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European Journal of Plant Pathology
Published in cooperation with the European Foundation for Plant Pathology

ISSN 0929-1873
Volume 152
Number 2

Eur J Plant Pathol (2018) 152:343-354
DOI 10.1007/s10658-018-1479-7

Springer

Eur J Plant Pathol (2018) 152:343–354
https://doi.org/10.1007/s10658-018-1479-7

Molecular diversity in *Fusarium oxysporum* isolates from common bean fields in Brazil

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Accepted: 26 March 2018 / Published online: 16 April 2018

Abstract The common bean (*Phaseolus vulgaris* L.) is widely cultivated in Brazil and is known as a very important crop for families in this country. Fusarium wilt severely harms common beans and has become a big issue for this crop. In order to assist the breeding programs that target resistance to this disease, the evaluation of genetic diversity of the pathogen and its molecular characterization are crucial. Thus, the present goal was to identify *Fusarium* isolates obtained from several places in Brazil using molecular tools; select molecular markers for these isolates; and analyze their diversity. All of isolates were molecularly identified as *Fusarium oxysporum* f. sp. *phaseoli* (*Fop*). By using seven selected SSR markers, the results of diversity obtained by the dendrogram and the Bayesian analysis formed four groups where a large diversity of this fungus was found within each state. However, the groups were more homogenous according to the collection source and the pathogenicity test. More specifically, group 2 was composed of the most virulent strains and originated from Minas Gerais State – UFV, and group 3 was mostly composed by isolates from Goias state. Group I was also more diverse in terms of location and virulence. The overall results indicated a positive correlation between *Fusarium* diversity and its virulence to common bean. Furthermore, the use of these markers was effective in molecular identification and in detecting polymorphism within *F. oxysporum* f. sp. *phaseoli*.

Keywords Diversity · *Fusarium* · Genetic · Molecular markers · *Phaseolus vulgaris*

Electronic supplementary material The online version of this article (https://doi.org/10.1007/s10658-018-1479-7) contains supplementary material, which is available to authorized users.

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Introduction

The annual production of common bean (*Phaseolus vulgaris* L.) in Brazil reaches more than 3.0 million tons, of which 15% is cultivated under irrigated conditions, especially in the Cerrado region (CONAB 2017). Yield losses due to Fusarium wilt caused by *F. oxysporum* f. sp. *phaseoli* (*Fop*) have been gradually

rising in irrigated areas because of successive plantings and the utilization of susceptible cultivars (Paula Junior et al. 2004; Paula Júnior et al. 2015). This fungus is ubiquitous in all regions that produce beans worldwide (Buruchara and Camacho 2000).

The genus *Fusarium* (anamorphs Giberella, Ascomycete) comprises several phytopathogenic species that infect many crops worldwide and is strongly present in pulse-growing areas, including among common bean (Nene et al. 1996). It usually occurs in plants individually, so the first symptoms in the crop area might not be easily noticed. The number of infected plants may increase until the disease spreads to the whole crop area (Paula Júnior et al. 2015).

This typically soil-borne and seed-borne pathogen infects the root system and causes significant reduction in nodulation and root mass in the case of leguminous plants (Hartman et al. 1997). The symptoms of *F. solani* f. sp. *phaseoli* are characterized by root-rot and lesions in plant stems. And for *Fop,* the symptoms in leaves are basically characterized by discoloration of the vascular tissue that leads to leaf necrosis and premature defoliation (de Farias Neto et al. 2006), with leaf abscission and plant death. Above-ground symptoms also include loss of turgor, starting from the lower leaves, and this may be manifested on only one side of the host plant (Schwartz et al. 2005). Infection may occur even at the seedling stage, impairing development and resulting in stunted plants. This pathogen is sometimes difficult to eradicate due to the ability of fungal chlamydospores to survive for a long time in soil in the absence of a host plant. This fungus is almost ubiquitous in soil worldwide, and its ecological activities indicate a much more diverse role in nature. Even though *Fusarium* communities are abundant in soils, their species diversity may vary, depending on several factors such as host, soil physicochemical properties, climatic conditions, especially humidity, and human activities (Summerell et al. 2010). The diversity of this fungus might be influenced by different geographic scales, in particular their location (Balmas et al. 2010; Laurence et al. 2012). However, the relative importance of environmental factors that might affect *Fusarium* communities is still unknown.

