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Characterization of mating type, spore killing, and pathogenicity of *Fusarium verticillioides* populations from maize in China

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Abstract

Sexual reproduction in fungi promotes genetic diversity and helps the fungus to adapt to environmental stresses. *Fusarium verticillioides* is a heterothallic filamentous ascomycete that is a major cause of maize ear and stalk rot worldwide, while also posing a threat to human and animal health by producing various mycotoxins. Sexual reproduction in *F. verticillioides* is controlled by the *MAT-1* and *MAT-2* loci, which mandate that only strains of opposite mating types can mate to yield perithecia and ascospores. Nevertheless, there exists a phenomenon called 'spore killing', in which only four typical ascospores appear in the asci following a cross between a strain carrying the spore killer allele (Sk^K) and one with the spore killer sensitive allele (Sk^S). In this study, 31 isolates of *F. verticillioides* collected from eight provinces in China during the maize growing season from 2014 to 2020 were compared based on their mating type, spore killing genotype, and pathogenicity. To determine the mating types and spore killing genotypes of these isolates, partial sequences were amplified from the *MAT* loci and the *SKC1* gene, respectively. The PCR results showed that out of the 31 isolates, 18 were *MAT-1* and 13 were *MAT-2*, and that 25 had Sk^K genotypes and 6 had Sk^S genotypes. Genetic crosses between LNF15-11 (*MAT-2*) and 18 *MAT-1* isolates produced normal perithecia with varying numbers. However, crosses between LNF15-11 and the 3 isolates (SDF18-36, HNF14-8, and GSF19-6) produced only four ascospores per ascus, while the remaining isolates except SDF18-28, yielded eight ascospores per ascus. These findings suggest that the *SKC1* amplicon variation can be used to differentiate Sk^K and Sk^S genotypes in the field and that the 3 isolates are truly Sk^S genotypes with the *MAT-1* allele. Altogether, this study contributes to our knowledge of the mating type and spore killing genotype of *F. verticillioides* in China and offers valuable strain resources for investigating heterothallic sexual reproduction.

Keywords *Fusarium verticillioides*, Sexual reproduction, Heterothallic ascomycete, Mating type, Spore killing

Background

Sexual reproduction is widespread in eukaryotes and is an important way to generate genetic diversity among offspring. The genetic materials from both parents undergo allele recombination, eliminating harmful mutations and resulting in more adaptable offspring to their environment (Goodenough and Heitman 2014; Sun et al. 2019). Fungi are ideal model organisms for investigating the molecular mechanisms of sexual reproduction in eukaryotes due to their ease of manipulation in the laboratory and short generation

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times (Wallen and Perlin 2018). Fungi utilize mating type loci to control their sexual reproduction, with two main mating type systems being identified: the bipolar mating system (e.g., ascomycetes) and the tetrapolar mating system (e.g., basidiomycetes). They employ various strategies for sexual reproduction, including homothallic, heterothallic, and pseudohomothallic. Selfing or outcrossing can be observed in homothallic (e.g., *Fusarium graminearum*) and pseudohomothallic (e.g., *Podospora anserina*) fungi, while heterothallic (e.g., *F. verticillioides*) fungi are only outcrossing due to self-sterility (Leslie et al. 2006; Grognet and Silar 2015; Heitman 2015; Gardiner et al. 2020). Theoretically, each allele has an equal chance to be passed to offspring during sexual reproduction. However, some selfish genes, known as meiotic drivers, can manipulate gamete production to better their chances of transmission in the next generation. Meiotic drive is widespread in eukaryotes and may play an important role in population dynamics, genome evolution, and the creation of new species (Werren 2011; Lindholm et al. 2016; Gardiner et al. 2020). In ascomycete fungi, meiotic products are packaged in individual sacs called asci. Normally, a given species generates a specific number of ascospores in each ascus, typically four or eight (Vogan et al. 2022). The occurrence of meiotic drive can be investigated by observing ascospores within asci. A meiotic drive phenomenon called spore killing has been observed in several ascomycetes, including *F. verticillioides* (van der Gaag et al. 2000; Zanders et al. 2014). While previous studies have shown that spore killing may be controlled by a single gene or multiple genes (Zanders and Johannesson 2021; Vogan et al. 2022), the evolution and driving forces underlying this phenomenon remain unknown.

F. verticillioides is a globally distributed ascomycete that can cause maize ear and stalk rot and can produce various mycotoxins to threaten human and animal health (Blacutt et al. 2018; Kamle et al. 2019). This fungus is self-sterile, meaning that only two strains with opposite mating types (*MAT-1* and *MAT-2*) can cross and produce asci usually containing eight normal ascospores (Yun et al. 2000; Leslie et al. 2006). However, *F. verticillioides* is also hermaphroditic, which coomplicates the mating process. When two strains containing spore killer allele (Sk^K) and spore killer sensitive allele (Sk^S) are crossed, only four normal ascospores in an ascus are produced (Kathariou and Spieth 1982; Xu and Leslie 1996). In the past several decades, the spore killer was located in a 102 kb segment of chromosome V, called the *Sk* region (Xu and Leslie 1996; Pyle et al. 2016). Recently, Spore Killer Candidate 1 (*SKC1*), an Sk^K strain-specific gene, was discovered to be required for spore killing in *F. verticillioides* (Lohmar et al. 2022b). However, it is still unknown whether there are other genes involved in the regulation spore killing in this fungus.

The objective of this study is to assign mating type, spore killing genotypes, and pathogenicity of the *F. verticillioides* isolates collected from maize in China and to provide valuable strain resources for investigating heterothallic sexual reproduction.

