Tri-	2	0.080	0.699	23.076
	Tetra-	3	0.120	1.049	65.732
	Penta-	6	0.240	2.098	57.690
	Hexa-	8	0.320	2.797	69.228
	Other repeats	1	0.040	0.350	12.587
	Compound repeats	2	0.080	0.699	39.509
Total		25		8.741	297.541
IX	Mono-	2	0.057	0.725	22.844
	Di-	2	0.057	0.725	31.909
	Tri-	1	0.029	0.363	11.909
	Tetra-	5	0.143	1.813	62.368
	Penta-	9	0.257	3.263	87.750
	Hexa-	10	0.286	3.626	121.835
	Other repeats	2	0.057	0.725	30.459
	Compound repeats	4	0.114	1.450	62.368
Total		35		12.691	431.499
X	Mono-	1	0.200	0.442	11.052
	Di-	-	-	-	-
	Tri-	1	0.200	0.442	14.589
	Tetra-	1	0.200	0.442	12.378
	Penta-	-	-	-	-
	Hexa-	2	0.400	0.884	21.220
	Other repeats	-	-	-	-
	Compound repeats	-	-	-	
Total		5		2.210	59.239
XI	Mono-	3	0.375	1.470	38.709
	Di-	1	0.125	0.490	11.760
	Tri-	-	-	-	-
	Tetra-	-	-	-	-
	Penta-	-	-	-	-
	Hexa-	3	0.375	1.470	41.159
	Other repeats	-	-	-	-
	Compound repeats	1	0.125	0.490	29.400
Total		8		3.920	121.028

[a] Frequency.
[b] Relative frequency.
[c] Relative abundance.
[d] Relative density.

dinucleotidic microsatellite marker Fv-269 were analyzed and only changes >3 bp in size were considered for the estimation of the genetic parameters. The QX Alignment Marker 15 bp/600 bp was used to compensate for variations in migration speed between different capillaries, and to align the first (15 bp) and last peak or band (600 bp) across all 12 channels. Allele sizing was determined with the ScreenGel software (QIAGEN, v1.0.2.0; Ambion Inc., Austin, TX) that is provided with the QIAxcel system, using the QX DNA Size Marker 25–500. This software displays one digital gel image per run of 12 samples and an electropherogram for each sample. To assure that alleles fit into the best resolution capability of the QIAxcel system, those varying in size by 3 bp were considered different, and were used for genetic analyses. GenAlex 6.5 (Peakall and Smouse, 2012) was used to calculate the number of effective alleles and Shannon's information content (I), and the polymorphic information content (PIC) was determined with the PowerMarker V3.25 software (Liu and Muse, 2005).

3. Results

3.1. Microsatellite genome survey

A total of 470 perfect microsatellites were found within the *F. verticillioides* genome using the TRF software (Tables 1 and S2); subsequently they were mapped to their respective chromosomes (Fig. S1). Ninety-four percent of these microsatellite loci were also detected with the SciRoKo software, which confirms the results obtained with the TRF software. The different repeat units had similar distributions among the chromosomes; hexanucleotide repeats were the most frequent, followed by penta- and tetranucleotide repeats (Table 1). Microsatellite classes rich in A/T predominated throughout the entire genome (Table 2). This microsatellite analysis revealed a sharp drop in the average number of penta- and hexanucleotide repetitions (6 and 5 repetitions, respectively) with respect to the mono-, di-, tri- and tetranucleotides (27, 18, and 12 repetitions, respectively).

Twenty-four repeat motifs longer than six nucleotides in size were also found, and these were most frequent in chromosomes II, IV and V (Table 1). Compound microsatellites (in which two or more repeated motifs are next to each other without interruption) were also detected. Nine of the 17 compound microsatellites were detected in chromosome V, whereas chromosomes II and X lack this type of repeat (Table 1).

3.2. Microsatellite validation

In order to validate the microsatellite predictions, a set of 11 primer pairs was selected to confirm usefulness and polymorphism among a collection of *F. verticillioides* isolates (Table S1).