The utilization of a resistant cultivar has been shown to be an efficient method for Fusarium wilt control (Carneiro et al. 2010). However, many growers still use susceptible cultivars, due to the preference of consumers (Toledo Souza et al. 2012). Moreover, the diversity of this fungus is larger than expected, although there is some specificity between the pathogen and the common bean (Paula Júnior et al. 2015; Ramalho et al. 2012). *F. oxysporum* often displays a high degree of host specificity, and it can be classified into *formae speciales* and races based on the plant species and on the host cultivars affected, respectively. The evaluation of this grouping is usually done by pathogenicity test, but this assay is not always conclusive and sometimes excludes nonpathogenic strains. To overcome these problems, DNA-based techniques have been used, particularly the molecular identification of isolates/races, which may develop an effective strategy for the management of wilt diseases (Panthee and Chen 2010; Tsui et al. 2011). Molecular methods are very precise for differentiation between species and identification of new isolates taken from infected plants, with special regard to the genomic sequences in internal transcribed spacer regions in the ribosomal repeat regions (ITS) (O'Donnell and Cigelnik 1997). Moreover, the sequencing with the translation elongation factor 1—alpha (TEF-1) is very precise and informative at the species level to identify the Fusarium (O'Donnell et al. 2015).

Molecular markers are very useful in verifying the intra and inter species relationships, which have been used to verify data that were not polymorphic enough or exhibited problems of paralogous sequences (Aoki and O'Donnell 1999; Costa et al. 2016; Petrov et al. 2016). DNA fingerprinting in *Fusarium* has been well applied to characterize data from individual isolates, and thus it is a tool that is very useful for identifying an unknown sample (Castellá et al. 1999; Silva et al. 2013). Knowledge about pathogen races and diversity is crucial for a breeding program that aims for resistant crop varieties, especially for pyramid resistance genes in an elite genotype.

Simple sequence repeats (SSRs) provide a powerful tool for population genetic studies. SSRs have been used as genetic markers in numerous DNA-fingerprinting experiments for strain typing of a variety of filamentous fungi. SSR markers targeting *Fusarium* genus were developed, such as for the case of *F. circinatum* (Britz et al. 2002) and *F. oxysporum* (Giraud et al. 2002; Bogale et al. 2005). SSR markers were able to distinguish four races of *F. oxysporum* f. sp. *ciceri* (Barve et al. 2001). Also the polymorphism revealed with seven SSR markers was sufficient for the study of genetic diversity in the *F. oxysporum* complex (Bogale et al. 2005). Genetic diversity studies can reveal the adaptive potential of pathogenic populations (Huertas-González

et al. 1999). However, research into the variability of *F. oxysporum* from common bean in Brazil is still limited, as most of the papers focus on a resistance program (Pereira et al. 2011) without deeply evaluating the fungus itself.

The aims of this study were to carry out a molecular identification of *F. oxysporum* isolates isolated from diseased common bean plants in Brazil, test some specific SSR markers on these isolates; and analyze their genetic diversity using the chosen markers.

Materials and methods

Sampling, isolation and DNA extraction

Fifty-three *F. oxysporum* isolates were collected from different bean producing regions of Brazil (Table 1) located in the states of Goiás, Mato Grosso do Sul, Minas Gerais, Pernambuco, Santa Catarina and Sao Paulo and used in this study. They were taken from plants exhibiting typical symptoms of Fusarium wilt as vascular discoloration and cultivated in Petri dishes with medium (PDA + streptomycin) and incubated at 25 °C. The mycelia were removed from the PDA and stored at 4 °C. The morphological criterion was used to identify the isolates, according to the hyphal observations, spores, and mycelium color (Leslie and Summerell 2006). Eighteen isolates were obtained and belong to the Universidade Federal de Viçosa (UFV) and the other isolates were provided by the Instituto Agronômico de Campinas (IAC), Universidade Federal de Lavras (UFLA, Laboratory of Seed Pathology - LAPS), and Embrapa Arroz e Feijão in Brazil.

The DNA extraction (Doyle 1990; Zolan and Pukkila 1986), with some modifications, was carried out by taking around 0.2 g of mycelia and grinding in nitrogen liquid. Then they were homogenized in 600 μl of extraction buffer (200 mM Tris HCl pH 8.0, 250 mM NaCl, 50 mM EDTA and 2% SDS) and kept in water bath (65 °C) for 30 min. Afterwards, this mix was homogenized in 600 μl of Phenol/Chloroform/Isoamil Alcohol (25:24:1) and centrifuged at 23000×g for 5 min, where the supernatant was transferred to another tube. A second cleaning with the same centrifugation was performed using 500 μl of Chloroform/Isoamil Alcohol (24:1). Then 400 μl of the supernatant was recovered, mixed with 400 μl of cooled isopropanol and 40 μl of ammonium acetate 7 M and centrifuged at 23000×g for 15 min. The supernatant was washed twice with 400 μl of Ethanol 70% by shaking and centrifuging for 5 min between each washing. The pellets were then completely dried at room temperature, and re-suspended in 100 μL of milli-Q water containing RNase at a final concentration of 40 μg/mL. The tubes were placed in a water bath at 37 °C for 30 min to dissolve the pellets. The DNA integrity was analyzed in agarose gel electrophoresis, and the concentration was checked with a Nanodrop 2000 spectrophotometer (Thermo Fisher Scientific).