Results

Identification and morphological comparison of different *F. verticillioides* isolates

Diseased maize ears and stalks were collected from eight provinces in China (Fig. 1a). A total of 31 *F. verticillioides* isolates were identified by morphological and molecular means. These isolates were cultured on PDA to compare their morphology, and it was observed that they showed polymorphism in mycelial density and pigmentation. Among them, the HNF14-8 isolate produced the most pigmentation, while the mycelia of the SHF19-7 isolate were denser than those of the other isolates. The rate of colony growth and conidial morphology did not show significant variation, except for the slower growth rate of the JLF19-28 and SHF19-63 isolates compared to the other isolates (Fig. 1b, c).

Classification of *F. verticillioides* isolates based on mating types and spore killers

The mating types of these isolates was determined by PCR. Among the 31 isolates tested, 18 were found to possess the *MAT-1* allele, as shown by the amplification of a 250 bp fragment. Meanwhile, 13 isolates, including the LNF15-11 isolate, were identified as carrying the *MAT-2* allele, with an 800 bp fragment being amplified. The ratio of *MAT-1*: *MAT-2* was 18:13, indicating 58% of all isolates possess the *MAT-1* allele (Fig. 2a and Table 1). Previous studies showed that the *SKC1* gene is required for spore killing in the *Sk* region of the Sk^K strain. To evaluate if the *Sk* region variation can differentiate between the Sk^K and Sk^S strains, all the isolates were evaluated by amplifying the *Sk* region with specialized primer pairs (Table 2). The results showed that 25 isolates were identified as Sk^K, with about 800 bp fragments being amplified, including the 10 *MAT-2* isolates and the 15 *MAT-1* isolates. Moreover, 6 isolates were identified as Sk^S, with about 300 bp fragments being amplified, including the 3 *MAT-2* isolates and the 3 *MAT-1* isolates. The proportion of Sk^K:Sk^S was 25:6, suggesting that the Sk^K genotype is more prevalent in China (Fig. 2b and Table 1).

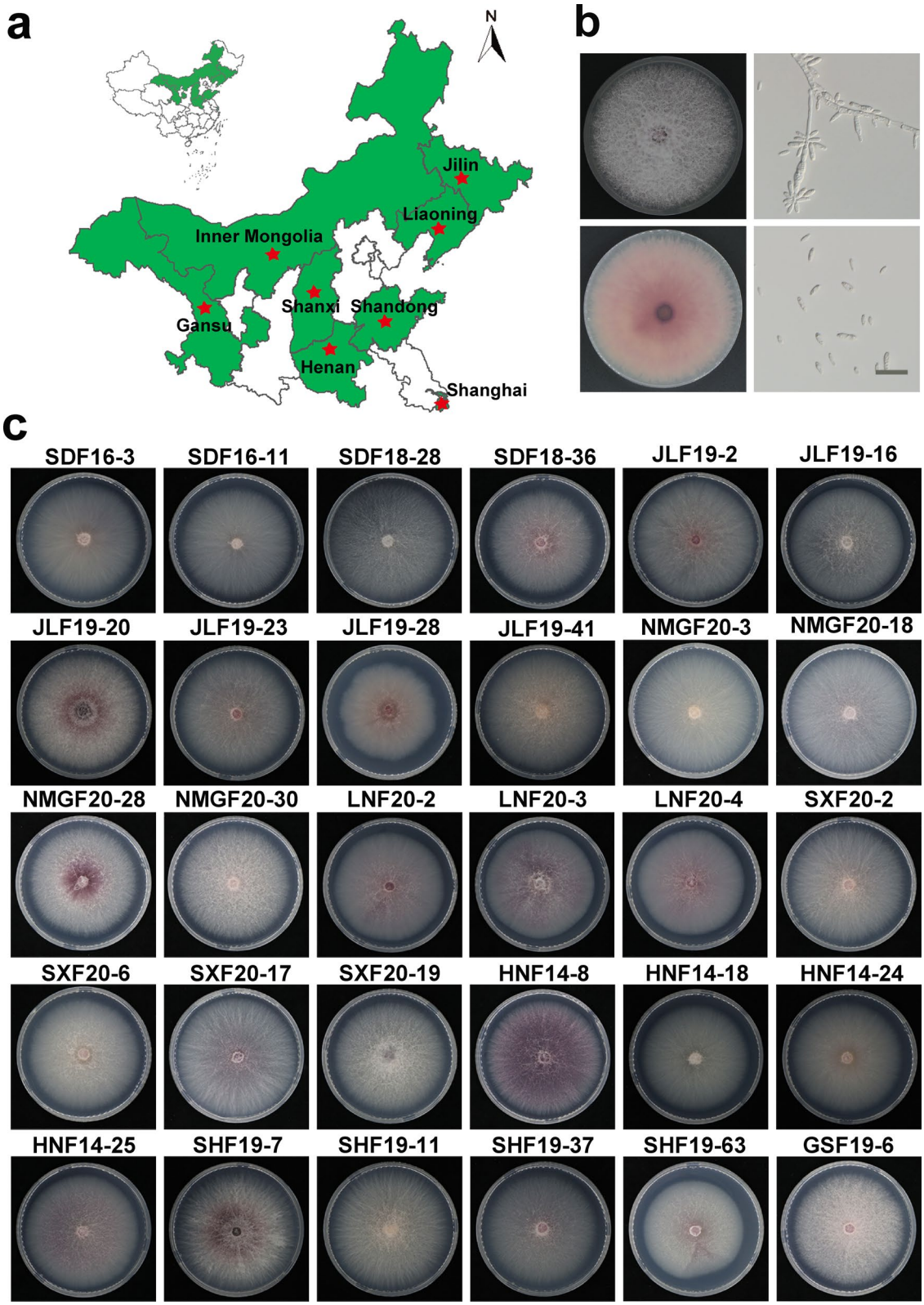

Fig. 1 Isolation and identification of different *Fusarium verticillioides* isolates. **a** A map of the geographic distribution of sample collection sites. **b** The morphological characteristics of the LNF15-11 isolate cultured on PDA at 6 days post-inoculation. Scale bar = 20 μm. **c** Comparison of colony morphology of different *F. verticillioides* isolates. All isolates were cultured on PDA at 25°C for 6 days

