Phytopathology • 2020 • 110:1093-1104 • https://doi.org/10.1094/PHYTO-09-19-0366-R

Mycology

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Identification of Pathogenicity Groups and Pathogenic Molecular Characterization of *Fusarium oxysporum* f. sp. *sesami* in China

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Accepted for publication 12 February 2020.

ABSTRACT

Fusarium oxysporum f. sp. *sesami* is an extremely destructive pathogen, causing sesame Fusarium wilt disease worldwide. To clarify the pathogenicity and the genetic characters of *F. oxysporum* f. sp. *sesami*, we systematically investigated 69 *F. oxysporum* isolates collected from major sesame-growing areas in China. Among these isolates, 54 isolates were pathogenic and 15 were nonpathogenic according to pathogenicity testing on sesame seedlings. For the pathogenic isolates, three *F. oxysporum* f. sp. *sesami* pathogenicity groups were defined based on the three differential sesame hosts for the first time. A translation elongation factor 1α gene tree was constructed to determine the genetic diversity of the *F. oxysporum* isolates but could not separate *F. oxysporum* f. sp. *sesami* isolates from the nonpathogenic isolates and other *F. oxysporum* formae speciales. Ten *secreted-in-xylem* (*SIX*) genes (one family of effectors) were identified in *F. oxysporum* f. sp. *sesami* isolates by a search with the genome data, and were subsequently screened in the 69 *F. oxysporum* isolates. Compared with the *SIX* gene profiles in other *F. oxysporum* formae speciales, the presence and sequence variations of the *SIX* gene homologs directly correlated with the specific pathogenicity of *F. oxysporum* f. sp. *sesami* toward sesame. Furthermore, eight of these *F. oxysporum* f. sp. *sesami SIX* genes were significantly expressed in sesame plants as infection of the *F. oxysporum* f. sp. *sesami* isolate. These findings have important significance for understanding the pathogenic basis of *F. oxysporum* f. sp. *sesami* isolates, and will contribute to improve the diagnostics to effectively control Fusarium wilt disease in sesame.

Keywords: *Fusarium oxysporum*, Fusarium wilt, pathogenicity, *secreted-in-xylem*, sesame

Sesame (*Sesamum indicum* L.) is one of the oldest oilseed crops and is widely cultivated in tropical and subtropical regions of Asia, Africa, and South America (Ashri 1998). Sesame production is seriously threatened by Fusarium wilt disease caused by *Fusarium oxysporum* f. sp. *sesami*, which was first found in the United States (Armstrong and Armstrong 1950) and was subsequently reported in China, Korean, Egypt, India, Sudan, and other main sesame production countries (Cho and Choi 1987; Li 1989; Verma et al. 2005). In China, *F. oxysporum* f. sp. *sesami* has been spread in most sesame-growing areas. The fungus commonly infects the roots of sesame plants, causing damping-off at the seedling stage, leaf chlorosis and abscission, stem necrosis, internal vascular browning, and eventually whole-plant wilting and death at the adult stage (Li et al. 2012; Su et al. 2012). Recently, a number of *F. oxysporum* f. sp. *sesami* isolates have been identified from wilted sesame plants and their morphological and pathogenic characteristics were further described (Li et al. 2012; Qiu et al. 2014; Su et al. 2012). However, only a single study has attempted to analyze the genetic diversity of *F. oxysporum* f. sp. *sesami* isolates by an amplified fragment length polymorphism (AFLP) method, which showed an ambiguous correlation between molecular characteristic and pathogenicity (Li et al. 2012). Thus far, a study on pathogenic differentiation, genetic diversity, and pathogenic basis of *F. oxysporum* f. sp. *sesami* isolates is not yet available.

F. oxysporum is well known as a ubiquitous soilborne plant pathogen that infects a wide range of plant hosts worldwide and always causes significant economic losses (Gordon and Martyn 1997; Michielse and Rep 2009). As a species complex, *F. oxysporum* comprises a diversity of morphologically indistinguishable nonpathogenic and pathogenic isolates (Snyder and Smith 1981). Conventionally, pathogenic isolates are assigned to intraspecific groups designated as formae speciales based on their host specificity (Snyder and Hansen 1940). Furthermore, several formae speciales can be subdivided into races based on their pathogenic specificity to different genotypes, lines, or cultivars of the same host species (Armstrong and Armstrong 1981).

To understand the pathogenic differentiation and genetic diversity in the *F. oxysporum* species complex (FOSC), numerous phylogenetic studies have been carried out using standard molecular approaches such as DNA fingerprinting by AFLP, restriction fragment length polymorphism, and random amplified polymorphic DNA; and locus genotyping based on sequences of internal transcribed spacer (ITS), intergenic spacer, and housekeeping genes, including translation elongation factor 1α (*EF-1a*), β-tubulin (*Tub*), RNA polymerase II, and so on (Baayen et al. 2000; Epstein et al. 2017; Lievens et al. 2008; Recorbet et al. 2003; Taylor et al. 2016). These studies revealed that the FOSC, including both phytopathogenic and clinical isolates, could be divided into four evolutionary clades (O'Donnell et al. 2004). Furthermore, the phylogenetic classification was attempted as the molecular method for identification of pathogenic *F. oxysporum* isolates, which was classically based on pathogenicity testing (Lievens et al. 2008). However, standard molecular approaches have not been successful in distinguishing between pathogenic and nonpathogenic isolates or between different formae speciales (Taylor et al. 2016).

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Funding: Key Laboratory of Specific Oilseed Crops Genomics of Henan Province, the earmarked fund for China Agriculture Research System (CARS-14), International Cooperation and Exchanges Project of Henan Province (182102410040), Science-Technology Foundation for Outstanding Youth Scientists of Henan Academy of Agricultural Sciences (2018YQ26), the Plan for Scientific Innovation Talent of Henan Province (184200510002), the Distinguished Professor Program of Institutions of Higher Learning in Henan Province (DPPIHL2017), and the Innovation Scientists and Technicians Troop Construction Projects of Henan Province (ISTTCPHP2016).

*The *e*-**X**tra logo stands for "electronic extra" and indicates that two supplementary figures and three supplementary tables are published online.

The author(s) declare no conflict of interest.

The genetic basis of pathogenicity in the FOSC has been found to involve specific virulence genes, which are required for virulence in host plants (Hogenhout et al. 2009). Recently, a number of putative effector genes were found to localize on the accessory lineage-specific chromosomes in *F. oxysporum* f. sp. *lycopersici* (Ma et al. 2010). Among them, 14 effector genes were identified and designated as *secreted-in-xylem* (*SIX*) genes. These genes encode small, cysteine-rich proteins, and are secreted into the xylem sap during infection of tomato plants (Houterman et al. 2007; Lievens et al. 2009; Rep and Kistler 2010; Rep et al. 2004; Schmidt et al. 2013). Moreover, five *F. oxysporum* f. sp. *lycopersici SIX* genes—*SIX1* (*Avr3*), *SIX3* (*Avr2*), *SIX4* (*Avr1*), *SIX5*, and *SIX6*—were found to contribute directly to host-specific pathogenicity (Gawehns et al. 2014; Houterman et al. 2009; Ma et al. 2015; Niu et al. 2016; Rep et al. 2004), while *SIX1*, *SIX3*, and *SIX4* were also recognized as avirulence genes by corresponding resistance genes *I-3*, *I-2*, and *I* in tomato (Houterman et al. 2009; Ma et al. 2015; Rep et al. 2004; Takken and Rep 2010). At present, a large number of homologs of *F. oxysporum* f. sp. *lycopersici SIX* genes have been subsequently detected in other *F. oxysporum* formae speciales, including *betae*, *canariensis*, *cepae*, *conglutinans*, *cubense*, *dianthi*, *fragariae*, *lilii*, *lycopersici*, *medicaginis*, *melonis*, *niveum*, *passiflorae*, *phaseoli*, *physali*, *pisi*, *raphani*, *vasinfectum*, and *zingiberi* (Chakrabarti et al. 2011; Covey et al. 2014; Czislowski et al. 2018; Fraser-Smith et al. 2014; Laurence et al. 2015; Lievens et al. 2009; Simbaqueba et al. 2018; Taylor et al. 2016; Thatcher et al. 2012; Williams et al. 2016). These homologs of *SIX* genes commonly form a particular combination within individual *F. oxysporum* formae speciales or races (Chakrabarti et al. 2011; Czislowski et al. 2018; Lievens et al. 2009). Several studies have also demonstrated that the presence and sequence variations of *SIX* genes could be used as ideal loci for pathogenicity-based molecular diagnostics to distinguish between *F. oxysporum* formae speciales or races (Chakrabarti et al. 2011; Fraser-Smith et al. 2014; Lievens et al. 2009).

In this study, we systematically investigated the pathogenicity of 69 *F. oxysporum* isolates collected from major sesame-growing areas in China, and subdivided them into three pathogenicity groups based on the three differential sesame hosts with different genetic backgrounds of resistance to Fusarium wilt, which were selected from more than 500 sesame germplasm accessions worldwide using resistance evaluation techniques (Miao et al. 2019; Qiu et al. 2014). To further explore the relationship between the pathogenicity and genetic diversity of *F. oxysporum* isolates from sesame, molecular characterization of *EF-1a* and several *SIX* genes was determined. Particularly, the homologs of 10 *SIX* genes were first identified in *F. oxysporum* f. sp. *sesami* isolates. The presence and sequence variations of these *SIX* gene homologs were demonstrated to correlate with specific pathogenicity of *F. oxysporum* f. sp. *sesami* toward sesame. The results revealed the availability of *SIX* genes for reliable discrimination of *F. oxysporum* f. sp. *sesami* isolates from nonpathogenic isolates and other formae speciales.

MATERIALS AND METHODS

Collection of *Fusarium* isolates. A large number of wilted sesame plants were collected from major sesame-growing areas in China between 2007 and 2011. The *Fusarium* isolates were separated and purified using the method described by Su et al. (2012). A small section, 2 to 3 cm in length, was cut from the infected tissues and surface sterilized in 70% ethanol for 1 min followed by 1.25% NaClO for 10 min, then rinsed in sterile distilled water three times. The vascular tissue were removed from the sterilized section and placed on potato dextrose agar (PDA) medium with streptomycin at 100 µg/ml, and incubated for 3 to 4 days at 28°C. After the morphological characteristics of *Fusarium*-like colonies, hyphae, and microconidia were observed, the isolates were purified by single-spore isolation, and maintained on PDA plates. A representative subset of 69 *F. oxysporum* isolates and three *F. solani* isolates from sesame was selected for subsequent pathogenicity testing, gene amplification, and phylogenetic analysis (Table 1).

Pathogenicity testing. The aforementioned 72 *Fusarium* isolates were assessed for their pathogenicity toward sesame seedlings as described by Qiu et al. (2014). Each isolate was cultured on PDA for 5 to 7 days at 28°C. One agar plug (5 mm) from the colony edge was placed in a culture flask containing potato dextrose broth (PDB) and incubated at 28°C and 120 rpm on a rotary shaker for 4 days. Microconidial suspensions were filtered through three layers of sterilized gauze and centrifuged at 5,000 rpm for 8 min. The microconidial pellet was washed three times in sterile distilled water to remove the PDB. Then, the spores were resuspended to a final concentration of 4×10^6 conidia/ml for inoculation.