Molecular identification and SSR analysis

An internal transcribed spacer (ITS) region of rDNA was amplified using the primers ITS-5 (GGAAGTAA AAGTCGTAACAAGG) and ITS-4 (TCCTCCGC TTTATTGATATG) (White et al. 1990). The *Fusarium* specific primers FC-1 (CATACCACTTGTTGCCTC) and FC-2 (ATTAACGCGAGTCCCACC) were also used (Zhang et al. 2012). Amplification was performed in total volume of 20 μl containing 0.6 U Taq DNA polymerase (Invitrogen), 0.15 mM dNTPs, 0.2 μM of each primer, 1 mM $MgCl_2$, and 2 μl of template containing 50 ng of genomic DNA. The PCR was conducted under the following conditions: an initial denaturation for 3 min at 94 °C followed by a cycle of 30 s denaturation at 94 °C, 1 min annealing at 55 °C, 1 min elongation at 72 °C repeated 35 times, and final elongation step of 7 min at 72 °C. The PCR products were subjected to electrophoresis in agarose gel (0.8%) and 1× TBE, and viewed under UV light after staining with Ethyl Bromide (100 ppm in 1X TBE); then they were submitted to sequencing (Genome Quebec, Co. Canada). The sequences were compared to those in the Fusarium MSLT database (http://www.westerdijkinstitute.nl/Fusarium/), whose similarity and identification number were recorded.

The PCR for SSR analysis was carried out with specific markers (Table 2) whose amplification was done with 0.6 U Taq DNA polymerase (Invitrogen), 0.15 mM dNTPs, 0.2 μM of each primer, 2 mM $MgCl_2$, and 50 ng template of genomic DNA. The PCR was conducted with the following thermocycler program: Initial denaturation for 2 min at 94 °C, Touchdown cycling repeated 10 times by 30 s denaturation at 94 °C, 30 s annealing from 59 to 50 °C, and 30 s elongation at 72 °C, then another cycling composed of 30 s denaturation at 94 °C, 30 s annealing at 50 °C, and

Table 1 *Fusarium oxysporum* f. sp. *phaseoli* isolates with their respective origins and codes

Number	Code	City	State	Institution
1	Fop14435	Pindorama	SP	IAC
2	Fop11173	Angatuba	SP	IAC
3	Fop14629	Cerqueira César	SP	IAC
4	Fop11472	Itararé	SP	IAC
5	FopUFV07	Coimbra	MG	UFV
6	FopUFV08	Unaí	MG	UFV
7	Fop14645	Unaí	MG	IAC
8	Fop11178	Taquarituba	SP	IAC
9	Fop11205	Casa Branca	SP	IAC
10	Fop14353	Campos Novos	SC	IAC
11	Fop11257	Capão Bonito	SP	IAC
12	Fop11299	Capão Bonito	SP	IAC
13	FopUFV03	Viçosa	MG	UFV
14	FopUFV05	Canaã	MG	UFV
15	FopUFV06	Coimbra	MG	UFV
16	FopUFV04	Viçosa	MG	UFV
17	Fop 46	Belém de São Francisco	PE	Embrapa Arroz e Feijão
18	FopUFV01	Viçosa	MG	UFV
19	FopUFV10	Viçosa	MG	UFV
20	FopUFV02	Viçosa	MG	UFV
21	FopUFV09	Vianópolis	GO	UFV
22	FopLAPS 163	Campinas	SP	UFLA
23	FopLAPS 161	Campinas	SP	UFLA
24	FopLAPS 507	Dourados	MS	UFLA
25	FopLAPS 160	Campinas	SP	UFLA
26	FopLAPS 164	Rio Verde	GO	UFLA
27	FopLAPS 505	Dourados	MS	UFLA
28	FopLAPS 503	Dourados	MS	UFLA
29	FopLAPS 502	Dourados	MS	UFLA
30	FopLAPS 509	Santo Antônio do Goiás	GO	UFLA
31	FopLAPS 166	Lavras	MG	UFLA
32	FopLAPS 510	Santo Antônio do Goiás	GO	UFLA
33	FopLAPS 506	Guaíra	SP	UFLA
34	FopLAPS 156	Lavras	MG	UFLA
35	FopLAPS 157	Lavras	MG	UFLA
36	FopLAPS 168	Patos de Minas	MG	UFLA
37	Fop 03	Anápolis	GO	Embrapa Arroz e Feijão
38	Fop 07	Santo Antônio do Goiás	GO	Embrapa Arroz e Feijão
39	Fop 15	Santo Antônio do Goiás	GO	Embrapa Arroz e Feijão
40	Fop 101	Santo Antônio do Goiás	GO	Embrapa Arroz e Feijão
42	Fop 106	Lavras	MG	Embrapa Arroz e Feijão
43	Fop 56	Santo Antônio do Goiás	GO	Embrapa Arroz e Feijão
44	Fop 103	Santo Antônio do Goiás	GO	Embrapa Arroz e Feijão
45	Fop 13	Santo Antônio do Goiás	GO	Embrapa Arroz e Feijão