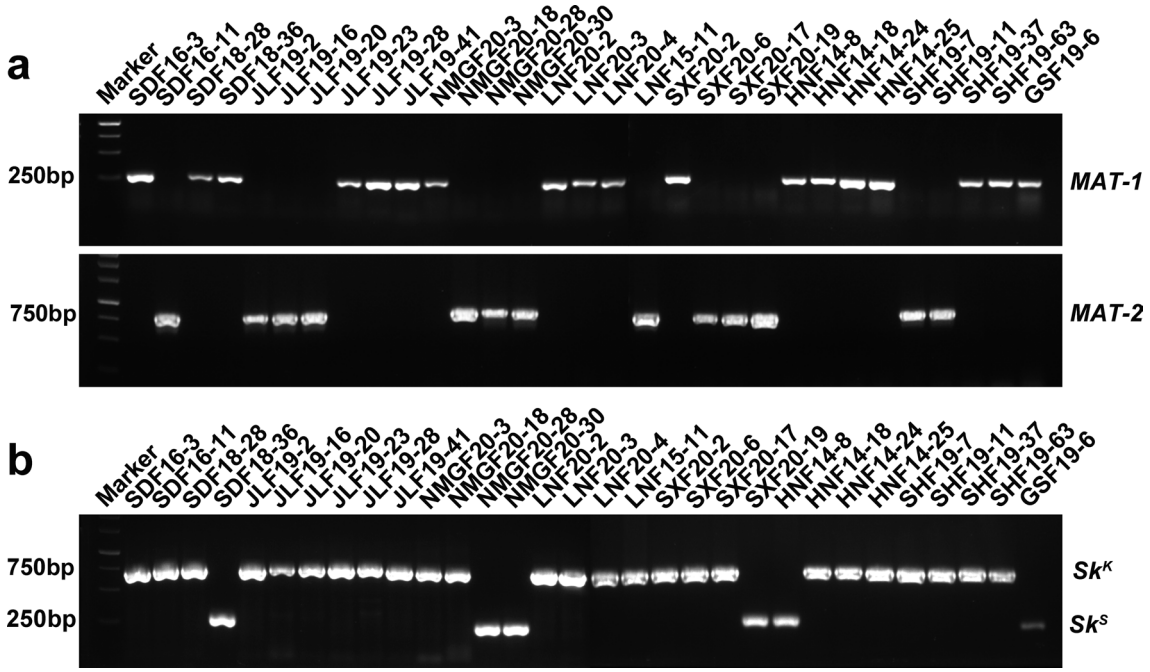

Fig. 2 Identification of mating types and spore killing genotypes in all *F. verticillioides* isolates. **a** The 250 bp fragment was amplified from the *MAT-1* isolates, and the 800 bp fragment was amplified from the *MAT-2* isolates. **b** The 800 bp fragment was amplified from the Sk^K strains, and the 300 bp fragment was amplified from the Sk^S strains

Observation of asci and spore killing in *F. verticillioides*

To further confirm the *SKC1* gene is necessary for spore killing in the Sk^K strain, the LNF15-11 isolates (*MAT-2* and SK^K) serving as the female parent was crossed with each of the 18 *MAT-1* isolates. After 4 weeks of mating, we observed that the 18 *MAT-1* isolates produced perithecia. The production of perithecia varied among the 18 *MAT-1* isolates, with JLF19-41, SXF20-2, and SHF19-37 producing the most perithecia and HNF14-24, HNF14-25, and GSF19-6 producing the least perithecia (Fig. 3a, b, and Table 1). These results indicate that the sexual reproductive capacity of each isolate was different and that the LNF15-11 isolate was female fertile. We also observed obvious differences in the sporulation of the 31 isolates, with SDF18-36, SXF20-2, GSF19-6, and JLF19-20 producing more conidia and LNF20-2, LNF20-4, HNF14-8, NMGF20-18, and SHF19-7 producing fewer (Fig. 3c). Therefore, a correlation analysis between the sporulation and production of perithecia the 18 *MAT-1* isolate were carried out. However, the result shows that there was no correlation between sporulation and the production of perithecia (Fig. 3d).

The mature perithecium was pressed with slide, and the number of asci and ascospores was observed to differentiate spore killing genotypes in these strains. Eight ascospores were observed in the asci produced by crossing LNF15-11 with SDF16-3, JLF19-23, JLF19-28, JLF19-41, NMGF20-3, LNF20-2, LNF20-3, LNF20-4, SXF20-2, HNF14-18, HNF14-24, HNF14-25, SHF19-37, and SHF19-63 isolates, while only four ascospores were observed in crossing LNF15-11 with SDF18-36, HNF14-8, and GSF19-6, indicating the three isolates contained *MAT-1* allele and SK^S genotype (Fig. 4 and Table 1). However, despite the emergence of perithecia, no asci and ascospore were observed when LNF15-11 was crossed with SDF18-28 (Table 1). The same results were observed in two repetitions. Taken together, these results further proved that there are 15 isolates (LNF15-11, SDF16-3, JLF19-23, JLF19-28, JLF19-41, NMGF20-3, LNF20-2, LNF20-3, LNF20-4, SXF20-2, HNF14-18, HNF14-24, HNF14-25, SHF19-37, and SHF19-63) carried Sk^K genotype and the SDF18-36, HNF14-8, and GSF19-6 isolates were Sk^S genotype (Table 1).