Three sesame cultivars (Yuzhi 11, Ji 9014, and HJ16) were chosen for the pathogenicity evaluation. Three independent replicates for each variety were set for each treatment. Ten seeds for each sesame cultivar were grown in 12-cm-diameter plastic pots with infected medium containing a mixture of conidial suspensions, sterilized vermiculite, and soil (1:3:3 [vol/vol/vol]). The final concentration of *F. oxysporum* f. sp. *sesami* isolates was set at 5 to 7×10^5 conidia/ml. Inoculated plants were positioned in growth chambers with day and night of 15 and 9 h at 28 and 23°C, respectively, and 70% relative humidity. The 10-fold-diluted liquid Murashige-Skoog medium was watered regularly for seedling growth. Sterile distilled water was used as control.

Fusarium wilt disease symptoms of each treatment were recorded after 28 days inoculation. The symptoms of roots, stems, and leaves were scored using a categorical disease severity scale, where 0 = no visible symptoms, 1 = slight wilting of seedling, 2 = severe browning of principal root, wilting of plant, and slow growth, and 3 = death of the whole plant. The disease index (DI) data were further calculated using the formula described by Qiu et al. (2014). The significant difference in pathogenicity between *Fusarium* isolates was analyzed using analysis of variance in SAS software (SAS Institute Inc.). The pathogenicity levels of *Fusarium* isolates were also evaluated using the grade scale as follows: $0 \leq DI \leq 20$ = nonpathogenicity or weak pathogenicity, $20 < DI \leq 50$ = moderate pathogenicity, and $50 < DI \leq 100$ = high pathogenicity.

DNA extraction. The mycelia of each *Fusarium* isolate were collected on three layers of sterilized gauze, and the excess liquid was removed using filter papers. Immediately, the mycelia were frozen in liquid nitrogen and stored at –80°C for genomic DNA extraction. Genomic DNA was extracted using a Qiagen DNeasy Plant Minikit (Qiagen) according to the manufacturer's instructions.

PCR amplification of *EF-1a* gene. The *EF-1a* gene was used to distinguish taxa at the infraspecies level within the 69 *F. oxysporum* isolates from sesame. The *EF-1a* gene sequences were amplified by PCR with a primer pair designed using Primer Premier 5.0 (PREMIER Biosoft) (Table 2). PCRs were set up in a 20-µl mixture containing 1 × PCR Buffer, 0.1 mM dNTPs, 0.2 µM of each primer, 1.0 U of Taq polymerase (Vazyme), and approximately 50 ng of DNA. PCR amplifications were performed on an Eppendorf Mastercycler (Eppendorf) under the following conditions: 95°C for 2 min; 35 cycles of 95°C for 20 s, 55°C for 30 s, and 72°C for 1 min; with a final 8-min extension at 72°C. The products were visualized by electrophoresis on 1% agarose gel using a Gel Doc XR+ Imaging System (Bio-Rad) and purified using a SanPrep Column DNA Gel Extraction Kit (Sangon Biotech), then sequenced on an ABI 3730 XL DNA Analyzer (Applied Biosystem) by forward and reverse primers. The *EF-1a* gene sequences in the 69 *F. oxysporum* isolates were deposited into GenBank with the accessions MN417138 to MN417206. Additionally, *EF-1a* gene sequences in other species were obtained from the publicly available genomes of *F. oxysporum* (https://www.ncbi.nlm.nih.gov/genome/genomes/707) and the NCBI nucleotide database through a BLAST search on a reference sequence in *F. oxysporum* f. sp. *sesami* isolate FS08027 (Supplementary Table S1).

TABLE 1. *Fusarium* isolates used for pathogenicity testing, phylogenetic analysis, and identification of *secreted-in-xylem* genes in this study

Fusarium spp.	Isolate code	Collection location	Year isolated
Fusarium oxysporum	FS09033	Anqing, Anhui	2009
F. oxysporum	FS09035	Chuzhou, Anhui	2009
F. oxysporum	FS09037	Fuyang, Anhui, site 1	2009
F. oxysporum	FS09038	Fuyang, Anhui, site 2	2009
F. oxysporum	FS09095	Fuyang, Anhui, site 3	2009
F. oxysporum	FS11608	Fuyang, Anhui, site 4	2011
F. oxysporum	FS10176b	Hefei, Anhui	2010
F. oxysporum	FS10133	Xuancheng, Anhui, site 1	2010
F. oxysporum	FS10175	Xuancheng, Anhui, site 2	2010
F. oxysporum	FS10134	Hengshui, Hebei	2010
F. oxysporum	FS09060	Shijiazhuang, Hebei, site 1	2009
F. oxysporum	FS10198	Shijiazhuang, Hebei, site 2	2010
F. oxysporum	FS11649	Shijiazhuang, Hebei, site 3	2011
F. oxysporum	FS07021	Pingdingshan, Henan	2007
F. oxysporum	FS07024	Xuchang, Henan	2007
F. oxysporum	FS08027	Luohe, Henan	2008
F. oxysporum	FS09086	Jiaozuo, Henan	2009
F. oxysporum	FS09076	Luoyang, Henan, site 1	2009
F. oxysporum	FS09078	Luoyang, Henan, site 2	2009
F. oxysporum	FS10112	Luoyang, Henan, site 3	2010
F. oxysporum	FS11597a	Luoyang, Henan, site 4	2011
F. oxysporum	FS07008	Nanyang, Henan, site 1	2007
F. oxysporum	FS09025	Nanyang, Henan, site 2	2009
F. oxysporum	FS10211	Nanyang, Henan, site 3	2010
F. oxysporum	FS09069	Xinxiang, Henan, site 1	2009
F. oxysporum	FS09100	Xinxiang, Henan, site 2	2009
F. oxysporum	FS09077	Zhengzhou, Henan	2009
F. oxysporum	FS09046	Zhoukou, Henan	2009
F. oxysporum	FS09012	Zhumadian, Henan, site 1	2009
F. oxysporum	FS10012	Zhumadian, Henan, site 2	2010
F. oxysporum	FS11603b	Zhumadian, Henan, site 3	2011
F. oxysporum	FS11641	Sanmenxia, Henan, site 1	2011
F. oxysporum	FS11719a	Sanmenxia, Henan, site 2	2011
F. oxysporum	FS11719b1	Sanmenxia, Henan, site 2	2011
F. oxysporum	FS08042	Xiangyang, Hubei, site 1	2008
F. oxysporum	FS09096	Xiangyang, Hubei, site 2	2009
F. oxysporum	FS10040	Xiangyang, Hubei, site 3	2010
F. oxysporum	FS11454a	Xiangyang, Hubei, site 3	2011
F. oxysporum	FS11473a	Xiangyang, Hubei, site 4	2011
F. oxysporum	FS11614	Xiangyang, Hubei, site 5	2011
F. oxysporum	FS10099	Huanggang, Hubei	2010
F. oxysporum	FS09016	Harbin, Heilongjiang	2009
F. oxysporum	FS09017	Baicheng, Jilin, site 1	2009
F. oxysporum	FS09018	Baicheng, Jilin, site 2	2009
F. oxysporum	FS10218	Shuangliao, Jilin	2010
F. oxysporum	FS10216	Songyuan, Jilin	2010
F. oxysporum	FS10173a2	Huaian, Jiangsu	2010
F. oxysporum	FS10090a	Jiangxi	2010
F. oxysporum	FS10184b1	Nanchang, Jiangxi	2010
F. oxysporum	FS10224	Chaoyang, Liangning	2010
F. oxysporum	FS10228	Chaoyang, Liangning	2010
F. oxysporum	FS09051	Liaoyang, Liaoning, Site 1	2009
F. oxysporum	FS10001	Liaoyang, Liaoning, Site 2	2010
F. oxysporum	FS10008	Liaoyang, Liaoning, Site 2	2010
F. oxysporum	FS10195	Shenyang, Liaoning	2010
F. oxysporum	FS10053	Fenyang, Shanxi, site 1	2010
F. oxysporum	FS10201	Fenyang, Shanxi, site 1	2010
F. oxysporum	FS11718a	Fenyang, Shanxi, site 2	2011
F. oxysporum	FS10044	Jinzhong, Shanxi	2010
F. oxysporum	FS10047	Jinzhong, Shanxi	2010
F. oxysporum	FS11458	Ankang, Shaanxi, site 1	2011
F. oxysporum	FS11476a	Ankang, Shaanxi, site2	2011
F. oxysporum	FS11527	Ankang, Shaanxi, site3	2011
F. oxysporum	FS11584a	Ankang, Shaanxi, site4	2011
F. oxysporum	FS11470	Hanzhong, Shaanxi	2011
F. oxysporum	FS11509	Hanzhong, Shaanxi	2011
F. oxysporum	FS11416	Shangluo, Shaanxi	2011
F. oxysporum	FS11710b	Weinan, Shaanxi	2011
F. oxysporum	FS11702	Yulin, Shaanxi	2011
F. solani	FS07029	Pingdingshan, Henan	2007
F. solani	FS08030	Luohe, Henan	2008
F. solani	FS09040	Shangqiu, Henan	2009

Identification of *SIX* genes. The *SIX* genes were identified in *F. oxysporum* f. sp. *sesami* by a BLAST search with E-value ≤ 1E-10 from our preliminary genome assemblies of the isolates FS08027, FS09095, and FS10175 (unpublished data) based on the reference sequences of *SIX1* to *-14* from *F. oxysporum* f. sp. *lycopersici* isolates 4287, MN25, and BFOL-51 (Lievens et al. 2009; Schmidt et al. 2013). All *F. oxysporum* f. sp. *sesami SIX* genes were further screened in the 69 *F. oxysporum* isolates and 3 *F. solani* isolates using PCR amplification. The specific primers of *F. oxysporum* f. sp. *sesami SIX* genes were designed (Table 2), and PCRs were set up as described above. PCR amplification was performed with standard conditions as follows: 95°C for 2 min; 35 cycles of 95°C for 20 s, 55 to 61°C annealing (see Table 2 for temperatures) for 30 s, and 72°C for 1 min; with a final cycle of 8 min at 72°C. The amplicons were visualized, purified, and sequenced as described above. The GenBank accessions MN417207 to MN417222 were provided for the *SIX1* to *-14* gene sequences in the *Fusarium* isolates from sesame. The homologs of *SIX* genes were also searched in other species by blasting the reference sequences of *F. oxysporum* f. sp. *lycopersici SIX1* to *-14* against the aforementioned nucleotide datasets (Supplementary Table S1). The sequences of the *SIX* genes in both *F. oxysporum* f. sp. *sesami* and the other species were aligned using DNAMAN 6 software (Lynnon Corporation), and the sequence similarities of *SIX* genes were outputted by the observed divergence method based on the sequence alignments.

Phylogenetic analysis. Phylogenetic analyses were performed on the *EF-1a* and *SIX* gene datasets. The sequences were aligned by the Muscle method, and the maximum-likelihood (ML) trees were constructed using MEGA 5.2 software (https://www.megasoftware.net/) with the computed most appropriate models: Kimura two-parameter for *EF-1a*, *SIX6*, *SIX11*, and *SIX14*; Kimura two-parameter plus invariant sites for *SIX1* and *SIX9*; Kimura two-parameter plus γ distribution for *SIX7*, *SIX8*, and *SIX13*; Jukes-Cantor for *SIX3*; and Jukes-Cantor plus γ distribution for *SIX10*. For the *EF-1a* tree, the *F. foetens* isolate NRRL31841 was used as the outgroup taxon (Schroers et al. 2004). Branches were tested for the inferred tree by bootstrap analysis on 1,000 random trees.

