Phytopathology Research

RESEARCH

Open Access

Genetic diversity of field *Fusarium asiaticum* and *Fusarium graminearum* isolates increases the risk of fungicide resistance

Ya-Zhou Zhang[1,2*†], Zhuo Li[1,2†], Jie Man[1,2], Dan Xu[1,2], Lan Wen[1,2], Chen Yang[1,2], Qiang Xu[1,2], Qian-Tao Jiang[1,2], Guo-Yue Chen[1,2], Mei Deng[1,2], Yun-Liang Peng[3], Kai Zhong[4], Peng-Fei Qi[1,2] and Yu-Ming Wei[1,2*]

Abstract

Fusarium head blight (FHB) caused by *Fusarium* species, seriously threatens the safety of wheat (*Triticum aestivum*) production. Resistant cultivars and fungicides are frequently used to control these FHB pathogens. However, *Fusarium* species have been adapting the current FHB control approaches in a manner that raises concern for future FHB control strategies, which could lead to a greater risk of FHB outbreaks. In this study, a total of 521 strains of *Fusarium* were isolated from Sichuan province of China, to investigate the diversity of *Fusarium* species and the genes associated with their adaptation. Seven species were identified based on molecular markers and morphological analysis. The virulence assays showed that *Fusarium asiaticum* (Fa) and *Fusarium graminearum* (Fg) were the two major causal agents of FHB, with high virulence and more frequent isolates. Fungicide resistance analysis showed that four isolates had developed the resistance to carbendazim, and four isolates had developed the resistance to tebuconazole. Of note, two point-mutation variants (F200Y and E198Q) occurred in the *β2-tubulin* gene, leading to the carbendazim resistance. The landscape of genomic diversity was analyzed through whole-genome sequencing, revealing a total of 182,811 and 430,733 variants (including: single nucleotide polymorphisms, SNP, insertion and deletion, Indel, and structure variation, SV) among the Fa and Fg isolates, respectively. In addition, potential alterations in gene function (15.22%) were predicted among Fg variants. These alterations offer potential helps for the *Fusarium* species to adapt to various managements of FHB, which may increase risks in developing fungicide-resistant isolates. However, these annotated genetic variants are valuable resources for further genetic and genomic studies, as well as potential markers to assist disease risk assessment.

Keywords Fusarium head blight, Virulence, Whole-genome sequencing, Genetic diversity, Fungicide

†Ya-Zhou Zhang and Zhuo Li contributed equally to this work.

*Correspondence:
Ya-Zhou Zhang
yazhou14716@sicau.edu.cn
Yu-Ming Wei
ymwei@sicau.edu.cn
[1] State Key Laboratory of Crop Gene Exploration and Utilization in Southwest China, Sichuan Agricultural University, Chengdu 611130, China
[2] Triticeae Research Institute, Sichuan Agricultural University, Chengdu 611130, China
[3] Institute of Plant Protection, Sichuan Academy of Agricultural Sciences, Chengdu 610066, China
[4] College of Biomass Science and Engineering, Sichuan University, Chengdu 610065, China

Background

Wheat (*Triticum aestivum*) is one of the most important staple crops in the world. However, Fusarium head blight (FHB) seriously threatens the safety of wheat production worldwide. It mainly infects the wheat head and absorbs nutrients, resulting in considerable yield reduction and the deterioration of flour quality (Bottalico and Perrone 2002; Salgado et al. 2015). After infection of FHB, mycotoxins, including NIV, DON, 3-ADON, and 15-ADON, are produced by *Fusarium* in wheat seeds (Cheat et al. 2015). These mycotoxins can cause anorexia, diarrhea, vomiting, and gastrointestinal bleeding in animals, and cannot be easily removed by temperature or chemical and physical treatments (Marin et al. 2013; Thapa et al. 2021). A great deal of efforts has been devoted to avoid the exposure of mycotoxin. However, human or animal consuming of food contaminated by mycotoxins still occasionally occurs (Al-Jaal et al. 2019). Due to global warming and cropping system changes, frequent FHB outbreaks have caused significant economic losses in wheat globally, and the situation appears getting worse (Vaughan et al. 2016; Xu et al. 2021).

FHB pathogens are complex and diverse. More than 19 phylogenetically distinct species have been reported to cause FHB on wheat (Leslie and Summerell 2006; Van der Lee et al. 2015). Different climatic conditions and crop rotations can lead to differences in the distribution and predominance of the pathogens. For instance, *Fusarium graminearum* (Fg) and *Fusarium asiaticum* (Fa) are the major pathogens under warmer climate conditions (Xu et al. 2021), while *Fusarium culmorum*, *Fusarium nivale*, and *Fusarium poae* are the major pathogens under cooler climate conditions (Dweba et al. 2017). Fa is the predominant species in the southern wheat-rice rotation regions of China, but Fg is the predominant species in the Northern China where wheat and maize rotation is very common (Zhang et al. 2012). Furthermore, *Fusarium* species isolated from different regions show differences in morphology, pathogenicity, and fungicide resistance (Xu et al. 2021; Wang et al. 2022). Therefore, an investigation of the diversity of *Fusarium* is valuable for evaluating the severity of FHB.