Table 1 (continued)

Number	Code	City	State	Institution
46	Fop 42	Santo Antônio do Goiás	GO	Embrapa Arroz e Feijão
47	FopUFV11	Presidente Bernardes	MG	UFV
48	FopUFV12	Presidente Bernardes	MG	UFV
49	FopUFV13	Presidente Bernardes	MG	UFV
50	FopUFV14	Presidente Bernardes	MG	UFV
51	FopUFV15	Presidente Bernardes	MG	UFV
52	FopUFV16	Presidente Bernardes	MG	UFV
53	FopUFV17	Porto Firme	MG	UFV
54	FopUFV18	Porto Firme	MG	UFV

30 s elongation at 72 °C repeated 30 times, followed by a final elongation of 8 min at 72 °C. Eighteen markers from Invitrogen were tested in agarose gel and seven were selected for the SSR analysis: SSR1, SSR5, SSR7, SSR9 (Bogale et al. 2005), FomSSR-2, FomSSR-6 and FomSSR-9 (Mahfooz et al. 2012). PCR products were analyzed in 6% polyacrylamide gels and visualized by autoradiography.

The primary function was to analyze the band size at a specific marker. The bands amplified by SSR markers (codominant) were coded according to the observed genotype and software requirements within the targeted band size. Alleles were recorded by band observation, using the haploid criteria, with each allele codified by one digit number (de Brito et al. 2010). For example, in the locus containing three alleles the samples were coded as 1, 2 and 3. The distance matrix was generated by the arithmetical complement of the weighted index (Cruz et al. 2011) implemented in the GENES software (Cruz 2013) according to the following equation:

$$D_{ii'} = 1 - \left(\frac{1}{2} \sum_{j=1}^{L} p_j c_j \right)$$

Where, Dii′ means the genetic distance between pairs of accessions i and i′; L is the total number of loci studied; and C_j is the number of common alleles between pairs of accessions i and i′. the PIC (Polymorphism Information

Table 2 Allele amplification of *Fusarium oxysporum* f. sp. *phaseoli* with seven primer

Primer	Primer sequence (5′-3′)	Number of Alleles	Amplified alleles size (bp)	F	PIC value	Gene diversity*
SSR 1	TGCTGTGTATGGATGGATGG CATGGTCGATAGCTTGTCTCAG	6	300	0.97	0.64	1.51
SSR 5	GTGGACGAACACCTGCATC AGATCCTCCACCTCCACCTC	4	400	1.0	0.70	1.44
SSR 7	CGTCTCTGAACCACCTTCATC TTCCTCCGTCCATCCTGAC	3	300	1.0	0.32	0.96
SSR 9	GGTAGGAAATGACGAAGCTGAC TGAGCACTCTAGCACTCCAAAC	3	350	1.0	0.34	0.88
FomSSR-2	TCATTCTCCATGTCCTCATC TCGTTCCGATAGTAATTCGTCA	3	180	1.0	0.68	1.44
FomSSR-6	ACACTCCAAGAACTCAGCATCA GACAAAACTCGCTATTCGTTCC	4	220	1.0	0.58	1.34
FomSSR-9	GCACACAATTCTATCCTCCTCC CTGAAAGTGCTGTTGATACGCT	3	350	0.93	0.55	1.32
Mean				0.98	0.53	1.07

*Gene diversity among individuals within the population calculated using Shannon-Weaver index

Content) was calculated to verify the differences among markers in their information systems (Nagy et al. 2012) by Popgenes software (Yeh 1997).

The dendrogram was constructed using the unweighted pair group method using the arithmetical average (UPGMA), and the clustering technique from the values of the distance matrix generated, which established genetic relationships among different isolates. This dendrogram was generated using the MEGA7 software (Kumar et al. 2016).

The matching between the distance matrices and the dendrogram was estimated with the cophenetic correlation coefficient. Genetically similar clusters were sought by applying Principal Component Analysis (PCA). Genotypic diversity was estimated by Shannon-Wiener measures, using the GENES software, and the N1 (Hill 1973), Alpha and Beta index with R (Vegan and Vegetarian packages).