Infection treatment and expression profile assay of *F. oxysporum* f. sp. *sesami SIX* genes. The sesame cultivar Yuzhi 11, susceptible to isolate FS08027, was used in the infection treatment. Seed were grown in pots with sterilized vermiculite in growth chambers, as described above. Four-week-old seedlings with two pairs of true leaves were gently pulled out of the potting substrate, and the roots were carefully washed in water. The root mass was dipped into a microconidial suspension of 4×10^6 conidia/ml for 10 min; then, the seedlings were planted in pots with sterilized vermiculite. The roots were sampled at 12, 24, 48, 72, 120, and 168 h postinoculation (hpi). The microconidia and mycelia of the FS08027 strain were also collected as described above. Three independent replicates were carried out in the experiment. All samples were frozen in liquid nitrogen and stored at –80°C for total RNA extraction.

Total RNA was extracted from samples using RNAiso Plus Reagent (TaKaRa), and genomic DNA was removed by DNase I treatment. First-strand cDNA was synthesized using a RevertAid First-Strand cDNA Synthesis Kit (Thermo Scientific) according to the manufacturer's instructions. Primer pairs of *F. oxysporum* f. sp. *sesami SIX* genes were designed using Primer Premier 5.0 for Real-time PCR detection. Real-time PCR was performed on a Mastercycler ep realplex (Eppendorf), with the PCR mixture in a total volume of 20 µl containing 10 µl of FastStart Essential DNA Green Master (Roche), 2.0 µl of fivefold-diluted first-strand cDNA, and 0.2 µM each primer. The cycling conditions were as follows: 95°C for 10 min and 40 cycles of 95°C for 15 s, 56 to 60°C for 20 s (Table 2), and 72°C for 15 s. The specific product of each gene was confirmed using the melt-curve analysis. The transcriptional level of *F. oxysporum* f. sp. *sesami SIX* genes was analyzed against the *F. oxysporum* f. sp. *sesami Tub* gene using the $2^{-\Delta CT}$ method (Pfaffl

2001). The significant difference between transcriptional levels in microconidia, mycelia, and infected roots was analyzed using Student's *t* test in SPSS 16.0 (SPSS Inc.).

RESULTS

Pathogenicity testing and classification of pathogenicity group of *F. oxysporum* f. sp. *sesami* isolates. On the basis of the geographical locations, morphological characteristics, and ITS sequences, 69 *F. oxysporum* isolates from sesame were selected for pathogenicity testing. Meanwhile, three *F. solani* isolates originally separated from diseased sesame plants were also evaluated for their ability to cause wilt disease. In the sesame seedling test, significantly different pathogenicity was observed in the *F. oxysporum* isolates on three sesame cultivars (Yuzhi 11, Ji 9014, and HJ16) (Fig. 1). Of the 69 *F. oxysporum* isolates, 50 isolates caused significant seedling wilt on all three sesame cultivars; 1 isolate (FS09095) was highly pathogenic to Ji 9014 and HJ16 but presented nonpathogenicity or weak pathogenicity to Yuzhi 11; and 3 isolates (FS09060, FS09069, and FS10175) were specifically pathogenic to HJ16. Therefore, in total, 54 *F. oxysporum* isolates could be assigned to f. sp. *sesami*. In addition, the remaining 15 *F. oxysporum* isolates and 3 *F. solani* isolates did not cause significant disease symptoms on sesame.

Based on the different pathogenicity toward three sesame cultivars, the *F. oxysporum* f. sp. *sesami* isolates were subdivided into three pathogenicity groups. *F. oxysporum* f. sp. *sesami* pathogenicity group 1 was defined for the isolates with pathogenicity specifically on HJ16, *F. oxysporum* f. sp. *sesami* pathogenicity group 2 was pathogenic to Ji 9014 and HJ16 but nonpathogenic to Yuzhi 11, and *F. oxysporum* f. sp. *sesami* pathogenicity group 3 was highly pathogenic to all three cultivars (Table 3). Surprisingly, 50 of the 54 *F. oxysporum* f. sp. *sesami* isolates were assigned to *F. oxysporum* f. sp. *sesami* pathogenicity group 3, which was distributed across almost all the collection locations in China between 2007 and 2011. In contrast, the three isolates of pathogenicity group 1 were collected from Hebei, Henan, and Anhui Provinces in 2009 and 2010, and the only isolate of pathogenicity group 2 was collected from Anhui Province in 2009.

Phylogenetic relationship of *F. oxysporum* f. sp. *sesami* isolates. To determine the phylogenetic relationship of *F. oxysporum* f. sp. *sesami* and nonpathogens from sesame, the ML tree was constructed based on *EF-1a* sequences, including those in *F. oxysporum* isolates from sesame and other plants and nonpathogenic isolate Fo47. The phylogenetic analyses showed that the FOSC isolates in this study were divided into four clades, and the 69 isolates from sesame were placed in clades I, III, and IV (Fig. 2). Clade I covered all 54 pathogenic isolates in *F. oxysporum*

TABLE 2. Primer pairs used for the amplification and quantitative detection of target sequences (genes) in this study

Target[a]	Primers	Sequences (forward primer/reverse primer, 5′–3′)	Size (bp)[b]	T (°C)[c]
PCR[d]				
EF-1a	EF-F/EF-R	ATGGGTAAGGAGGACAAGAC/GCCATCCTTGGAGATACCAGC	~784	55
SIX1	SIX1-F/SIX1-R	GCTACCATTATTTACAATCCCCATC/CGAAGCCGATAGTAAATGAGTGAC	1,062	60
SIX3	SIX3-F/SIX3-R	ATGCGTTTCCTTCTGCTTATCG/TTCGCTTGGGATTCCAGACAT	559	61
SIX6	SIX6-F/SIX6-R	AACTTCAGCCCAATCTTCACG/CATCCCTACTGGGCTCAGAACT	771	59
SIX7	SIX7-F/SIX7-R	CAGAGTCAGTTATGTGGCACAAG/GACTTGCTACTAACTCTGCTGTGTT	705	57
SIX8a1	SIX8a1-F/SIX8a1-R	ACCTGTATCAAAAGAACAGCTCTG/TATCCCTATCGGGCCTGAT	778	56
SIX8a2	SIX8a2-F/SIX8a2-R	GCCGATTTTATTTACAGTATGTTAC/ACACAACACACAACAATAGCTACTA	814	55
SIX8a3	SIX8a3-F/SIX8a3-R	CCCGTATCAAAAGAACAGCTCTT/CCTTATCCCTATCAGGCCTAAC	784	57
SIX8a4	SIX8a4-F/SIX8a4-R	CCCGTATCAAAAGAACAGCTCTC/TTATCCCTATCGGGCCTCAT	1,733	58
SIX8a5	SIX8a1-F/SIX8a1-R	ACCTGTATCAAAAGAACAGCTCTG/TATCCCTATCGGGCCTGAT	772	56
SIX8b	SIX8b-F/SIX8b-R	GCTTTTAAGAGAGATTTAAGCGGG/GATGGGACTGCCTATGTGCC	735	60
SIX9	SIX9-F/SIX9-R	GAGCGCTGATCACAGCTTTTAG/TTAATTGACGGTGCATTGTCC	676	58
SIX10	SIX10-F/SIX10-R	ATACCAAGCGATAACCCGTCT/TCAATAAAGAGGGGGATGGTAG	657	58
SIX11	SIX11-F/SIX11-R	ATGATGTTCTCAAAGGCCATCTC/TCAAATGCAGGGTCTATTGAGAAT	336	60
SIX13	SIX13-F/SIX13-R	CTGCCCACTATGACTCGGTTTC/CAGGATTATTTTCTGGCGTCTAG	1,027	58
SIX14	SIX14-F/SIX14-R	ATGCATTTCCACTATGTATTTCTTC/TATTGTCCAAGCTAACTTTAACAGC	334	57
Homologs[e]				
SIX13a	S-SIX13a-F/S-SIX13a-R	ATGAAGATAGGTCATGCAACGC/CGTACACATGATGTATAAGGTACCAGT	316	59
SIX13b	S-SIX13b-F/S-SIX13b-R	TAGATGAAGATACGTCATGCAACAG/CGTACACATGATGTATGAGGTACCATA	319	59
RT-PCR[f]				
SIX1	RT-SIX1-F/RT-SIX1-R	TCCTTGGGTTTGGGGCTTA/TGGGACAACATCGTCCGTC	185	56
SIX3	RT-SIX3-F/RT-SIX3-R	TGCGTTTCCTTCTGCTTATCG/GGAGGGTCTCTGGGTATTGTTC	214	59
SIX6	RT-SIX6-F/RT-SIX6-R	TCACTATGTTGATGAAGGTCCCA/GTTTTCCGTTGCTAAGGTTGC	251	59
SIX7	RT-SIX7-F/RT-SIX7-R	CTTTACCTCCTTTTCCATTTCG/AGAGTCCTACACCCTGAGTCCA	208	57
SIX8a1	RT-SIX8a1-F/RT-SIX8a1-R	TGGCGTTTCAGGCATATGCT/GCACGATCTGGACTACTTCCC	168	58
SIX8a2	RT-SIX8a2-F/RT-SIX8a2-R	GTGGCGTTTCAGGCATATCA/CACGATCTGGACTACTTCCCG	168	59
SIX8a3	RT-SIX8a3-F/RT-SIX8a3-R	ACGGCGTTTCAGGCATATCA/GCGATTTGGACTACCTCCGAC	167	60
SIX8a4	RT-SIX8a4-F/RT-SIX8a4-R	CTGCATAACAGGTGCCGGT/GCACGATTTGGACTACCTCGT	188	59
SIX8b	RT-SIX8b-F/RT-SIX8b-R	GGCGGTAACTGAAGTGGTGG/GATGGGACTGCCTATGTGCC	108	60
SIX9	RT-SIX9-F/RT-SIX9-R	CGGTTGCTACGCTGTTGATT/GTTCCGGTGCTCGTTTTTG	213	58
SIX10	RT-SIX10-F/RT-SIX10-R	GTGTTACCCCACTTGTTCGTTC/CTCGTAGATTAGTCTGCCGTCC	185	58
SIX11	RT-SIX11-F/RT-SIX11-R	CCGCCAGAATGTTATCTTGAA/GCAGGGTCTATTGAGAATGCC	211	58
SIX13a	RT-SIX13a-F/RT-SIX13a-R	GCAGCACTACAGAGGCTCCA/GTTGCATGACCTATCTTCATCTGA	99	58
SIX13b	RT-SIX13b-F/RT-SIX13b-R	CCTGGTTCTCCTATTGCTTATGA/GTTGCATGACGTATCTTCATCTAC	272	56
SIX14	RT-SIX14-F/RT-SIX14-R	TGCCTAATGGCTCCCTGAAC/CAACAGAACCGCCGTTATCAT	162	60
β-*tubulin*	FoTub-F/FoTub-R	GCCTTTCCCTCGTCTACACTT/AGTTCTTGTTCTGGACGTTGC	239	57

[a] *EF-1a = translation elongation factor 1a* and *SIX = secreted-in-xylem.*
[b] Amplicon size.
[c] Annealing temperature.
[d] PCR amplification and sequencing.
[e] Specific homologs of SIX13 screening.
[f] Real-time reverse-transcription PCR detection.

f. sp. *sesami* pathogenicity groups 1, 2, and 3 as well as the 2 nonpathogenic isolates, FS09046 and FS10184b1. Isolate FS09046 presented an *EF-1a* sequence identical to that of the *F. oxysporum* f. sp. *sesami* isolates, which was also identical to the isolates of ff. spp. *vasinfectum*, *niveum*, and *fragariae*. Additionally, 1 nonpathogenic isolate, FS11476a, shared an identical *EF-1a* sequence with the biocontrol isolate Fo47 and was placed in clade III (Fuchs et al. 1997), while the other 12 nonpathogenic isolates from sesame were clustered in clade IV with the isolates of f. sp. *cubense* race 4 isolates.