Pathogenicity tests of different *F. verticillioides* strains on maize kernels

To compare the pathogenicity of the *F. verticillioides* isolates with different mating types and spore killing genotypes, maize kernels were inoculated with 11 representative *F. verticillioides* isolates. The symptoms and severity of the disease were recorded at 7 dpi. The results showed that all isolates were pathogenic to maize kernels, regardless of their mating types and spore killing genotypes (Fig. 5a). The fungal biomass in the maize kernels was analyzed by

Table 1 Summary of the *Fusarium verticillioides* isolates with mating type and spore killing genotype identified in this study

Number	Isolates	Origin	Year	Mating type	Production of perithecia[#]	Number of ascospores	Spore killer genotype
1	SDF16-3	Shandong	2016	*MAT-1*	17.00 ± 2.00^{f}	8	Sk^{K}
2	SDF16-11	Shandong	2016	*MAT-2*	N.T	N.T	Sk^{K}
3	SDF18-28	Shandong	2018	*MAT-1*	23.67 ± 2.52^{bc}	N.A	Sk^{K}
4	SDF18-36	Shandong	2018	*MAT-1*	15.00 ± 2.00^{f}	4	Sk^{S}
5	JLF19-2	Jilin	2019	*MAT-2*	N.T	N.T	Sk^{K}
6	JLF19-16	Jilin	2019	*MAT-2*	N.T	N.T	Sk^{K}
7	JLF19-20	Jilin	2019	*MAT-2*	N.T	N.T	Sk^{K}
8	JLF19-23	Jilin	2019	*MAT-1*	17.67 ± 2.52^{ef}	8	Sk^{K}
9	JLF19-28	Jilin	2019	*MAT-1*	23.00 ± 2.00^{bcd}	8	Sk^{K}
10	JLF19-41	Jilin	2019	*MAT-1*	38.00 ± 3.61^{a}	8	Sk^{K}
11	NMGF20-3	Inner Mongolia	2020	*MAT-1*	18.00 ± 2.00^{def}	8	Sk^{K}
12	NMGF20-18	Inner Mongolia	2020	*MAT-2*	N.T	N.T	Sk^{K}
13	NMGF20-28	Inner Mongolia	2020	*MAT-2*	N.T	N.T	Sk^{S}
14	NMGF20-30	Inner Mongolia	2020	*MAT-2*	N.T	N.T	Sk^{S}
15	LNF20-2	Liaoning	2020	*MAT-1*	24.00 ± 2.00^{b}	8	Sk^{K}
16	LNF20-3	Liaoning	2020	*MAT-1*	25 ± 2.00^{b}	8	Sk^{K}
17	LNF20-4	Liaoning	2020	*MAT-1*	23.67 ± 2.08^{bc}	8	Sk^{K}
18	LNF15-11	Liaoning	2015	*MAT-2*	N.T	N.T	Sk^{K}
19	SXF20-2	Shanxi	2020	*MAT-1*	39.00 ± 3.61^{a}	8	Sk^{K}
20	SXF20-6	Shanxi	2020	*MAT-2*	N.T	N.T	Sk^{K}
21	SXF20-17	Shanxi	2020	*MAT-2*	N.T	N.T	Sk^{K}
22	SXF20-19	Shanxi	2020	*MAT-2*	N.T	N.T	Sk^{S}
23	HNF14-8	Henan	2014	*MAT-1*	18.67 ± 1.53^{cdef}	4	Sk^{S}
24	HNF14-18	Henan	2014	*MAT-1*	22.33 ± 1.53^{bcde}	8	Sk^{K}
25	HNF14-24	Henan	2014	*MAT-1*	7.33 ± 1.53^{g}	8	Sk^{K}
26	HNF14-25	Henan	2014	*MAT-1*	7.33 ± 2.52^{g}	8	Sk^{K}
27	SHF19-7	Shanghai	2019	*MAT-2*	N.T	N.T	Sk^{K}
28	SHF19-11	Shanghai	2019	*MAT-2*	N.T	N.T	Sk^{K}
29	SHF19-37	Shanghai	2019	*MAT-1*	41.67 ± 4.51^{a}	8	Sk^{K}
30	SHF19-63	Shanghai	2019	*MAT-1*	22.33 ± 1.53^{bcde}	8	Sk^{K}
31	GSF19-6	Gansu	2019	*MAT-1*	8.00 ± 1.73^{g}	4	Sk^{S}

N.T. not tested, *N.A.* not available, Sk^{K} spore killer, Sk^{S} spore killer sensitive

[#] Production of Perithecia: count the number of asci per plate. Data represent the mean±SE of three replicates. The letters a to g indicate that columns with different letters represent a statistical significance of $P < 0.05$ according to Waller–Duncan test

qPCR, with the LNF15-11 isolate as a control. The SXF20-2, NMGF20-28, and GSF19-6 isolates had a biomass similar to that of the LNF15-1 isolate, while the JLF19-16, JLF19-41, SHF19-37, SXF20-19, and SDF18-36 isolates had higher biomass than the LNF15-11 isolate.The NMGF20-30 and HNF14-8 isolates, on the other hand, exhibited lower biomass than the LNF15-11 isolate and the lowest levels biomass overall (Fig. 5b). In summary, these findings suggest that pathogenicity is independent of mating type, spore-killing genotype, and conidia production capacity.