Evaluation of virulence

The pathogenicity test for each isolate was performed first by inserting bean roots (ten plants) from a susceptible cultivar (BRS Valente) in a suspension of fungal conidia, which was obtained from PDA medium for seven days at 25 °C, 12 h dark and 12 h light. After the pathogenicity test, 45 isolates of *Fop* were used to evaluate the virulence in four common bean cultivars (BRS Valente, Manteigao Fosco, RP-1 and Perola). The experimental design was completely randomized with three replicates (pots with three plants) for each isolate totalizing nine plants per treatment. After the appearance of the first leaf pair (around 10 days) the inoculation was done. The plants were removed from the substrate and their roots were washed, cut at about 1/3 of their length and immersed in the conidial suspension (10^6 conidia/mL) for 5 min. The control plants were immersed in water. After the inoculation, the seedlings were transplanted to 1-L pots with Tropstrato® (Vida Verde Co., Mogi Mirim, Brazil) and grown in the greenhouse for 25 days. After that, the severity was visually evaluated using the scale 1–9 (Pastor-Corrales and Abawi 1987). Both experiments on pathogenicity test and virulence were performed once, where the average of severity scale was recorded.

The correlation between virulence of the *Fop* and the diversity was also determined by the Mantel test after obtaining both matrices by Euclidian analysis and Gower distance, respectively, with GENES software.

Results

Identification of the isolates

All isolates were identified as *F. oxysporum*, (escept for the isolate no. 40 (Fop 101) that matched to *F. solani*), according to the Fusarium MSLT database with similarity ranging between 99 and 99.65% (Suppl. Table 1). Furthermore observations on fungal morphological features (microconidia oval, elliptical or kidney shaped, usually without septate and conidiogenous cells on short monophialides), colony features on PDA (mycelia floccose, sparse or abundant and range in color from white to pale violet), isolation site of plants exhibiting symptoms of Fusarium wilt as vascular discoloration (Suppl. Fig. 1) and the results of the pathogenicity and virulence test in common bean confirmed the causal agent (*F. oxysporum* f. sp. *phaseoli*), responsible of the disease, whose sequences with around 400 bp were deposited in the DNA Data Bank of Japan (http://www.ddbj.nig.ac.jp).

SSR marker analysis

The polymorphism analysis of isolates was performed using SSR molecular markers. Among the 18 markers tested, seven were chosen based on clear bands at their respective size.

After verifying the temperatures with touchdown system and the concentrations of $MgCl_2$, the ideal amplification conditions for SSR analysis were optimized as described in the methodology. The main advantage of the single temperature range was that it can fit all markers, and it is unnecessary to run a different PCR for each marker. Amplified fragment size of these markers ranged from 100 to 400 bp, and 26 alleles were amplified, with an average of 3.7 alleles per locus. A maximum of six alleles were amplified by SSR1, whereas a minimum of three alleles came from SSR7, SSR9, FomSSR-2 and FomSSR-9 (Table 2). The PIC (Polymorphism Informative Content) value had the mean of 0.53, where the highest one was reached by the SSR5. However, there was a disparity between the means of these parameters, in particular for the primers SSR-7 and FomSSR-2. The diversity of genes, evaluated by the Shannon-Wiener index, reached the maximum value with the SSR1, SSR5 and FomSSR-2 markers, whereas the lowest one was the SSR9 (Table 2).

Genetic diversity based on SSR markers and correlation with virulence

The seven optimized markers were analyzed in a fungi collection containing 53 isolates. The analysis of fragment patterns generated by each primer led to the construction of a genetic similarity matrix according to the arithmetic complement of weighted index, which was used to construct the dendrogram based on UPGMA methodology (Fig. 1).

All isolates are remarkably distinguished by their genotypes, showing the absence of replicates in the collection. They were separated into major clusters and the number IV cluster was subdivided into three ones (a, b and c). The first cluster contained one isolate from Goias state. Cluster II had one from Sao Paulo and another from Minas Gerais, whereas sub-cluster IVa contained one from Pernambuco and Sao Paulo. Clusters III and IV comprised 18 and 32 isolates, respectively, from several states. All isolates molecularly identified within *Fusarium* were not grouped homogeneously in their respective single clusters based on location and virulence as demonstrated by the dendrogram (Fig. 1).

The population diversity among the states demonstrated that Minas Gerais was the highest, according to the Shannon Weaver index. However, the top value in Hill's index was found in Goias state, and Sao Paulo showed the lowest one. Alpha and Beta index showed Goias and Minas Gerais as the highest and lowest value in terms of diversity respectively (Table 3).

A PCA graph, with two main axes, showed a light contrast between clusters III and IV, with a 26% of variability. The separation in these groups followed the dendrogram with their respective locations (Fig. 2). All bean cultivars showed different levels of susceptibility to *Fop*, for which Manteigão Fosco and Valente were the most and least resistant, respectively. The average severity indicated that isolates 22, 23 and 54 had the highest severity ratings, whereas 1, 3, 11 and 16 had the lowest (Suppl. Table 2). Also the virulence of *Fop* was positively correlated to the diversity at 99% according to the Mantel test (Fig. 3).