Identification of *SIX* genes in *F. oxysporum* f. sp. *sesami*. The genome sequences of the three *F. oxysporum* f. sp. *sesami*

Fig. 1. Pathogenicity of 69 *Fusarium oxysporum* isolates and 3 *F. solani* isolates toward the seedlings of three sesame cultivars: Yuzhi 11, Ji 9014, and HJ16. Data on the disease index (DI) were calculated from three independent biological replications at 28 days postinoculation. Error bars indicate the standard error of the mean. The least significant difference ($\alpha = 0.05$) was analyzed using analysis of variance in SAS software. The dotted line separates the DI values into two parts, and the upper is significantly different from the uninoculated control (CK).

isolates (FS10175, FS09095, and FS08027) were queried based on *F. oxysporum* f. sp. *lycopersici SIX1* to *-14* sequences to search for *SIX* genes in *F. oxysporum* f. sp. *sesami*. As a result, the homologies of 10 *F. oxysporum* f. sp. *lycopersici SIX* genes—*SIX1*, *SIX3*, *SIX6*, *SIX7*, *SIX8*, *SIX9*, *SIX10*, *SIX11*, *SIX13*, and *SIX14*—were identified in the *F. oxysporum* f. sp. *sesami* isolates. Furthermore, the presence and sequence of these *SIX* gene homologs were verified by PCR screening in the 69 *F. oxysporum* isolates and 3 *F. solani* isolates from sesame (Table 3). All 10 *F. oxysporum* f. sp. *sesami SIX* genes were present in the 3 isolates in *F. oxysporum* f. sp. *sesami* pathogenicity group 1 and 29 isolates in *F. oxysporum* f. sp. *sesami* pathogenicity group 3, and the majority of the *SIX* genes (except one or two of the *SIX1*, *SIX3*, *SIX9*, *SIX10*, and *SIX14* genes) were present in the other 21 isolates in *F. oxysporum* f. sp. *sesami* pathogenicity group 3 and the 1 isolate in *F. oxysporum* f. sp. *sesami* pathogenicity group 2. Noticeably, five *SIX* genes (*SIX6*, *SIX7*, *SIX8*, *SIX11*, and *SIX13*) were identified in all 54 *F. oxysporum* f. sp. *sesami* isolates. In contrast, all *SIX* genes were absent in the 15 nonpathogenic *F. oxysporum* and 3 *F. solani* isolates, with the exception of two nonpathogenic isolates, FS10090a and FS10176b, which possessed the *SIX8* gene.

No polymorphisms in eight *F. oxysporum* f. sp. *sesami SIX* genes were individually detected either within an *F. oxysporum* f. sp. *sesami* isolate or between the different *F. oxysporum* f. sp. *sesami* isolates, with the exception of *SIX8* and *SIX13* (Table 3). The sequence variations of *SIX8* and *SIX13* were observed in a single *F. oxysporum* f. sp. *sesami* isolate, which led to the identification of five distinct *SIX8* gene homologs (*SIX8a1*, *SIX8a2*, *SIX8a3*, *SIX8a4*, and *SIX8b*) and two *SIX13* gene homologs (*SIX13a* and *SIX13b*) (Supplementary Figs. S1 and S2). Another *SIX8* gene homolog (*SIX8a5*) was identified in the nonpathogenic isolates FS10090a and FS10176b. Similar to other *F. oxysporum* f. sp. *sesami SIX* genes, each homolog of *F. oxysporum* f. sp. *sesami SIX8* and *SIX13* had an identical sequence between the different *F. oxysporum* f. sp. *sesami* isolates.

The homologs of *SIX* genes were also identified from another 26 *F. oxysporum* formae speciales isolates and *Verticillium dahliae* isolate JR2 (Supplementary Table S2) (Czislowski et al. 2018). The sequence alignments generated from the coding DNA sequences of these *SIX* genes showed that *SIX* genes in *F. oxysporum* f. sp. *sesami* shared high sequence similarity to those in other formae speciales, ranging from 63.4 to 100% nucleotide identity (Supplementary Table S3). Notably, *F. oxysporum* f. sp. *sesami SIX8a4* and *SIX14* shared identical sequences with corresponding *SIX* genes in *F. oxysporum* f. sp. *niveum*, and *F. oxysporum* f. sp. *sesami SIX9* also had an identical sequence with the *SIX9* genes in ff. spp. *vasinfectum*, *niveum*, and *raphanin* and Fo5176. However,

TABLE 3. Homologs of *secreted-in-xylem* (*SIX*)*1* to *-14* identified in *Fusarium oxysporum* and *F. solani* isolates from sesame

		Pathogenicity[c]			*SIX* gene[d]													
Species, code[a]	PG[b]	Yu	Ji	HJ	1	2	3	4	5	6	7	8[e]	9	10	11	12	13	14
F. oxysporum f. sp. *sesame*																		
FS09060	1	–	–	+	+	–	+	–	–	+	+	a1, a2, a3, a4, b	+	+	+	–	a, b	+
FS09069	1	–	–	+	+	–	+	–	–	+	+	a1, a2, a3, a4, b	+	+	+	–	a, b	+
FS10175	1	–	–	+	+	–	+	–	–	+	+	a1, a2, a3, a4, b	+	+	+	–	a, b	+
FS09095*	2	–	+	+	+	–	+	–	–	+	+	a1, a2, b	+	+	+	–	a	–
FS10133	3	+	+	+	–	–	–	–	–	+	+	a1, a2, a3, a4, b	+	+	+	–	a, b	+
FS10134	3	+	+	+	–	–	–	–	–	+	+	a1, a2, a3, a4, b	+	+	+	–	a, b	+
FS09017*	3	+	+	+	–	–	+	–	–	+	+	a1, a2, a3, a4, b	+	+	+	–	a, b	+
FS09018	3	+	+	+	–	–	+	–	–	+	+	a1, a2, a3, a4, b	+	+	+	–	a, b	+
FS09051	3	+	+	+	–	–	+	–	–	+	+	a1, a2, a3, a4, b	+	+	+	–	a, b	+
FS09096*	3	+	+	+	–	–	+	–	–	+	+	a1, a2, a3, a4, b	+	+	+	–	a, b	+
FS10001*	3	+	+	+	–	–	+	–	–	+	+	a1, a2, a3, a4, b	+	+	+	–	a, b	+
FS10198	3	+	+	+	–	–	+	–	–	+	+	a1, a2, a3, a4, b	+	+	+	–	a, b	+
FS11649	3	+	+	+	–	–	+	–	–	+	+	a1, a2, a3, a4, b	+	+	+	–	a, b	+
FS10040*	3	+	+	+	+[f]	–	+	–	–	+	+	a1, a2, a3, a4, b	+	+	+	–	a, b	+
FS10211	3	+	+	+	+[f]	–	+	–	–	+	+	a1, a2, a3, a4, b	+	+	+	–	a, b	+
FS10216	3	+	+	+	+[f]	–	+	–	–	+	+	a1, a2, a3, a4, b	+	+	+	–	a, b	+
FS07024	3	+	+	+	+	–	–	–	–	+	+	a1, a2, a3, a4, b	+	+	+	–	a, b	+
FS09016	3	+	+	+	+	–	–	–	–	+	+	a1, a2, a3, a4, b	+	+	+	–	a, b	+
FS10012	3	+	+	+	+	–	–	–	–	+	+	a1, a2, a3, a4, b	+	+	+	–	a, b	+
FS10053	3	+	+	+	+	–	–	–	–	+	+	a1, a2, a3, b	+	+	+	–	a, b	+
FS10224	3	+	+	+	+	–	–	–	–	+	+	a1, a2, a3, a4, b	+	+	+	–	a, b	–
FS11527	3	+	+	+	+	–	–	–	–	+	+	a1, a2, a3, a4, b	+	+	+	–	a, b	+
FS09025	3	+	+	+	+	–	+[g]	–	–	+	+	a1, a2, a3, a4, b	+	+	+	–	a, b	+
FS10099	3	+	+	+	+	–	+[g]	–	–	+	+	a1, a2, a3, a4, b	+	+	+	–	a, b	+
FS10218	3	+	+	+	+	–	+	–	–	+	+	a1, a2, a3, b	+	+	+	–	a, b	+
FS10228	3	+	+	+	+	–	+	–	–	+	+	a1, a2, a3, b	+	+	+	–	a, b	+
FS11597a	3	+	+	+	+	–	+	–	–	+	+	a1, a2, a3, a4, b	–	+	+	–	a, b	+
FS11509	3	+	+	+	+	–	+	–	–	+	+	a1, a2, a3, a4, b	+	–	+	–	a, b	+
FS11603b	3	+	+	+	+	–	+	–	–	+	+	a1, a2, a3, a4, b	+	–	+	–	a, b	+
FS11608	3	+	+	+	+	–	+	–	–	+	+	a1, a2, a3, a4, b	+	–	+	–	a, b	+

(*Continued on next page*)

[a] *Fusarium* spp. and isolate code. The 11 *Fusarium* isolates indicated by an asterisk (*) have been previously assessed for their pathogenicity on two sesame cultivars, Yuzhi 11 and Ji 9014, by Qiu et al. (2014).

[b] Pathogenicity group.

[c] Pathogenicity of the 69 isolates on sesame cultivars Yuzhi 11 (Yu), Ji 9014 (Ji), and HJ16 (HJ): + indicates highly pathogenic to seedlings of the corresponding sesame cultivar, presenting 50 < disease index (DI) ≤ 100, and – indicates nonpathogenic or weakly pathogenic to the corresponding sesame cultivar, presenting 0 ≤ DI ≤ 20.

[d] Symbols: + indicates the presence of one *secreted-in-xylem* (*SIX*) gene homolog in the corresponding isolate, – indicates the absence of a *SIX* gene in the corresponding isolate, and single letters indicate the homologs of one *F. oxysporum* f. sp. *sesami SIX* gene in the corresponding isolate.

[e] The 5′ untranslated region of *F. oxysporum* f. sp. *sesami SIX8a4* is interrupted by a transposon.

[f] Coding sequences of *F. oxysporum* f. sp. *sesami SIX1* are interrupted by the transposons.

[g] Coding sequences of *F. oxysporum* f. sp. *sesami SIX3* are interrupted by the transposons.

comparing the presence and sequence variations of *SIX* genes between different *F. oxysporum* isolates, *F. oxysporum* f. sp. *sesami* exhibited a specific combination of *SIX* genes, which was distinguishable from the *SIX* gene profiles in other formae speciales screened in this study (Table 3; Supplementary Table S2). In addition, among the *F. oxysporum* f. sp. *sesami* isolates, *F. oxysporum* f. sp. *sesami* pathogenicity group 2 was distinguishable from *F. oxysporum* f. sp. *sesami* pathogenicity groups 1 and 3 by the absence of both *SIX8a3* and *SIX13b*, whereas *F. oxysporum* f. sp. *sesami* pathogenicity groups 1 and 3 exhibited a similar profile of *SIX* genes.