Discussion

F. verticillioides greatly affects maize yield in maize-growing regions globally and produces fumonisins that pose a serious threat to human and animal health (Blacutt et al. 2018). As a heterothallic ascomycete, sexual development is controlled by two mating-type loci, termed *MAT-1* and *MAT-2* idiomorphs, respectively. Although the reproductive biology of *F. verticillioides* has been studied in many maize-growing areas, there is currently no information about the mating behavior and spore killing genotype

Table 2 Primer sequences used in this study

Name	Sequence (5′–3′)	Purpose
EF-1 F	ATGGGTAAGGARGACAAGAC	Primer pair for the identification of *F.verticillioides*
EF-1 R	GGARGTACCAGTSATCATGTT	
GFmat1a	GTTCATCAAAGGGCAAGCG	Primer pair for the identification of the mating type
GFmat1b	TAAGCGCCCTCTTAACGCCTTC	
GFmat2c	AGCGTCATTATTCGATCAAG	Primer pair for the identification of the mating type
GFmat2d	CTACGTTGAGAGCTGTACAG	
SkF	CGAATGACCTGGGGAGCCATAA	Primer pair for the identification of the spore killing genotype
SkR	TCTCTCCACCACCTCCATCAGC	
Maize_EF-1α_F	TGGGCCTACTGGTCTTACTACTGA	Primer pair for detection of relative biomass in maize kernels
Maize_EF-1α_R	ACATACCCACGCTTCAGATCCT	
Fv_Tub_F	CCCCGAGGACTTACGATGTC	Primer pair for detection of relative biomass of *F. verticillioides* in maize kernels
Fv_Tub_R	CGCTTGAAGAGCTCCTGGAT	

of this plant pathogen in China. In this study, we evaluated 31 *F. verticillioides* isolates for their mating type, spore killing genotype, and pathogenicity. In heterothallic ascomycetes, the mating type should ideally be a 1:1 ratio since this feature is known to be controlled by a single Mendelian locus (Leslie and Klein 1996). In this study, 18 isolates were identified as *MAT-1* type, and 13 isolates were identified as *MAT-2* type. The ratio of *MAT-1:MAT-2* was 1.38:1, a little higher than the theoretical proportion. These results were in agreement with those obtained by Cumagun (2008), but differed from those reported by Leslie and Klein (1996) and Qiu et al. (2015). This could be due to the geographical locations of the sample and its size. Additionally, we performed a genetic cross between the isolate LNF15-11 as a female and 18 isolates of *MAT-1* as males separately. In all cases, the perithecia were observed; however, we noted differences in the number of perithecia produced in each isolate, suggesting that the sexual reproductive capacity of each isolate was variable. Since perithecia development is an intricate process directed by multiple genes and signaling pathways, further investigation is needed to understand the difference in perithecia formation among the isolates.

A phenomenon called spore killing occurs in the Sk^K strain carrying the spore killing element when the Sk^K strain is crossed with the Sk^S strain with a sensitive element. The *Sk* region of the Sk^K strain carries a unique gene *SKC1*, which produces SKC1b through A-to-I editing, which is required for spore killing (Lohmar et al. 2022a). Therefore, we amplified the fragment of *SKC1* by PCR to determine spore killing genotypes of the 31 isolates. Of the 31 isolates, 25 were Sk^K (81%), and 6 were Sk^S (19%). These results indicate that the Sk^K strains may have more advantages than the Sk^S strains in nature owing to spore killing. In this study, we also observed the spore killing phenomenon in the LNF15-11 isolate when crossed with the SDF18-36, HNF14-8, and GSF19-6 isolates. However, asci and ascospores were not observed in the perithecia produced by the cross between the LNF15-11 and SDF18-28 isolates, and the reason behind this remains unclear. In *Neurospora*, there are three known spore killers: *Sk-1*, *Sk-2,* and *Sk-3*. No live spores are observed in the cross between an *Sk-2* strain and an *Sk-3* strain (Turner and Perkins 1979). Spore killing of the *Sk-1* strain is regulated by a single gene *Spk-1* (Svedberg et al. 2021), while spore killing of the *Sk-2* strain is regulated by two genes: a resistance gene called *rsk* and a killer gene called *rfk* (Hammond et al. 2012; Harvey et al. 2014). At present, the mechanism of how resistance to spore killing in the Sk^K strain is unknown in *F. verticillioides*, but it might be similar to *rsk* in *Neurospora*.. Further analysis of the Sk^K strains will be needed to shed light on this mechanism.

(See figure on next page.)