Discussion

The molecular identification of all the isolates as F. oxysporum with 100% identity can indicate their similarity within the *Fusarium* genus, however, after the confirmation of the morphological features and

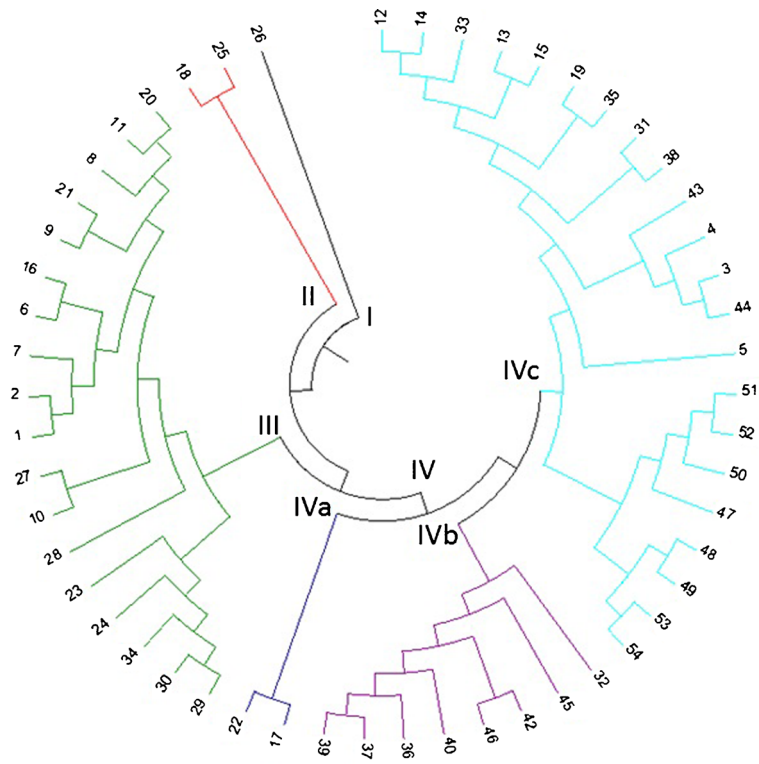

Fig. 1 Dendrogram showing the genetic relationship among *Fusarium oxysporum f. sp. phaseoli* isolates from common bean based on 7 SSR markers

Table 3 Diversity index for the *Fusarium oxysporum* f. sp. *phaseoli* populations according to the state (Only those states containing more than 10 individuals were considered)

	Sao Paulo	Minas Gerais	Goias
Sample size*	11	24	12
SW index#	0.86	0.96	0.91
Hill's index@	4.51	4.55	4.65
Alpha index$	0.15	0.14	0.16
Beta index&	0.89	0.92	0.90

*Number of individuals sampled

@ Hill's diversity index

Shannon Weaver diversity index

$ Alpha diversity index

& Beta diversity index

symptoms, the sequences were registered as *F. oxysporum* f. sp. *phaseoli*. In fact, the ITS region alone is not strong enough to confirm the *Fusarium* at species level (O'Donnell et al. 2012), therefore the further confirmation by morphology, pathogenicity test and isolation source (Common bean - *P. vulgaris* L.) was necessary.

Among the eighteen tested markers, it was demonstrated that seven were enough for the current study of genetic diversity in *Fop* from bean, as they discriminated all isolates. The SSR primers were able to amplify single bands that showed enough polymorphism, with a range of allele sizes and diversities clearly evident in each locus. By using 10 selected markers, the relative abundance and density of microsatellites were higher in *F. oxysporum* f. sp. *melonis* than in *F. oxysporum* f. sp. *lycopersici* (Mahfooz et al. 2012). In fact, high levels of polymorphism associated with SSR were due to the unique mechanism to generate allelic diversity by replication slippage (Tautz 1989). The knowledge of abundance and distribution of genetic variability within