Evolutionary relationship of *SIX* genes in *F. oxysporum* f. sp. *sesami*. The phylogenetic trees were constructed for each of 10 *SIX* genes identified in *F. oxysporum* f. sp. *sesami* to further determine the evolutionary relationships of the *SIX* genes. The results showed that the *SIX* gene sequence divergence was clearly observed between the different *F. oxysporum* formae speciales, and *F. oxysporum* f. sp. *sesami* was also separated from the other formae speciales in the *SIX1*, *SIX3*, *SIX6*, *SIX7*, *SIX10*, *SIX11*, and *SIX13* gene trees but not in the *SIX8*, *SIX9*, and *SIX14* gene trees owing to the identical *SIX* gene sequences described above between *F. oxysporum* f. sp. *sesami* and several other formae speciales (Fig. 3). In addition, the five homologs of *F. oxysporum* f. sp. *sesami SIX8* were clustered into four subclades in the *SIX8* gene tree, while *F. oxysporum* f. sp. *sesami SIX8a1* and *SIX8a2* was placed in a subclade including *SIX8a5* in the nonpathogenic isolates FS10090a and FS10176b and the homologs of *SIX8* in f. sp. *cubense* race 4 isolates (Fig. 3E; Supplementary Fig. S1), and presented more distant evolutionary relationships with one another compared with the *SIX8* homologs in other formae speciales. Unlike *F. oxysporum* f. sp. *sesami SIX8*, the two homologs of *F. oxysporum* f. sp. *sesami SIX13* were sorted into one subclade, and *F. oxysporum* f. sp. *sesami SIX13a* seemed to be more similar to *F. oxysporum* f. sp. *medicaginis SIX13b* than to *F. oxysporum* f. sp. *sesami SIX13b* (Fig. 3I; Supplementary Fig. S2).

The topologies of the *SIX* gene trees were further compared with the *EF-1a* tree. *F. oxysporum* f. sp. *sesami* and other formae speciales were clustered together in the *SIX1*, *SIX3*, *SIX10*, *SIX11*, and *SIX13* gene trees but were placed into distant clades in the *EF-1a* tree (Figs. 2 and 3). For example, *F. oxysporum* f. sp. *sesami* presented closer relationships to *F. oxysporum* f. sp. *melonis* in the *SIX1* gene tree but they were separated into clades I and II in the *EF-1a* tree. The results indicate that the *SIX* gene phylogeny is discordant to the *EF-1a* phylogeny.

Expression profiles of *F. oxysporum* f. sp. *sesami SIX* genes upon infection with *F. oxysporum* f. sp. *sesami*. To explore the expression profiles of the 10 *F. oxysporum* f. sp. *sesami*

TABLE 3. (*Continued from previous page*)

Species, code[a]	PG[b]	Pathogenicity[c]			*SIX* gene[d]													
		Yu	Ji	HJ	1	2	3	4	5	6	7	8[e]	9	10	11	12	13	14
FS10044*	3	+	+	+	+	–	+	–	–	+	+	a1, a2, a3, a4, b	+	+	+	–	a, b	–
FS11702	3	+	+	+	+	–	+	–	–	+	+	a1, a2, a3, a4, b	+	+	+	–	a, b	–
FS08027	3	+	+	+	+	–	+	–	–	+	+	a1, a2, a3, a4, b	+	+	+	–	a, b	+
FS08042	3	+	+	+	+	–	+	–	–	+	+	a1, a2, a3, a4, b	+	+	+	–	a, b	+
FS09012	3	+	+	+	+	–	+	–	–	+	+	a1, a2, a3, a4, b	+	+	+	–	a, b	+
FS09033	3	+	+	+	+	–	+	–	–	+	+	a1, a2, a3, a4, b	+	+	+	–	a, b	+
FS09035	3	+	+	+	+	–	+	–	–	+	+	a1, a2, a3, a4, b	+	+	+	–	a, b	+
FS09037	3	+	+	+	+	–	+	–	–	+	+	a1, a2, a3, a4, b	+	+	+	–	a, b	+
FS09038	3	+	+	+	+	–	+	–	–	+	+	a1, a2, a3, a4, b	+	+	+	–	a, b	+
FS09076	3	+	+	+	+	–	+	–	–	+	+	a1, a2, a3, a4, b	+	+	+	–	a, b	+
FS09077	3	+	+	+	+	–	+	–	–	+	+	a1, a2, a3, a4, b	+	+	+	–	a, b	+
FS09100*	3	+	+	+	+	–	+	–	–	+	+	a1, a2, a3, a4, b	+	+	+	–	a, b	+
FS10112	3	+	+	+	+	–	+	–	–	+	+	a1, a2, a3, a4, b	+	+	+	–	a, b	+
FS10173a2	3	+	+	+	+	–	+	–	–	+	+	a1, a2, a3, a4, b	+	+	+	–	a, b	+
FS10195	3	+	+	+	+	–	+	–	–	+	+	a1, a2, a3, a4, b	+	+	+	–	a, b	+
FS11416	3	+	+	+	+	–	+	–	–	+	+	a1, a2, a3, a4, b	+	+	+	–	a, b	+
FS11454a	3	+	+	+	+	–	+	–	–	+	+	a1, a2, a3, a4, b	+	+	+	–	a, b	+
FS11473a	3	+	+	+	+	–	+	–	–	+	+	a1, a2, a3, a4, b	+	+	+	–	a, b	+
FS11614	3	+	+	+	+	–	+	–	–	+	+	a1, a2, a3, a4, b	+	+	+	–	a, b	+
FS11641	3	+	+	+	+	–	+	–	–	+	+	a1, a2, a3, a4, b	+	+	+	–	a, b	+
FS11710b	3	+	+	+	+	–	+	–	–	+	+	a1, a2, a3, a4, b	+	+	+	–	a, b	+
FS11718a	3	+	+	+	+	–	+	–	–	+	+	a1, a2, a3, a4, b	+	+	+	–	a, b	+
FS11719a	3	+	+	+	+	–	+	–	–	+	+	a1, a2, a3, a4, b	+	+	+	–	a, b	+
FS11719b1	3	+	+	+	+	–	+	–	–	+	+	a1, a2, a3, a4, b	+	+	+	–	a, b	+
F. oxysporum																		
FS10090a*	–	–	–	–	–	–	–	–	–	–	–	a5	–	–	–	–	–	–
FS10176b	–	–	–	–	–	–	–	–	–	–	–	a5	–	–	–	–	–	–
FS07008	–	–	–	–	–	–	–	–	–	–	–	–	–	–	–	–	–	–
FS07021	–	–	–	–	–	–	–	–	–	–	–	–	–	–	–	–	–	–
FS09046	–	–	–	–	–	–	–	–	–	–	–	–	–	–	–	–	–	–
FS09078	–	–	–	–	–	–	–	–	–	–	–	–	–	–	–	–	–	–
FS09086*	–	–	–	–	–	–	–	–	–	–	–	–	–	–	–	–	–	–
FS10008*	–	–	–	–	–	–	–	–	–	–	–	–	–	–	–	–	–	–
FS10047*	–	–	–	–	–	–	–	–	–	–	–	–	–	–	–	–	–	–
FS10184b1	–	–	–	–	–	–	–	–	–	–	–	–	–	–	–	–	–	–
FS10201	–	–	–	–	–	–	–	–	–	–	–	–	–	–	–	–	–	–
FS11458	–	–	–	–	–	–	–	–	–	–	–	–	–	–	–	–	–	–
FS11470	–	–	–	–	–	–	–	–	–	–	–	–	–	–	–	–	–	–
FS11476a	–	–	–	–	–	–	–	–	–	–	–	–	–	–	–	–	–	–
FS11584a	–	–	–	–	–	–	–	–	–	–	–	–	–	–	–	–	–	–
F. solani																		
FS07029	–	–	–	–	–	–	–	–	–	–	–	–	–	–	–	–	–	–
FS08030	–	–	–	–	–	–	–	–	–	–	–	–	–	–	–	–	–	–
FS09040	–	–	–	–	–	–	–	–	–	–	–	–	–	–	–	–	–	–

SIX genes identified in this study, the transcriptional levels of all of the *F. oxysporum* f. sp. *sesami SIX* gene homologs were evaluated in sesame roots infected by *F. oxysporum* f. sp. *sesami* pathogenicity group 3 isolate FS08027 using real-time reverse-transcription PCR (Fig. 4). The expression of each *F. oxysporum* f. sp. *sesami SIX* gene was not different between microconidia and mycelia cultured in vitro. Compared with that in microconidia, the expression of *F. oxysporum* f. sp. *sesami SIX8a1*, *SIX8a2*, *SIX8b*, *SIX13a*, and *SIX14* was significantly upregulated at 24 to 168 hpi and *F. oxysporum* f. sp. *sesami SIX7*, *SIX10*, and *SIX13b* were upregulated at 48 to 168 hpi, whereas *F. oxysporum* f. sp. *sesami SIX6*, *SIX9,* and *SIX11* were upregulated after 72 hpi. Furthermore, *F. oxysporum* f. sp. *sesami SIX6*, *SIX8a1*, *SIX8a2*, *SIX8b*, *SIX9*, *SIX10*, *SIX11*, *SIX13a*, and *SIX14* showed more transcript accumulation as compared with *F. oxysporum* f. sp. *sesami SIX7* and *SIX13b*. In contrast, the low expression level of *F. oxysporum* f. sp. *sesami SIX1* did not significantly change at any time point, and *F. oxysporum* f. sp. *sesami SIX3*, *SIX8a3,* and *SIX8a4* had no transcripts detected in planta during *F. oxysporum* f. sp. *sesami* infection.