Ten out of 11 primer pairs amplified fragments with an expected size range (Table 3). A representative digital gel image and electropherograms generated by the ScreenGel software are presented in Fig. S2; allele frequencies and sizes are noted in Table S3. All reported microsatellite loci were polymorphic, and the number of detected alleles across all *F. verticillioides* isolates ranged from 7 to 17 (Table 3). In order to quantify the informative nature of the microsatellite data, the PIC value was calculated, which ranged from 0.65 to 0.91 (Table 3). Obtained I values ranged from 1.61 to 2.65. Cross-transferability was possible in all species tested. Eight microsatellite markers were transferable to *F. andiyazi*, six were transferable to *F. thapsinum* and *F. nygamai*, and only two were transferable to *F. oxysporum* (Table 4).

4. Discussion

Microsatellites in fungal genomes are less frequent and shorter than those in other taxa, including primates, rodents, non-mammalian vertebrates, arthropods and plants (Tóth et al., 2000). In this study, we identified a total of 470 perfect microsatellites in the genome of *F. verticillioides*, which is considerably lower than that previously reported for *F. graminearum* (Karaoglu et al., 2005: 2746 microsatellites; Lim et al., 2004: 2896 microsatellites) and *F. verticillioides* (Xu et al., 2012: 751 microsatellites). These variations are mainly due to the selected parameter settings and the differences in the search algorithms employed by the different software (Kofler et al., 2007; Leclercq et al., 2007). TRF is a widely used program that has demonstrated good performance regarding microsatellite detection (Kofler et al., 2007; Leclercq et al., 2007), and has proven its usefulness in studies pertaining to microsatellite distribution, characterization and abundance, as well as in exploring genome architecture (Merkel and Gemmell, 2008).

Fungal microsatellites are predominated by mono-, di- and trinucleotides (Tóth et al., 2000; Lim et al., 2004; Karaoglu et al., 2005). However, our analysis revealed a high frequency of tetra-, penta- and hexanucleotides in the *F. verticillioides* genome. These results are in partial agreement with a previous microsatellite survey of the *F. verticillioides* genome (Santana et al., 2009). The overrepresentation of these repeats could result from a tendency of the TRF software to detect microsatellites smaller than 15 bp, especially for tetra-, penta- and hexanucleotides (Leclercq et al., 2007).

Table 2
Most frequent microsatellite classes in the genome of *F. verticillioides*.

Mono-	Di-	Tri-	Tetra-	Penta-	Hexa-
A (54)	AG (20)	AAG (13)	ACAG (7)	GCACA (7)	TCTTCC (3)
G (2)	AC (13)	AGG (10)	TCCA (7)	GAAAA (4)	CCAGAG (3)
	AT (3)	ACG (6)	GAAA (7)	GGAAT (3)	CTCAGA (3)
		ACC (5)	GCAA (5)	TCTCA (3)	CTGCCT (3)
			GTGA (5)	TTCAG (2)	
			GTCA (5)		
			GTTT (4)		

Numbers in brackets represent the total occurrence of each class.

As with other ascomycetes and basidiomycetes, the *F. verticillioides* genome contains a high number of microsatellite motifs rich in A/T (Lim et al., 2004; Karaoglu et al., 2005; Dutech et al., 2007). Additionally, a sharp drop in the number of microsatellite tandem repeats and class frequency was observed as the repeat unit increased. This is a common phenomenon in fungal genomes that impacts the selection of these molecular markers for population genetics research. Indeed, it has been well established that longer microsatellites are more likely to be polymorphic than shorter ones due to a higher rate of DNA replication slippage or unequal crossing-over, which causes loss or addition of repeat units (Wierdl et al., 1997; Wöstemeyer and Kreibich, 2002; Lim et al., 2004; Karaoglu et al., 2005; Dutech et al., 2007; Santana et al., 2009). Di- and trinucleotides are the longest microsatellites in fungal genomes, and therefore the most widely used in population genetics studies.