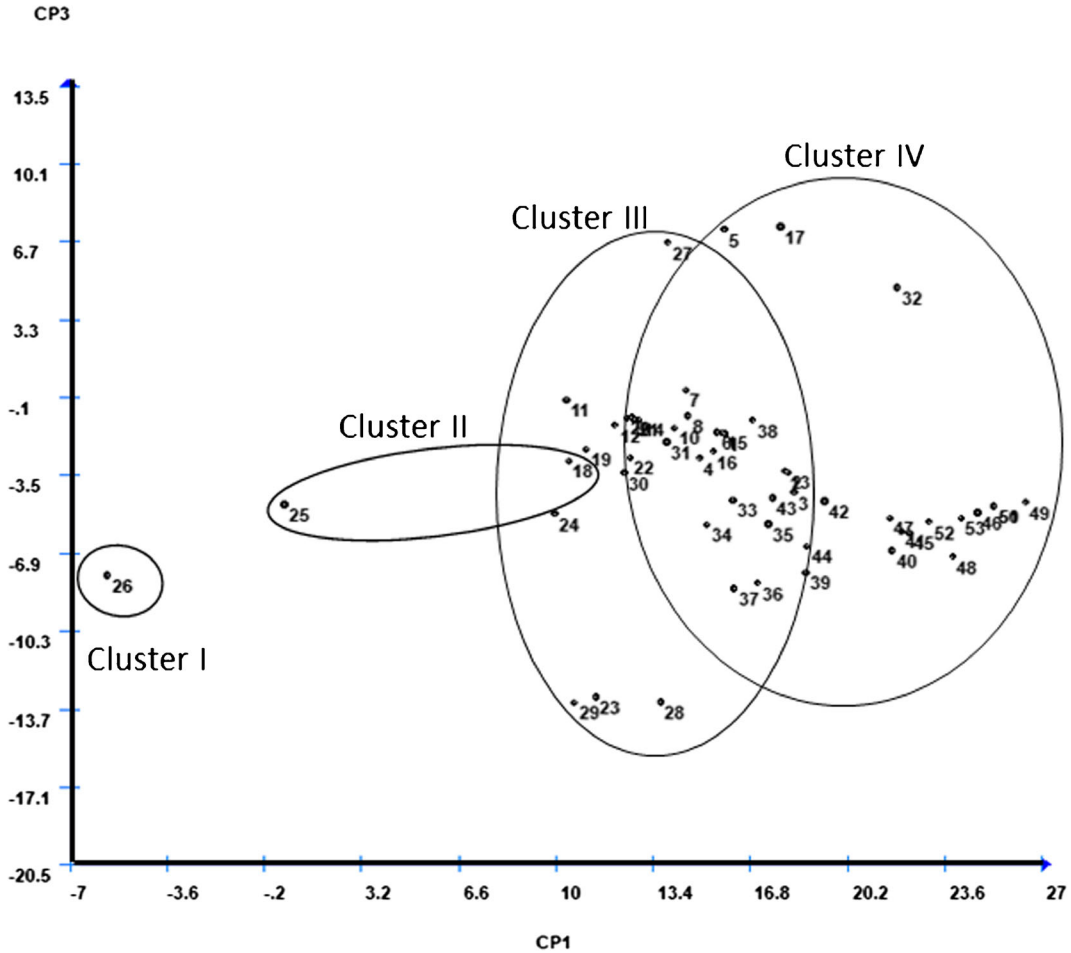

Fig. 2 Principal Component Analysis (PCA) of *Fusarium oxysporum f. sp. phaseoli* isolates from common bean. Clusters are separated by circles according to the Bayesian criterion analysis

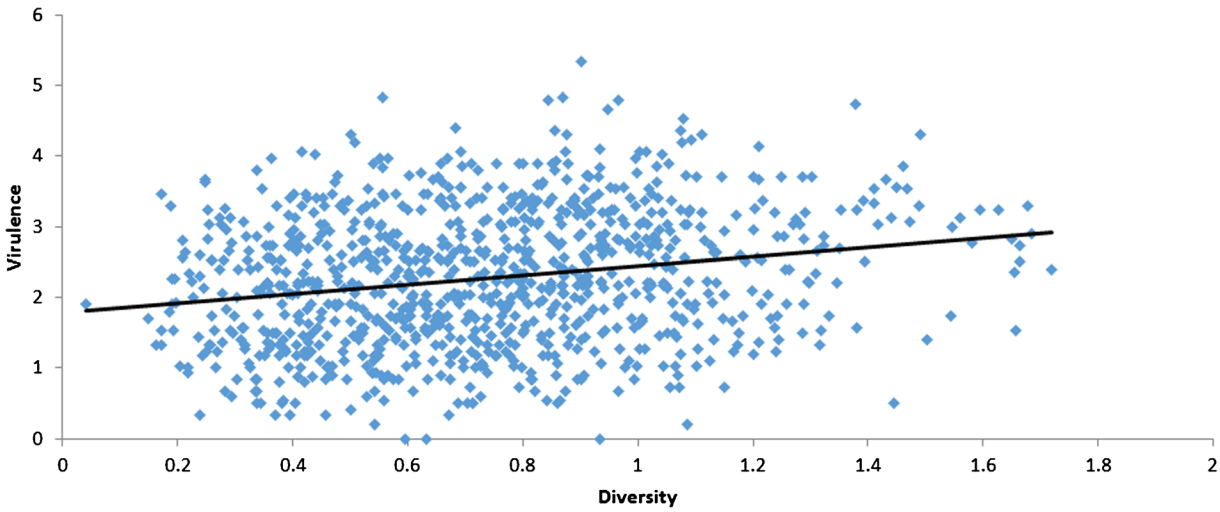

Fig. 3 Correlation between the virulence and diversity of *Fusarium oxysporum f. sp. phaseoli* isolates from common bean

Fusarium is crucial in understanding their genetic relationship (Bruns et al. 1991). The SSR technique is able to amplify small fragments of DNA and is a very strong tool for population genetic studies (Britz et al. 2002), and its advantages are due to the hypervariablity and abundance in eukaryotic genomes and co-dominance (Datta et al. 2011). Microsatellites have been applied to polymorphism studies of many plant pathogens, including *Sclerotinia* (Lehner et al. 2015) and *Rhizoctonia solani* (MwangOmbe et al. 2007).