DISCUSSION

Sesame Fusarium wilt is one of the major problems facing sesame producers in China owing to its economically destructive effects (Jyothi et al. 2011). Current effective control means are involved in the disease prevention prior to infection with pathogenic

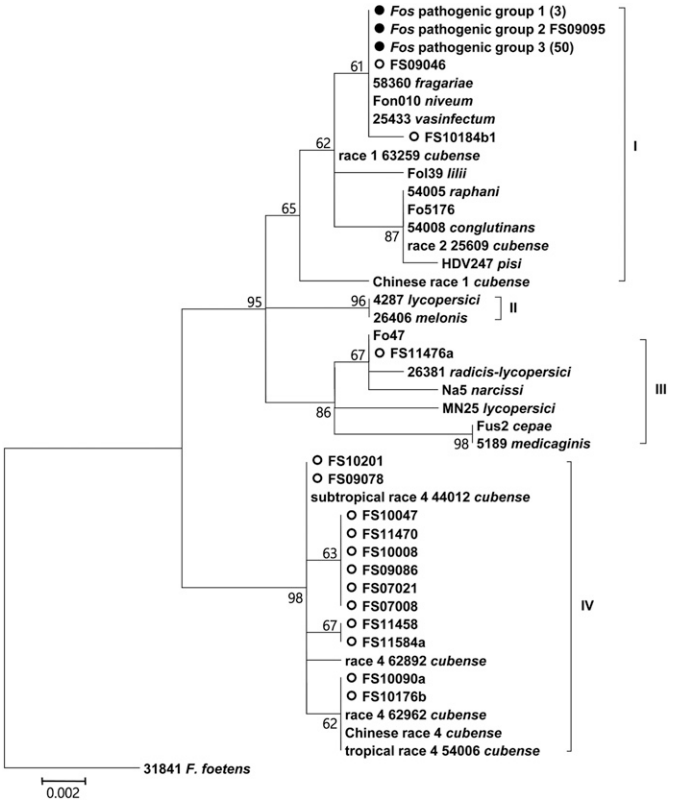

Fig. 2. Maximum-likelihood tree inferred from the translation elongation factor 1a (*EF-1a*) gene sequences in *Fusarium* isolates from sesame and other hosts. Scale bars indicate the number of substitutions per site. Numbers above the branch nodes represent bootstrap values from 1,000 replications. The sequence of *EF-1a* from *Fusarium foetens* isolate NRRL 31841 acts as an outgroup to root the tree. The isolates in the same pathogenicity group of *F. oxysporum* f. sp. *sesami* (Fos) with an identical *EF-1a* sequence are compressed into a branch, and their number is indicated in parentheses. The *F. oxysporum* f. sp. *sesami* isolates are indicated by solid black circles and nonpathogenic isolates from sesame are indicated by open circles.

F. oxysporum isolates (Lievens et al. 2008). The ability to reliably identify and distinguish pathogenic *F. oxysporum* isolates from sesame is of key importance for implementation of disease management. In this study, we determined the structure of pathogenic differentiation of *F. oxysporum* f. sp. *sesami* and demonstrated the correlation between the presence of *SIX* genes and the pathogenicity of *F. oxysporum* f. sp. *sesami* on sesame.

To systematically investigate the pathogenicity of *F. oxysporum* isolates from sesame, three sesame cultivars were selected as the differential hosts from more than 500 worldwide sesame germplasms, which were previously assessed for levels of resistance to Fusarium wilt under natural field and greenhouse conditions (data not shown). Based on the significantly different pathogenicity toward the differential cultivars, three pathogenicity groups were defined for *F. oxysporum* f. sp. *sesami* isolates for the first time. Of three *F. oxysporum* f. sp. *sesami* pathogenicity groups, the large number of isolates in pathogenicity group 3 with a high level of pathogenicity were the most geographically widespread across China, in contrast to the few isolates in *F. oxysporum* f. sp. *sesami* pathogenicity groups 1 and 2. Therefore, *F. oxysporum* f. sp. *sesami* pathogenicity group 3 was indisputably prevalent in past years in China.

Our phylogenetic tree based on *EF-1a* sequences divided the FOSC isolates into four clades, which showed that there was considerable genetic diversity between the isolates from sesame. In contrast, the nonpathogenic isolates from sesame were demonstrated to be phylogenetically diverse. *F. oxysporum* f. sp. *sesami* isolates were topologically monophyletic, because all of the *F. oxysporum* f. sp. *sesami* isolates were placed in one clade with an identical *EF-1a* sequence. In addition, *F. oxysporum* f. sp. *sesami* isolates also shared the identical *EF-1a* sequence with two nonpathogenic isolates from sesame and another three formae speciales isolates. This suggested that the tree inferred from the *EF-1a* gene failed to distinguish between the pathogenic and nonpathogenic isolates from sesame and between isolates of different formae speciales. In previous reports, poor correlation has been found between sequence variation of housekeeping genes and host-specific pathogenicity in other *F. oxysporum* isolates (O'Donnell et al. 1998).

Recent findings indicated that pathogenicity in *F. oxysporum* f. sp. *lycopersici* was conferred by multiple effector genes on a small accessory chromosome (Ma et al. 2010). Among these effector genes, several *SIX* genes have been further demonstrated to facilitate the pathogenicity of *F. oxysporum* f. sp. *lycopersici* (Gawehns et al. 2014; Houterman et al. 2009; Ma et al. 2015; Niu et al. 2016; Rep et al. 2004). The hypothesis of the current study was that homologs of *SIX* genes might exist in *F. oxysporum* f. sp. *sesami* with a conserved function, as proposed in other formae speciales (Simbaqueba et al. 2018; Taylor et al. 2016). For the first time, 10 *SIX* genes were identified in *F. oxysporum* f. sp. *sesami* isolates. *F. oxysporum* f. sp. *sesami SIX* gene sequences were identical and highly similar within and between *F. oxysporum* f. sp. *sesami* isolates, respectively, as well as highly conserved with those in other formae speciales. Previously, the high conservation of *SIX* gene sequences in formae speciales suggested that *SIX* genes on the accessory chromosome were gained by horizontal gene transfer between *F. oxysporum* strains (Czislowski et al. 2018; Fraser-Smith et al. 2014; Ma et al. 2010). In our gene trees, further evidence was that there was discordance between the evolutionary relationships of the *SIX* genes and *EF-1a* gene within the FOSC, which supported horizontal gene transfer as the evolutionary origin of *F. oxysporum* f. sp. *sesami SIX* genes.

The *SIX* gene profiles in *F. oxysporum* f. sp. *sesami* isolates were significantly different from the combinations of *SIX* genes in other formae speciales and absence of *SIX* genes in the nonpathogenic isolates from sesame. This revealed that there was a clear correlation between the *F. oxysporum* f. sp. *sesami SIX* gene profile and pathogenicity toward sesame, suggesting that *F. oxysporum*

f. sp. *sesami SIX* genes could be used as candidate loci for the molecular differentiation of *F. oxysporum* f. sp. *sesami* isolates from nonpathogenic isolates and the isolates of other formae speciales. Previously, gene *SIX6* was demonstrated to distinguish *F. oxysporum* f. sp. *vasinfectum* isolates from related colocalized nonpathogenic *F. oxysporum* isolates and from nonnative countries (Chakrabarti et al. 2011). The polymorphisms of *SIX3* and the presence of *SIX4* were used to distinguish *F. oxysporum* f. sp.

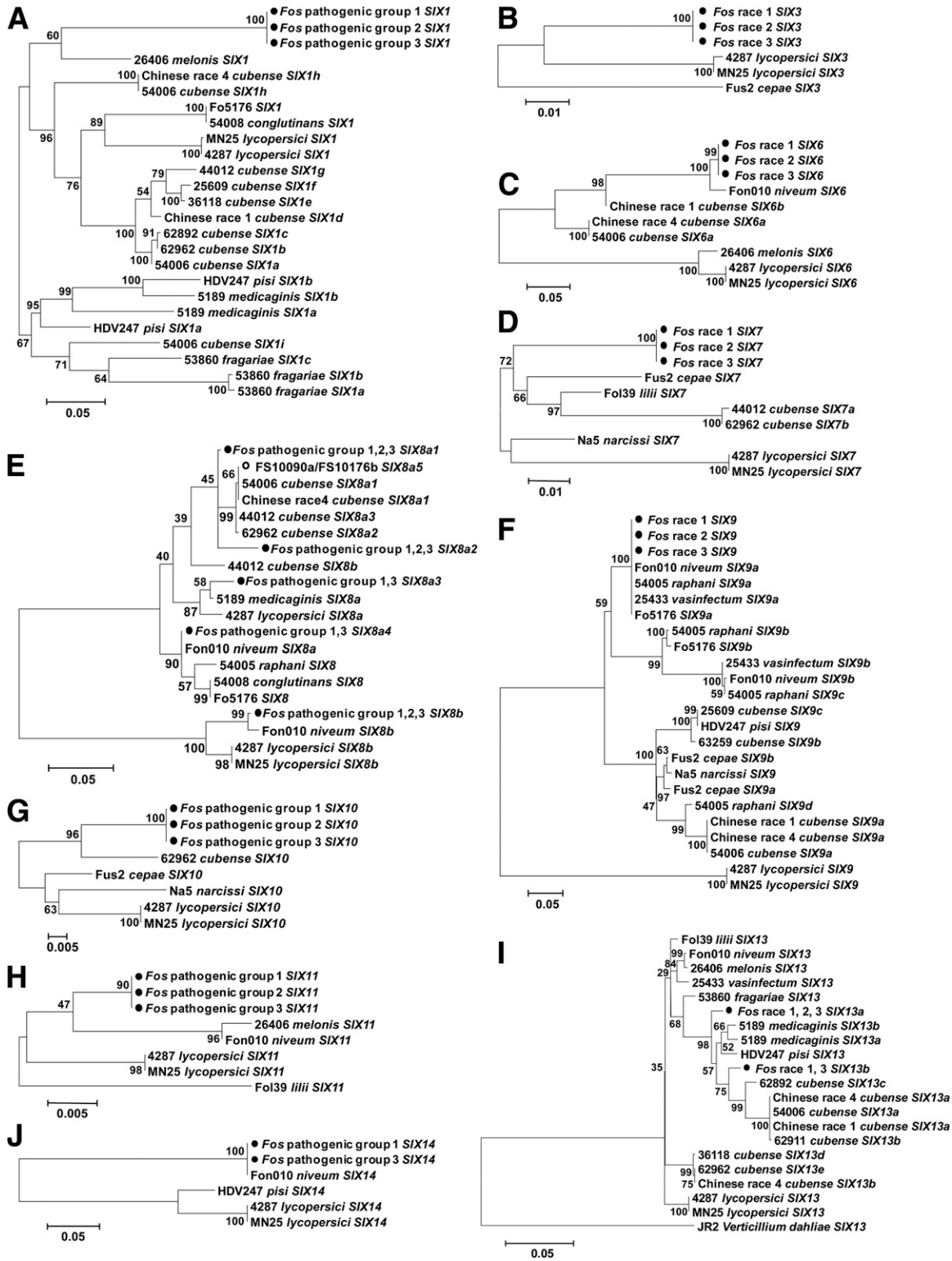

Fig. 3. Maximum-likelihood trees showing the sequences of *secreted-in-xylem* (*SIX*) gene sequences in *Fusarium oxysporum* isolates from sesame, other hosts, and a *Verticillium dahliae* isolate. **A,** *SIX1*; **B,** *SIX3*; **C,** *SIX6*; **D,** *SIX7*; **E,** *SIX8*; **F,** *SIX9*; **G,** *SIX10*; **H,** *SIX11*; **I,** *SIX13*; and **J,** *SIX14*. Isolates in other *F. oxysporum* and *V. dahliae* used in this study are listed in Supplementary Table S2, and the homologs of *SIX* genes were obtained for each isolate by a BLAST search from the public nucleotide datasets, including the nucleotide database in GenBank and the selected genome data described in Supplementary Table S1. Isolates in a pathogenicity group of *F. oxysporum* f. sp. *sesami* (Fos) with an identical sequence of *SIX* gene homologs are compressed into a branch. The *F. oxysporum* f. sp. *sesami* isolates are indicated by solid black circles and the nonpathogenic isolate from sesame is indicated by an open circle.

lycopersici races 1, 2, and 3 (Lievens et al. 2009), and the variation of the *SIX* gene profile could also reflect the differences in pathogenicity of *F. oxysporum* f. sp. *cubense* races (Czislowski et al. 2018; Fraser-Smith et al. 2014). In this study, *F. oxysporum* f. sp. *sesami* pathogenicity group 2 was distinguished from *F. oxysporum* f. sp. *sesami* pathogenicity groups 1 and 3 based on the absence of both *SIX8a3* and *SIX13b* but *F. oxysporum* f. sp. *sesami* pathogenicity groups 1 and 3 seemed to be indistinguishable owing to their similar *SIX* gene profiles. Therefore, the suitability of *SIX* genes to distinguish *F. oxysporum* f. sp. *sesami* pathogenicity groups should be further assessed in a wider range of *F. oxysporum* f. sp. *sesami* isolates.