The penta- and hexanucleotides are the most abundant microsatellites in the *F. verticillioides* genome, although their potential use in population genetics of *Fusarium* species was never previously explored. Here, we have validated six microsatellite *loci* of penta-, hexa- and heptanucleotides and five *loci* of di-, tri- and tetranucleotides from 62 *F. verticillioides* isolates. Ten out of eleven microsatellite *loci* were amplified, and all were found to be highly polymorphic. The number of detected alleles for all markers ranged from 7 to 17 with a mean of 11.7, which is greater than the number of alleles previously detected with published *F. verticillioides* markers (Saharan and Naef, 2008; Xu et al., 2012).

We also detected a greater number of alleles, using two of the microsatellite loci previously reported by Xu et al. (2012), than could be detected in Chinese isolates from the aforementioned study. Specifically, Fv-114 and Fv-312 registered 17 and 13 alleles in Mexican isolates, whereas these microsatellites respectively detected five and seven alleles in the Chinese isolates. PIC values for the 10 evaluated microsatellite loci range from 0.65 to 0.91 with a mean of 0.77. Our values were higher than the PIC values of the six-microsatellite set reported by Saharan and Naef (2008), which range from 0.34 to 0.74 with a mean of 0.57; this was calculated in PowerMarker using the data from Table 3 in Saharan and Naef (2008). It is important to mention that only six *F. verticillioides* isolates were used in the report of Saharan and Naef (2008), whereas our work compared 62 isolates. This greater number of isolates suggests that we could be detecting more alleles and more informative PIC values. The mean I value (1.96) observed in our study was greater than the reported value (1.30) in a similar study (Xu et al., 2012) that used a collection of 66 *F. verticillioides* isolates from China and 22 microsatellite markers. The values obtained in both studies are highly dependent on the studied population, and differences between them are expected to be mainly due to the geographic region of each of the populations. Nonetheless, we were able to detect a high level of polymorphism while analyzing a panel of isolates from a reduced geographic area, and even between isolates of the same plant (data not shown). The Mexican isolates could exhibit a higher diversity than the Chinese isolates, since Mexico is the center of origin and diversification of maize (Ortiz-García and Otero-Arnaiz, 2006; Ceecam, Centro de Estudios para el Cambio en el Campo Mexicano, 2012), which has resulted in a higher number of endemic maize races, varieties and wild relatives. Thus, co-evolution between *F. verticillioides* and this crop could explain diversification of this fungus.

One advantage of microsatellite markers is that some are transferable across species, particularly between closely related species. Indeed, several *Fusarium* species have been studied using microsatellite markers of phylogenetically close species, as well as from distant species belonging to different families (Bahar and Shahab, 2012; Kumar et al., 2013). However, cross-transferability is not always possible, and it can result in detection of fewer alleles (Bahar and Shahab, 2012) or fewer polymorphic loci (Kumar et al., 2013). In one study, 34% of microsatellite markers (on average) were observed to be transferable within different

Table 3
Characterization of 11 microsatellite loci isolated from the genome of *F. verticillioides*.