Fig. 3 Production of perithecia in *F. verticillioides*. **a** The LNF15-11 isolate (*MAT-2*) was used as the female parent and crossed with the 18 *MAT-1* isolates. The mature perithecia formation on carrot agar media after 4 weeks of fertilization. The sexual reproductive capacity of each isolate was variable. Among them, the JLF19-41, SXF20-2, and SHF19-37 isolates generate the most perithecia. **b** Statistical analysis of the number of perithecia in each case. **c** Statistical analysis of conidia production of different *F. verticillioides* isolates. The isolates were cultured on PDA for 6 days. One agar plug (1 cm in diameter) was collected from the edge of the colony and placed in 1 mL of sterile water. Conidia were released by vortexing and counted with a hemacytometer. **d** Correlation analysis between the sporulation and production of perithecia. Data represent the mean of three independent biological replicates. Bars indicate standard error. One-way ANOVA was used for statistical analysis. Columns with different letters represent a statistical significance of $P < 0.05$ according to the Waller-Duncan test

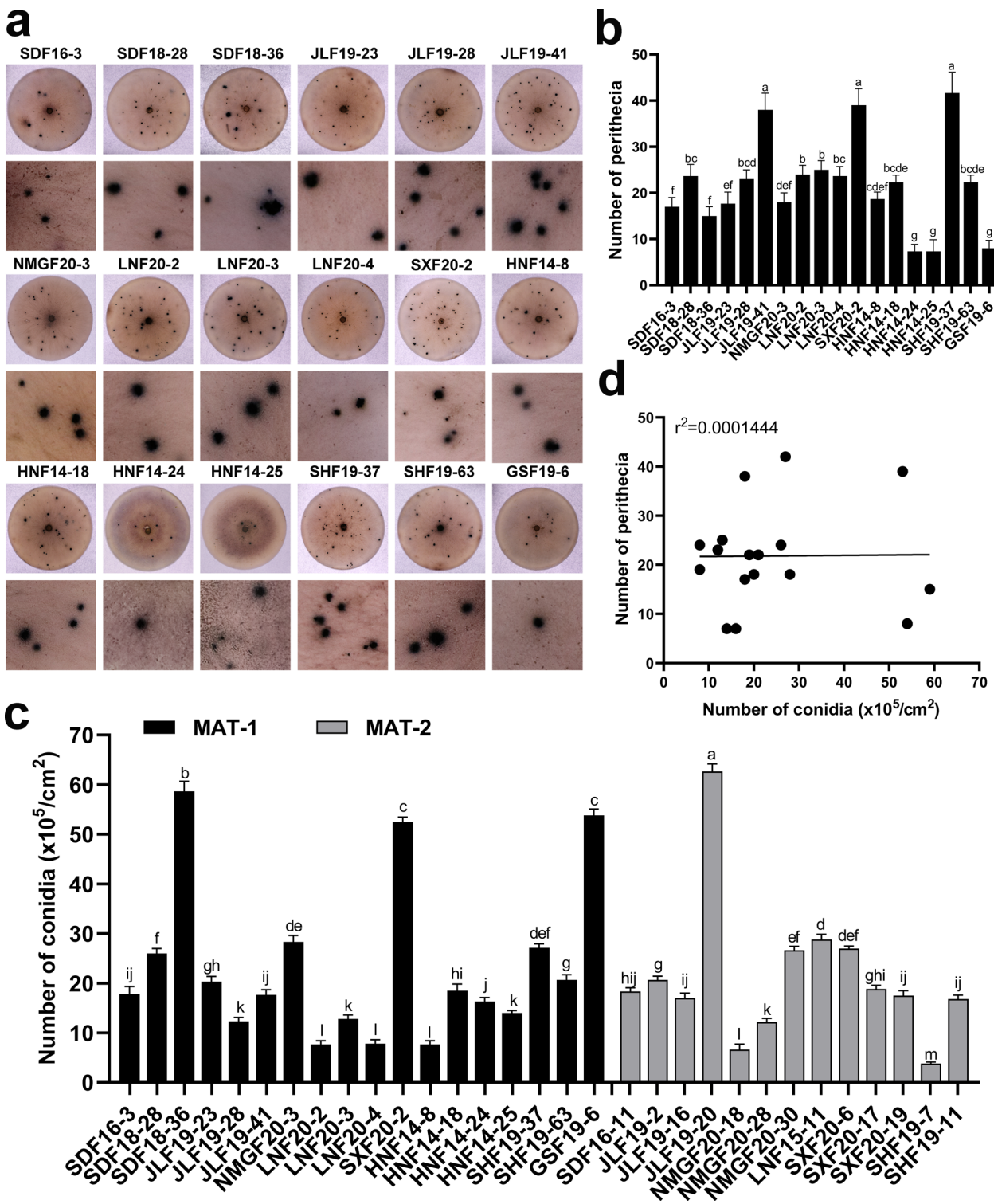

Fig. 3 (See legend on previous page.)