The *F. oxysporum* f. sp. *lycopersici* race evolution has been reported in a stepwise manner, in which one race evolves from another and the virulence of a race accumulates sequentially in clonal lineages (Biju et al. 2017). Point mutation and gene deletion as well as transposon movement in *SIX* genes played major roles in the *F. oxysporum* f. sp. *lycopersici* race evolution by the loss of the

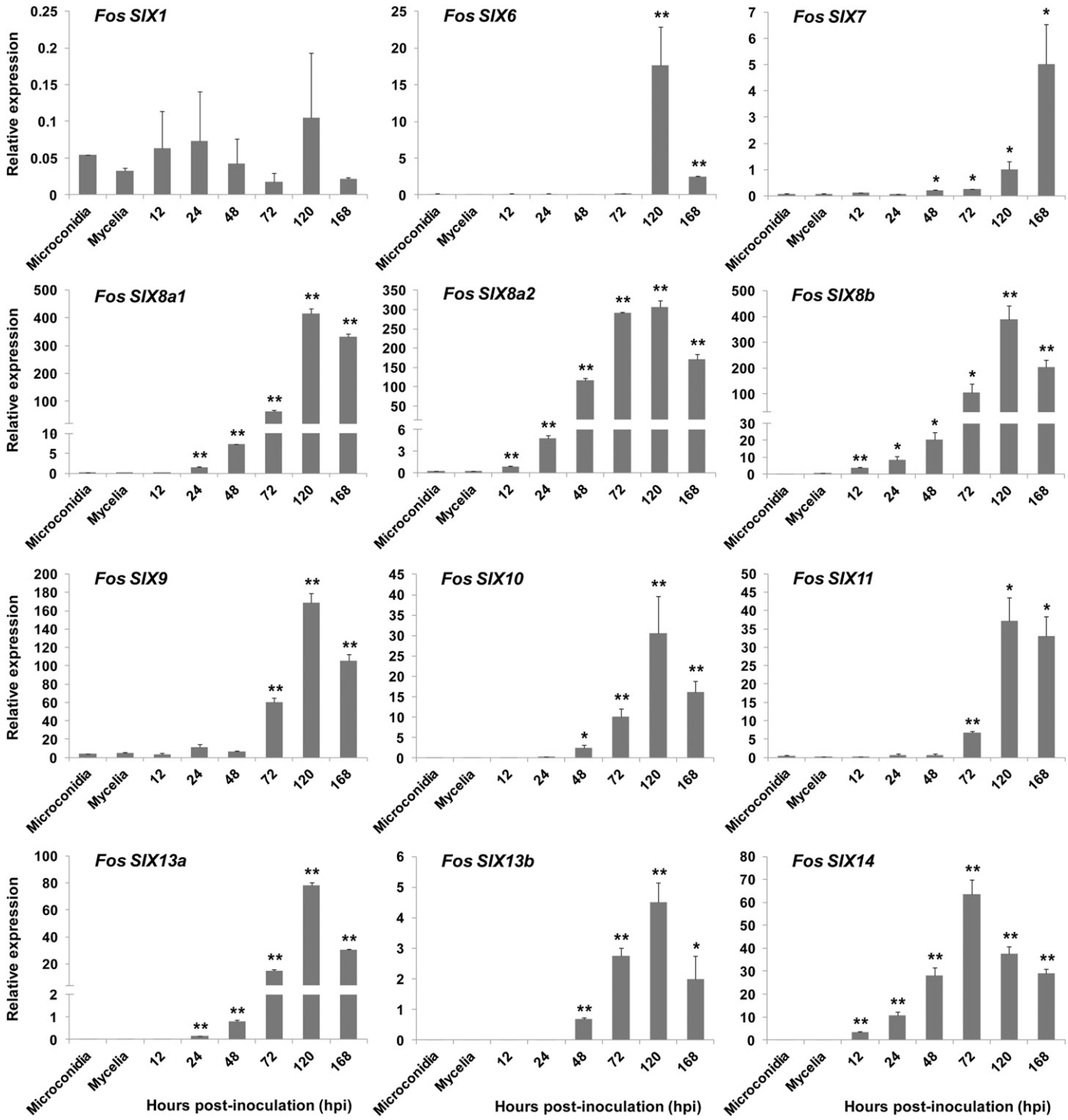

Fig. 4. Expression profiles of the homologs of *Fusarium oxysporum* f. sp. *sesami* (Fos) *secreted-in-xylem* (*SIX*) genes in sesame roots inoculated with *F. oxysporum* f. sp. *sesami* isolate FS08027. *F. oxysporum* f. sp. *sesami* β-*tubulin* gene was used as a reference gene. Transcriptional levels of *F. oxysporum* f. sp. *sesami SIX* genes were calculated relative to β-*tubulin*. Error bars indicate the standard errors of three biological replications. Asterisks indicate significant differences (* and ** indicate $P < 0.05$ and 0.01, respectively) compared with microconidia using Student's *t* test.

avirulence function to evade resistance-gene-mediated resistance in a gene-for-gene relationship (Biju et al. 2017). For *F. oxysporum* f. sp. *sesami* isolates, pathogenicity groups 1, 2, and 3 were not only considered to originate from an ancestor because of their monophyletic evolution but also presented gradually higher virulence. Meanwhile, the gene-for-gene relationship was also observed between the differential cultivars and the *F. oxysporum* f. sp. *sesami* pathogenicity groups that could be indicated as the *F. oxysporum* f. sp. *sesami* races, because one dominant resistance gene locus was identified in Yuzhi 11 contributing to resistance to the isolates FS10175 and FS09095, respectively (data not shown). Therefore, the underlying hypothesis was that the *F. oxysporum* f. sp. *sesami* races had evolved in a similar manner to the *F. oxysporum* f. sp. *lycopersici* races. In this hypothesis, although the essential virulence genes in *F. oxysporum* f. sp. *sesami* functioned in host-specific pathogenicity, the sequence variations of avirulence genes were necessary for the emergence of diverse *F. oxysporum* f. sp. *sesami* races. Considering the direct correlation between the *SIX* genes and the specific pathogenicity of *F. oxysporum* f. sp. *sesami*, *F. oxysporum* f. sp. *sesami SIX* genes were inferred to be recognized as candidates for effector genes. As expected, the homologs of *F. oxysporum* f. sp. *sesami SIX* genes were significantly expressed in sesame plants during the *F. oxysporum* f. sp. *sesami* infection, with the exception of *F. oxysporum* f. sp. *sesami SIX1*, *SIX3*, *SIX8a3*, and *SIX8a4*. This supported the idea that most of the *F. oxysporum* f. sp. *sesami SIX* genes played important roles in the virulence genotype. However, the concrete roles of these *F. oxysporum* f. sp. *sesami SIX* genes were unknown. More studies such as gene knockout and complementation could be performed in order to reveal the role of *SIX* genes. In addition, owing to the indistinguishable *SIX* gene profiles between *F. oxysporum* f. sp. *sesami* pathogenicity groups 1 and 3, novel effectors should be expected to be found in *F. oxysporum* f. sp. *sesami*, because the putative effectors *C5* and *CRX1* were associated with pathogenicity in *F. oxysporum* f. sp. *cepae* (Taylor et al. 2016).

Overall, the results of the current study subdivided *F. oxysporum* f. sp. *sesami* isolates into three pathogenicity groups in China. In *F. oxysporum* f. sp. *sesami*, the *SIX* genes were demonstrated to be associated with the specific pathogenicity toward sesame, and can be used as available candidate loci for molecular diagnosis on the basis of pathogenicity. Further studies are required to clearly define the *F. oxysporum* f. sp. *sesami* races and rigorously validate the hypothesis that *SIX* genes or novel genes in *F. oxysporum* f. sp. *sesami* function as effectors to facilitate pathogenicity on sesame and that their sequence variations have resulted in the emergence of diverse *F. oxysporum* f. sp. *sesami* races by the loss of the avirulence function or other evolutionary pathways.

ACKNOWLEDGMENTS

We thank Y. Su from Yunnan Academy of Agricultural Sciences for assisting us in the identification and classification of *Fusarium* isolates.

LITERATURE CITED

Armstrong, G. M., and Armstrong, J. K. 1981. *Formae speciales* and races of *Fusarium oxysporum* causing wilt diseases. Pages 391-399 in: *Fusarium*: Disease, Biology, and Taxonomy. P. E. Nelson, T. A. Toussoun, and R. J. Cook, eds. The Pennsylvania State University Press, University Park, PA, U.S.A.

Armstrong, J. K., and Armstrong, G. M. 1950. A Fusarium wilt of sesame in United States. Phytopathology 40:785.

Ashri, A. 1998. Sesame breeding. Plant Breed. Rev. 16:179-228.

Baayen, R. P., O'Donnell, K., Bonants, P. J., Cigelnik, E., Kroon, L. P., Roebroeck, E. J., and Waalwijk, C. 2000. Gene genealogies and AFLP analysis in the *Fusarium oxysporum* complex identify monophyletic and nonmonophyletic formae speciales causing wilt and rot disease. Phytopathology 90:891-900.

Biju, V. C., Fokkens, L., Houterman, P. M., Rep, M., and Cornelissen, B. J. C. 2017. Multiple evolutionary trajectories have led to the emergence of races in *Fusarium oxysporum* f. sp. *lycopersici*. Appl. Environ. Microbiol. 83: e02548-16.

Chakrabarti, A., Rep, M., Wang, B., Ashton, A., Dodds, P., and Ellis, J. 2011. Variation in potential effector genes distinguishing Australian and non-Australian isolates of the cotton wilt pathogen *Fusarium oxysporum* f. sp. *vasinfectum*. Plant Pathol. 60:232-243.

Cho, E. K., and Choi, S. H. 1987. Etiology of half stem rot in sesame caused by *Fusarium oxysporum*. Kor. J. Plant Prot. 26:25-30.

Covey, P. A., Kuwitzky, B., Hanson, M., and Webb, K. M. 2014. Multilocus analysis using putative fungal effectors to describe a population of *Fusarium oxysporum* from sugar beet. Phytopathology 104:886-896.

Czislowski, E., Fraser-Smith, S., Zander, M., O'Neill, W. T., Meldrum, R. A., Tran-Nguyen, L. T. T., Batley, J., and Aitken, E. A. B. 2018. Investigation of the diversity of effector genes in the banana pathogen, *Fusarium oxysporum* f. sp. *cubense*, reveals evidence of horizontal gene transfer. Mol. Plant Pathol. 19:1155-1171.

Epstein, L., Kaur, S., Chang, P. L., Carrasquilla-Garcia, N., Lyu, G., Cook, D. R., Subbarao, K. V., and O'Donnell, K. 2017. Races of the celery pathogen *Fusarium oxysporum* f. sp. *apii* are polyphyletic. Phytopathology 107:463-473.