Locus name	Repeat sequence	Primer name and sequence (5′–3′)	Tm (°C)	No. of alleles	Allelic size range (bp)	PIC[b]	Shannon's information index (I)
Fv-47	$(TGGTGC)_{13}$	FV-47F GCTGCTTAGTGGACCGTTTC Fv-47R AATTGTTGGTGGAGGTGGAG	59	11	248–380	0.73	1.78
Fv-98[a]	$(ATCC)_9$	Fv-98F AAACAAGATGCGGTCCATTC Fv-98R GGATCGGAGGAGAATCAACA	59	7	178–218	0.76	1.70
Fv-114[a]	$(GTCT)_{35}$	Fv-114F CGAATGCCTTGATCTGCTTC Fv-114R GAGAATCCTGTTTGCGTGGT	59	17	154–314	0.91	2.65
Fv-120[a]	$(TTG)_{24}$	Fv-120F GTAGCGCGGTAAGAAGATGC Fv-120R AGTCGAAGCCCAACTGAAGA	59	16	212–359	0.85	2.31
Fv-140	$(CTCTG)_9$	Fv-140F AGGCCAGAGGGAAAGAGGTA Fv-140R AGTTGGAAGGAAGCCCAGAG	57	10	224–324	0.75	1.78
Fv-210	$(AACCCCC)_4$	Fv-210F AGTTTGGAGAAAGGGGAGGA Fv-210R AACAGGAACAGAGGGCTTGA	ND	ND	ND	ND	ND
Fv-269	$(TA)_{31}$	Fv-269F TGTAGAGCGTGTTCGCTTGT Fv-269R CGTCGGAGTTGAACGATGAT	59	8	188–219	0.72	1.85
Fv-284	$(AAGAA)_{12}$	Fv-284F TCGGCGGGAGATTATACAAG Fv-284R ATGGTGAACAGGAGGGACAG	59	10	198–468	0.73	1.68
Fv-312[a]	$(CAGA)_{14}$	Fv-312F TTTCCGAATTCCTGGATCTG Fv-312R GACGCAGTTTGCACAAGGTA	59	13	233–337	0.78	1.95
Fv-338	$(AGCAG)_{11}$	Fv-338F TAGACCAGGCAGACGAGACA Fv-338R TGTGAGTGGGTGAGAGTGGA	59	14	212–312	0.86	2.30
Fv-403	$(GTGCT)_7$	Fv-403F GGTGTTGAGAGCGAGTGTGA Fv-403R AGACAAGGCAAGGCAAGGTA	59	11	215–320	0.65	1.61
		Average		11.7		0.77	1.96

[a] Microsatellites that match with previously reported microsatellites by Xu et al. (2012). Fv-98 = 2H08, Fv-114 = 2H17, Fv-120 = 2H15, Fv-312 = 6H03. Sequence of microsatellite motif may vary due to the software used in the genome survey [e.g. Fv-98 $(ATCC)_9$ and 2H08 $(CCAT)_9$].
[b] Polymorphic information content.

fungal genera (Dutech et al., 2007). Our results indicate that 80% of the microsatellite markers were able to amplify with *F. andiyazi*, 60% amplified with *F. thapsinum* and *F. nygamai*, and only 20% amplified with *F. oxysporum* (Table 4) without any modification to the PCR conditions; this clearly represents a technical advantage when manipulating several different species simultaneously. The higher percentage of transferability observed in *F. andiyazi*, *F. thapsinum* and *F. nygamai* as compared to *F. oxysporum* could be due to the close phylogenetic relationship of these species with *F. verticillioides*. While these results represent a substantial progress in population studies of these fungi, further studies using a broader collection of these microorganisms with other *Fusarium* species, especially those belonging to the *Gibberella fujikuroi* clade (e.g. *Fusarium udum*), will be necessary to assess questions of transferability and polymorphism.

5. Conclusions

Microsatellite distribution in the *F. verticillioides* genome is similar to other ascomycetes, but unlike other previously analyzed fungal genomes, the *F. verticillioides* genome has a high frequency of hexanucleotides.

We have demonstrated that the microsatellite markers developed in this study are highly polymorphic and thus useful for *F. verticillioides* population studies. Our results show that the examined tetra-, penta- and hexanucleotide microsatellites are more informative than previously reported microsatellites. Future studies of these fungal pathogens that have significant effects on sorghum and maize will benefit from successful cross-amplification of these markers in other species of the *Gibberella fujikuroi* clade (*F. thapsinum* and *F. nygamai*).

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Table 4
Transferable microsatellite loci among *Fusarium* species.

Species	PTL[a]	Microsatellite loci/allelic size (bp)							
		Fv-47	Fv-98	Fv-120	Fv-140	Fv-284	Fv-312	Fv-338	Fv-403
F. thapsinum	60%	237	203	NA	229	243	NA	183	322
		240	203		229	243		183	322
F. nygamai	60%	220	183	NA	240	243	NA	179	187
		215	187		240	243		179	NA
F. andiyazi	80%	226	187	214	257	243	NA	NA	218
		226	195	228	257	243	211	206	203
F. oxysporum f.sp lycopersici	20%	NA	NA	NA	224	240	NA	NA	NA

Microsatellite loci Fv-114 and Fv-269 are not shown since no amplification was recorded in any species.
[a] Percentages of transferable loci.

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