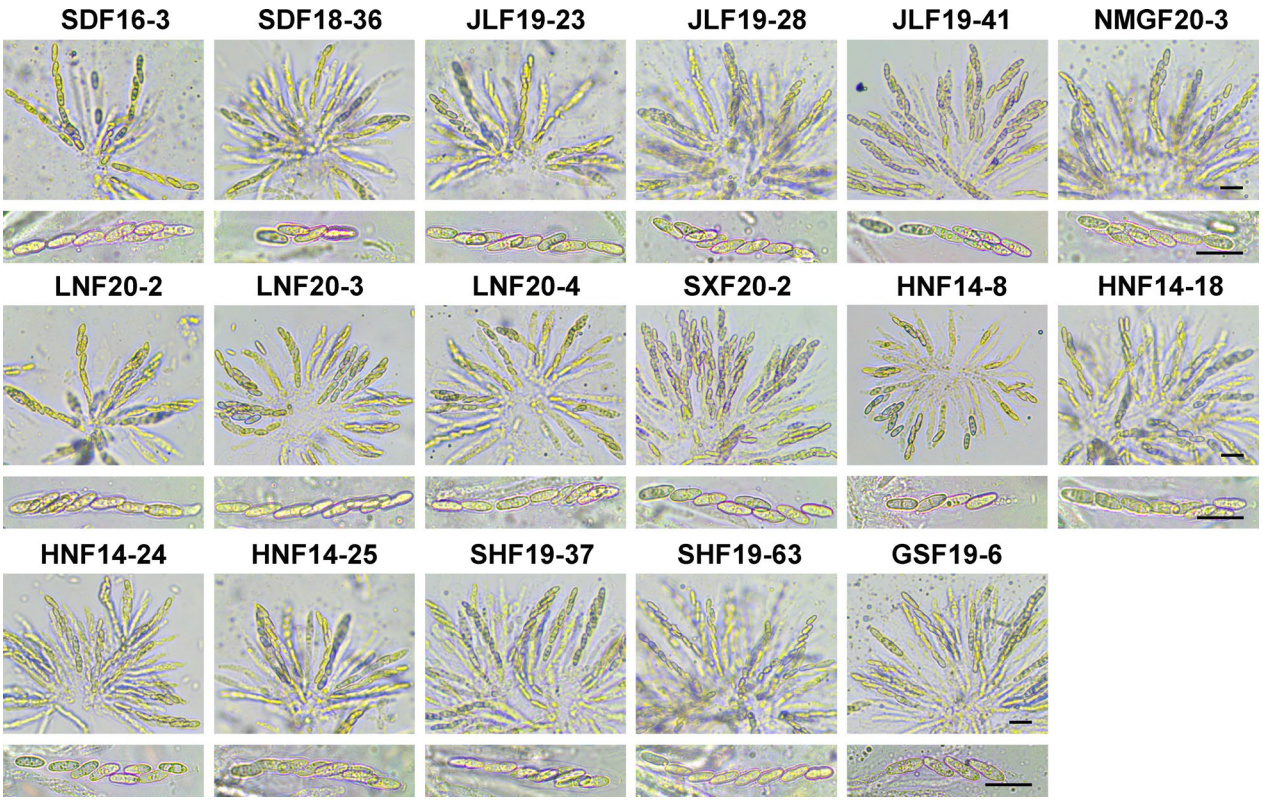

Fig. 4 Observation of spore killing phenomenon in *F. verticillioides*. The mature perithecium was pressed with a slide. The number of asci and ascospores was observed using the microscope. Eight ascospores were observed in most of the asci produced by crossing the LNF15-11 isolate with the SDF16-3, JLF19-23, JLF19-28, JLF19-41, NMGF20-3, LNF20-2, LNF20-3, LNF20-4, SXF20-2, HNF14-18, HNF14-24, HNF14-25, SHF19-37, and SHF19-63 isolates. Only four ascospores in an ascus were observed by crossing the LNF15-11 isolate with SDF18-36, HNF14-8, and GSF19-6 isolates. The asci and ascospores were not observed in the perithecia by crossing the LNF15-11 isolate with the SDF18-28 isolate. Scale bars = 20 µm

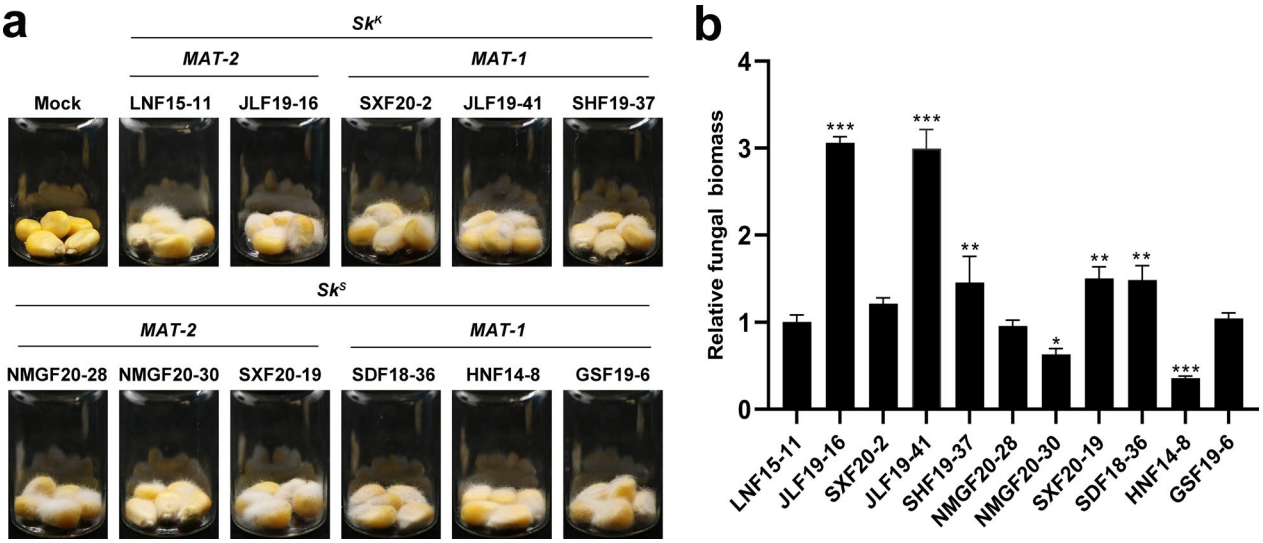

Fig. 5 Comparison of pathogenicity of different *F. verticillioides* isolates. **a** The maize kernels were inoculated with conidia from each isolate with different spore killing genotypes and mating types. The maize kernels were photographed at 7 days post-inoculation. The maize kernels were inoculated with sterile water as the negative control. **b** The fungal relative biomass of *F. verticillioides* in maize kernels was determined by qPCR. The LNF15-11 isolate was used as a control. Data represent the mean of three replicates. Bars indicate standard error. The *, **, and *** indicate, respectively, significant differences at $P < 0.05$, $P < 0.01$, and $P < 0.001$ according to a one-way analysis of variance (ANOVA)