Fraser-Smith, S., Czislowski, E., Meldrum, R. A., Zander, M., O'Neill, W., Balali, G. R., and Aitken, E. A. B. 2014. Sequence variation in the putative effector gene *SIX8* facilitates molecular differentiation of *Fusarium oxysporum* f. sp. *cubense*. Plant Pathol. 63:1044-1052.

Fuchs, J. G., Moënne-Loccoz, Y., and Défago, G. 1997. Nonpathogenic *Fusarium oxysporum* strain Fo47 induces resistance to Fusarium wilt in tomato. Plant Dis. 81:492-496.

Gawehns, F., Houterman, P. M., Ichou, F. A., Michielse, C. B., Hijdra, M., Cornelissen, B. J., Rep, M., and Takken, F. L. 2014. The *Fusarium oxysporum* effector Six6 contributes to virulence and suppresses *I-2*-mediated cell death. Mol. Plant-Microbe Interact. 27:336-348.

Gordon, T. R., and Martyn, R. D. 1997. The evolutionary biology of *Fusarium oxysporum*. Annu. Rev. Phytopathol. 35:111-128.

Hogenhout, S. A., van der Hoorn, R. A. L., Terauchi, R., and Kamoun, S. 2009. Emerging concepts in effector biology of plant-associated organisms. Mol. Plant-Microbe Interact. 22:115-122.

Houterman, P. M., Ma, L., Van Ooijen, G., De Vroomen, M. J., Cornelissen, B. J. C., Takken, F. L. W., and Rep, M. 2009. The effector protein Avr2 of the xylem-colonizing fungus *Fusarium oxysporum* activates the tomato resistance protein I-2 intracellularly. Plant J. 58:970-978.

Houterman, P. M., Speijer, D., Dekker, H. L., de Koster, C. G., Cornelissen, B. J. C., and Rep, M. 2007. The mixed xylem sap proteome of *Fusarium oxysporum*-infected tomato plants. Mol. Plant Pathol. 8:215-221.

Jyothi, B., Ansari, N. A., Vijay, Y., Anuradha, G., Sarkar, A., Sudhakar, R., and Siddiq, E. A. 2011. Assessment of resistance to Fusarium wilt disease in sesame (*Sesamum indicum* L.) germplasm. Australas. Plant Pathol. 40: 471-475.

Laurence, M. H., Summerell, B. A., and Liew, E. C. Y. 2015. *Fusarium oxysporum* f. sp. *canariensis*: Evidence for horizontal gene transfer of putative pathogenicity genes. Plant Pathol. 64:1068-1075.

Li, D. H., Wang, L. H., Zhang, Y. X., Lv, H. X., Qi, X. Q., Wei, W. L., and Zhang, X. R. 2012. Pathogenic variation and molecular characterization of *Fusarium* species isolated from wilted sesame in China. Afr. J. Microbiol. Res. 6:149-154.

Li, L. L. 1989. The kinds of diseases and studies in sesame in China. Chin. J. Oil Crop Sci. 1:11-16.

Lievens, B., Houterman, P. M., and Rep, M. 2009. Effector gene screening allows unambiguous identification of *Fusarium oxysporum* f. sp. *lycopersici* races and discrimination from other formae speciales. FEMS Microbiol. Lett. 300:201-215.

Lievens, B., Rep, M., and Thomma, B. P. 2008. Recent developments in the molecular discrimination of formae speciales of *Fusarium oxysporum*. Pest Manage. Sci. 64:781-788.

Ma, L., Houterman, P. M., Gawehns, F., Cao, L., Sillo, F., Richter, H., Clavijo-Ortiz, M. J., Schmidt, S. M., Boeren, S., Vervoort, J., Cornelissen, B. J., Rep, M., and Takken, F. L. 2015. The *AVR2-SIX5* gene pair is required to activate *I-2*-mediated immunity in tomato. New Phytol. 208:507-518.

Ma, L.-J., van der Does, H. C., Borkovich, K. A., Coleman, J. J., Daboussi, M.-J., Di Pietro, A., Dufresne, M., Freitag, M., Grabherr, M., Henrissat, B., Houterman, P. M., Kang, S., Shim, W.-B., Woloshuk, C., Xie, X., Xu, J.-R., Antoniw, J., Baker, S. E., Bluhm, B. H., Breakspear, A., Brown, D. W., Butchko, R. A. E., Chapman, S., Coulson, R., Coutinho, P. M., Danchin, E. G. J., Diener, A., Gale, L. R., Gardiner, D. M., Goff, S., Hammond-Kosack, K. E., Hilburn, K., Hua-Van, A., Jonkers, W., Kazan, K., Kodira, C. D., Koehrsen, M., Kumar, L., Lee, Y.-H., Li, L., Manners, J. M., Miranda-Saavedra, D., Mukherjee, M., Park, G., Park, J., Park, S.-Y., Proctor, R. H., Regev, A., Ruiz-Roldan, M. C., Sain, D., Sakthikumar, S., Sykes, S., Schwartz, D. C., Turgeon, B. G., Wapinski, I., Yoder, O., Young, S., Zeng, Q., Zhou, S., Galagan,

J., Cuomo, C. A., Kistler, H. C., and Rep, M. 2010. Comparative genomics reveals mobile pathogenicity chromosomes in *Fusarium*. Nature 464:367-373.

Miao, H. M., Chang, S. X., Zhang, H. Y., Huang, J. Y., Duan, Y. H., Qu, W. W. 2019. An evaluation technique of sesame resistance to Fusarium wilt disease at vegetative stage. J. Plant Genet. Resour. doi:10.13430/j.cnki.jpgr.20190428003

Michielse, C. B., and Rep, M. 2009. Pathogen profile update: *Fusarium oxysporum*. Mol. Plant Pathol. 10:311-324.

Niu, X., Zhao, X., Ling, K. S., Levi, A., Sun, Y., and Fan, M. 2016. The *FonSIX6* gene acts as an avirulence effector in the *Fusarium oxysporum* f. sp. *niveum*-watermelon pathosystem. Sci. Rep. 6:28146.

O'Donnell, K., Kistler, H. C., Cigelnik, E., and Ploetz, R. C. 1998. Multiple evolutionary origins of the fungus causing Panama disease of banana: Concordant evidence from nuclear and mitochondrial gene genealogies. Proc. Natl. Acad. Sci. U.S.A. 95:2044-2049.

O'Donnell, K., Sutton, D. A., Rinaldi, M. G., Magnon, K. C., Cox, P. A., Revankar, S. G., Sanche, S., Geiser, D. M., Juba, J. H., van Burik, J. A. H., Padhye, A., Anaissie, E. J., Francesconi, A., Walsh, T. J., and Robinson, J. S. 2004. Genetic diversity of human pathogenic members of the *Fusarium oxysporum* complex inferred from multilocus DNA sequence data and amplified fragment length polymorphism analyses: Evidence for the recent dispersion of a geographically widespread clonal lineage and nosocomial origin. J. Clin. Microbiol. 42:5109-5120.

Pfaffl, M. W. 2001. A new mathematical model for relative quantification in real-time RT-PCR. Nucleic Acids Res. 29:e45.

Qiu, C. P., Zhang, H. Y., Chang, S. X., Wei, L. B., and Miao, H. M. 2014. Laboratory detecting method for pathogenicity of *Fusarium oxysporum* Schl. f. sp. *sesami* isolates. Acta Phytopathol. Sin. 44:26-35.

Recorbet, G., Steinberg, C., Olivain, C., Edel, V., Trouvelot, S., Dumas-Gaudot, E., Gianinazzi, S., and Alabouvette, C. 2003. Wanted: Pathogenesis-related marker molecules for *Fusarium oxysporum*. New Phytol. 159:73-92.

Rep, M., and Kistler, H. C. 2010. The genomic organization of plant pathogenicity in *Fusarium* species. Curr. Opin. Plant Biol. 13:420-426.

Rep, M., van der Does, H. C., Meijer, M., van Wijk, R., Houterman, P. M., Dekker, H. L., de Koster, C. G., and Cornelissen, B. J. C. 2004. A small, cysteine-rich protein secreted by *Fusarium oxysporum* during colonization of xylem vessels is required for *I-3*-mediated resistance in tomato. Mol. Microbiol. 53:1373-1383.

Schmidt, S. M., Houterman, P. M., Schreiver, I., Ma, L., Amyotte, S., Chellappan, B., Boeren, S., Takken, F. L., and Rep, M. 2013. MITEs in the promoters of effector genes allow prediction of novel virulence genes in *Fusarium oxysporum*. BMC Genomics 14:119.

Schroers, H. J., Baayen, R. P., Meffert, J. P., De Gruyter, J., Hooftman, M., and O'Donnell, K. 2004. *Fusarium foetens*, a new species pathogenic to *Begonia elatior* hybrids (*Begonia* × *hiemalis*) and the sister taxon of the *Fusarium oxysporum* species complex. Mycologia 96:393-406.

Simbaqueba, J., Catanzariti, A. M., González, C., and Jones, D. A. 2018. Evidence for horizontal gene transfer and separation of effector recognition from effector function revealed by analysis of effector genes shared between cape-gooseberry- and tomato-infecting formae speciales of *Fusarium oxysporum*. Mol. Plant Pathol. 19:2302-2318.

Snyder, W. C., and Hansen, H. N. 1940. The species concept in *Fusarium*. Am. J. Bot. 27:64-67.

Snyder, W. C., and Smith, S. N. 1981. Current status. Pages 25-50 in: Fungal Wilt Diseases of Plants. M. E. Mace, A. A. Bell, and C. H. Beckman, eds. Academic Press, New York, NY, U.S.A.

Su, Y. L., Miao, H. M., Wei, L. B., and Zhang, H. Y. 2012. Study on separation and purification techniques of *Fusarium oxysporum* in sesame (*Sesamum indicum* L.). J. Henan Agric. Sci. 41:92-95.

Takken, F., and Rep, M. 2010. The arms race between tomato and *Fusarium oxysporum*. Mol. Plant Pathol. 11:309-314.

Taylor, A., Vágány, V., Jackson, A. C., Harrison, R. J., Rainoni, A., and Clarkson, J. P. 2016. Identification of pathogenicity-related genes in *Fusarium oxysporum* f. sp. *cepae*. Mol. Plant Pathol. 17:1032-1047.

Thatcher, L. F., Gardiner, D. M., Kazan, K., and Manners, J. M. 2012. A highly conserved effector in *Fusarium oxysporum* is required for full virulence on *Arabidopsis*. Mol. Plant-Microbe Interact. 25:180-190.

Verma, M. L., Mehta, N., and Sangwan, M. S. 2005. Fungal and bacterial diseases of sesame. Pages 269-303 in: Diseases of Oilseed Crops. G. S. Saharan, N. Mehta, and M. S. Sangwan, eds. B. B. N Printers, New Deli, India.

Williams, A. H., Sharma, M., Thatcher, L. F., Azam, S., Hane, J. K., Sperschneider, J., Kidd, B. N., Anderson, J. P., Ghosh, R., Garg, G., Lichtenzveig, J., Kistler, H. C., Shea, T., Young, S., Buck, S. A., Kamphuis, L. G., Saxena, R., Pande, S., Ma, L. J., Varshney, R. K., and Singh, K. B. 2016. Comparative genomics and prediction of conditionally dispensable sequences in legume-infecting *Fusarium oxysporum* formae speciales facilitates identification of candidate effectors. BMC Genomics 17:191.