In the current study, the diversity of *F. oxysporum* collected from common bean in different Brazilian states was high, so it was not possible to group them by collected location, or by their pathogenicity, where the most virulent isolates were in group IV. These data corroborate the positive correlation between the virulence and diversity. This high variability might be derived from genetically distinct clones, and the exchange of contaminated seeds and cultures could contribute to a variable population of *Fop* in wider geographic areas. This discrepancy may occur due to transfer of lineage-specific genomic regions in this fungus, which includes four chromosomes (Ma et al. 2010), and also the distribution of SSR in the genome is not random (Mahfooz et al. 2012). Many regions of rDNA have been used to study the diversity and phylogeny of *Fusarium* spp. (Alves-Santos et al. 2002; Oliveira et al. 2014). Usually these regions are well conserved and can be investigated with PCR amplification (Datta et al. 2011).

In contrast to the current results, the virulence level of *Sclerotinia sclerotiorum* from common bean was very similar, and no correlation was found between mycelial compatibility group and SSR, represented by genetic distance (Lehner et al. 2016). In the case of *S. sclerotiorum*, the markers are selectively neutral, but the SSR loci sometimes have functional roles (Li et al. 2002). The pathogenic loci of this fungus are usually independent of SSR loci, which could explain the non-association between virulence and SSR (Goodwin et al. 1995). Pathogenicity is a multigene trait, suggesting the unexpected association between this characteristic and SSR locus (Lehner et al. 2016).

In other studies, with *F. oxysporum* and *F. udum*, the fingerprinting-based group was totally different from those of virulence and geography. Large variations in symptoms are sometimes correlated to location and polymorphisms, and the genome analysis of *F. udum* by SSR clearly proved the high genetic variability of this fungus, which should be considered in breeding programs targeting wilt resistance (Datta et al. 2011). *F. udum* from the same or different sites can show high variability in terms of cultural characteristics and pathogenicity on pigeon pea (Gaur and Sharma 1989). Using the same fungi, other studies of genetic diversity with isoenzyme markers demonstrated low variation, followed by lack of correlation between molecular diversity and location of origin (Shit and Sen 1978).

Considering the location, this research showed that there was no relationship between the location and the genetic groups. The association between the geographic origins of the isolates and their respective measures of severity is usually very uncommon (Oliveira et al. 2014); indeed, the most virulent strains sometimes originate from many places in the same country. However, SSR markers were able to group *Fusarium* isolates from

lentil into two major groups based on their geographic location (Barve et al. 2001; Datta et al. 2011). These authors recorded different allele sizes using SSR markers, and the variable number of alleles per loci indicates a high level of polymorphism. In the case of the current study, the average PIC value of 0.55 could indicate a high degree of diversity. In early studies, the SSR markers for *Fusarium* placed isolates from lentil in two groups separated by 27% (Datta et al. 2011) and 25% with *F. oxysporum* f. sp. *ciceris* (Dubey and Singh 2008) of genetic similarity. Considerable variability was also found in some isolates of *Fop* (Woo et al. 1996) and *F. oxysporum* f. sp. *ciceris* (del Mar Jiménez-Gasco et al. 2001) belonging to the same agro climatic regions.

The high degrees of polymorphism generated by SSR markers in this study suggest that this technique should be sufficient to analyze the genetic diversity among *Fusarium* isolates from common bean. Many *Fusarium formae speciales* have high levels of genetic variability among the same species (Aoki et al. 2005), which suggests sexual recombination in the field. The genetic variability in *Fop* detected by SSR markers suggests the ability of the pathogen to adapt to different geographic conditions and cultural practices, knowledge which could be applied in the development of bean cultivars and location-specific resistant plants. This information has become a challenge in breeding programs that seek resistance genes against pathogenic races. These genes should be focused due to their ability to resist infection and their suitability for agro-climatic regions.

Acknowledgements This research was supported by Research Support Foundation of Minas Gerais (FAPEMIG) - Brazil and the National Council for Scientific and Technological Development (CNPq) - Brazil. Thanks also to laboratory of Coffee Biotechnology (Universidade Federal de Vicosa - UFV) for their assistance during the experiment. The authors also thank the Instituto Agronomico de Campinas (IAC), Universidade Federal de Lavras (UFLA) - Laboratory of Seed Pathology, and Embrapa Arroz e Feijao for provide the isolates. Finally the authors would like to express their gratitude to Prof, Dr. Cosme Damiao da Cruz and the laboratory of Bioinformatics for help with the statistical analysis.

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