Conclusions

In summary, this study suggests that the Chinese population of *F. verticillioides* is highly fertile, indicating a significant potential for recombination through sexual cycles in this population. These characteristics enable this population to produce offspring through sexual reproduction in the field and generate highly aggressive strains with a high capacity to produce fumonisins. In addition, this is the first contribution to spore killing genotypes and mating behavior of *F. verticillioides* in China. In the future, it would be beneficial to investigate the population genetic structure of *F. verticillioides* from other geographical locations and different host sources in China to clarify the genetic variation within and between populations.

Methods

Collection of samples, and isolation and purification of isolates

Maize tissues with typical symptoms of ear and stalk rot from maize fields were collected in the Inner Mongolia Autonomous Region, Jilin Province, Liaoning Province, Shanxi Province, Shandong Province, Henan Province, Gansu Province, Shanghai of China during the maize cropping season from 2014 to 2020. The isolates were purified and identified as described previously (Xi et al. 2021). The identified fusarium strains were stored in 30% glycerol at −80°C.

DNA extraction and species determination

All isolates were cultured on a potato dextrose agar medium (PDA) and incubated at 25°C for 5 days. The collected fungal mycelia were ground to powder in liquid nitrogen and suspended in 600 µL 2% CTAB buffer. The mixture was incubated at 65°C for 40 min, extracted with an equal volume of chloroform-isoamyl alcohol (24:1), and centrifugated at 13,000×*g* for 10 min. The nucleic acids were precipitated with 0.6 volumes of isopropanol from the supernatant after centrifugation (13,000×*g*, 10 min). The DNA pellet was washed twice in 70% ethanol and resuspended in 50 µL sterile distilled water. The quality and quantification of the extracted genomic DNA were determined by the microvolume spectrophotometers P200/P200+(Pluton Technology, California, USA). The extracted DNA was used as a template for PCR amplification using specific primer pairs EF-1F and EF-1R to amplify the partial translation elongation factor alpha gene (*EF-1α*) (Table 2). The amplified fragment was cloned into the vector pMD-18 T and sequenced. The resulting sequences were compared with the NCBI database and *Fusarium* database (FUSARIUM-ID v.3.0 database, http://isolate.fusariumdb.org/blast.php) for species determination (Torres-Cruz et al. 2022).

Identification of mating type and spore killing genotype

The mating types of the 31 *F. verticillioides* isolates were evaluated by PCR using specific primer pairs, amplifying distinct mating type (*MAT)* regions. The GFmat1a and GFmat1b primer pairs amplify a 250 bp fragment from the *MAT-1* α-domain, and the GFmat2c and GFmat2d primer pairs amplify an 800 bp fragment from the *MAT-2* HMG domain (Steenkamp et al. 2000). A unique gene *SKC1* is present in the Sk^K strains but absent in the Sk^S strains (Pyle et al. 2016). Therefore, the expected 800 bp and 300 bp fragments were amplified from the Sk^K and Sk^S strain using the specific primer pairs SkF and SkR, respectively (Table 2).

Genetic crosses, formation of perithecia, and observation of ascospores

To determine the reproductive ability of different isolates and observe the phenomenon of spore killing, crosses of the *F. verticillioides* isolates were conducted as described by Klittich and Leslie (1988). The LNF15-11 isolate with the completed genomic sequence was used as the female parent to cross with the male parent isolates of the opposite mating type. The LNF15-11 isolate was cultured on carrot agar medium (CA), and the male parent isolates were cultured on complete medium (CM) at 25°C. After 7 days, the conidia of the male parent isolates were collected from the CM plate, resuspended in 2.5% Tween 60 solution (Macklin, Shanghai, China) and adjusted to a concentration of 1×10^8 spores/mL. The 1 mL of the conidia suspension was spread on the surface of the female parent isolates. The fertilized plates were incubated at 25°C with 12 h light/12 h dark cycles. The asci can be observed oozing from mature perithecium after 4 weeks of fertilization. Then the perithecia were picked onto a slide dripped with sterile distilled water, pressed with a coverslip to release ascospores, and observed using the microscope at 10× and 40× magnification.

Pathogenicity assay of different isolates

Conidia were collected from 5-day-old PDA plates and adjusted to a concentration of 1×10^6 spores/mL with sterile distilled water. Maize kernels were sterilized sequentially using 75% alcohol for 5 min, 6% sodium hypochlorite for 3 min, and sterile water 3 times. Each sterilized maize kernel was inoculated with 10 µL conidia suspension and placed at 25°C. At 7 days after inoculation, disease symptoms were recorded, and the relative biomass of different isolates on maize kernels was determined by qPCR using the primer pairs Maize_EF-1α_F/R and Fv_Tub _F/R (Table 2).

Abbreviations

CA	Carrot agar medium
CM	Complete medium
PDA	Potato dextrose agar medium
Sk^K	Spore killer allele
Sk^S	Spore killer sensitive allele

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Author contributions
WG conceived and designed the experiments. FZ and TT performed the experiments. FZ and WG analysed the data and wrote the manuscript. FL reviewed and edited the original manuscript. All authors read and approved the final manuscript.

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Declarations

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The authors declare that they have no competing interests.

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