Various control strategies have been used to prevent Fa and Fg from infecting wheat, including timely fungicide application, resistance germplasm deployment, and special cultural practices. However, the genetic diversity of Fa and Fg can counteract the efficiency of these control strategies (Zeller et al. 2004; Talas et al. 2015a; Yang et al. 2020). There is a line of evidence that some Fa and Fg have adapted to FHB control strategies, especially fungicides (e.g., Tebuconazole, TEC, Carbendazim, MBC) (de Chaves et al. 2022). For example, point mutations at *β1-tubulin* (*FGSG_09530*) and *β2-tubulin* (*FGSG_06611*) have been reported to be associated with resistance to MBC in *Fusarium* (Chen et al. 2015). Spolti et al. (2014) isolated a Fg strain (Gz448NY11) from Steuben county, New York, USA, showing a resistance to TEC. In Anhui province of China, 8.23% of single-spore isolates of Fa were found to be resistant to MBC, and five types of point mutations (F167Y, E198L, E198K, F200Y, and E198Q) in the *β2-tubulin* gene conferred resistance to MBC (Chen et al. 2015). Previous studies have reported that *CYP51-A* (*FGSG_04092*) and *CYP51-B* (*FGSG_01000*) genes are related to the resistance to TEC in *Fusarium* by point mutations that cause overexpression of *CYP51-A* and *CYP51-B* genes (Ma et al. 2006; Qian et al. 2018). Qian et al. (2018) confirmed that the point mutation (Y137H) of *CYP51-B* led to the resistance to TEC by site-directed mutagenesis in Fg strain PH-1. A total of 150 TEC-resistant Fg strains were obtained from different areas of Henan province in China in 2018–2020, and six resistant strains possessed an amino acid mutation (S169T) in *CYP51-B* (Chen et al. 2021). Notably, there are no reports showing that overexpression of *CYP51-A* or *CYP51-B* could cause the resistance to TEC in *Fusarium*.

Whole-genome sequencing can provide information on genetic variation, such as single nucleotide polymorphisms (SNP), insertion and deletion (Indel), and structure variation (SV). Since the genome information of Fg is available, more than 10,000 SNPs have been identified, which are preferentially located at the ends of chromosomes or in inner chromosomal locations (Cuomo et al. 2007). Walkowiak et al. (2016) found 704,566 SNPs and Indels among 10 closely related members of the Fg species complexed with different mycotoxin genotypes. Similarly, Laurent et al. (2017) found 242,756 high-confidence genetic variants in six French isolates of Fg via whole-genome sequencing. Nevertheless, a large number of genetic variants suggests that a small number of isolates is not sufficient to explain all phenotypic, pathogenicity, and fungicide resistance variants. Hence, more isolates of Fa and Fg are required for discovering the genetic diversity, and the variants are potential markers for tracking the spreading of *Fusarium* populations, and aiding the assessing of the risk of FHB outbreaks (Oghenekaro et al. 2021).

The complex climate conditions and cultural practices in Sichuan province are conducive to the emergence of various *Fusarium* species (Huang and Ye 2005), which makes fungicide control strategies being less effective. In this study, we aimed to survey *Fusarium* species in Sichuan province, and to identify major pathogens of FHB using virulence assays. All *Fusarium* isolates can be used to investigate genetic variations by whole-genome sequencing. The fungicides MBC and TEC were used to verify the link between genetic variation and the diversity

of biological functions, which could help us understand the diversity of *Fusarium* species and the genes associated with their adaptations to counteract current and future FHB control strategies, and for building the prediction model for potential FHB outbreaks.

Results

Identification of *Fusarium* species from wheat spikelet with visible FHB signs

A total of 521 strains of *Fusarium* species were obtained from wheat spikelet with visible FHB symptoms (Additional file 1: Table S1). The distribution of all isolates was shown in Fig. 1a. One or more molecular markers from each isolate were successfully obtained by PCR amplification and sequencing. These isolates had more than 98% sequence similarity with Fa, Fg, *F. meridionale*, *F. avenaceum*, *F. tricinctum*, *F. flocciferum*, or *F. proliferatum* (Additional file 1: Table S1). The identities of these seven *Fusarium* species were further confirmed by morphological analyses based on the descriptions of Leslie and Summerell (2006) (Fig. 1b). The frequency assays for each *Fusarium* species showed that 68.58% of them were Fa species and 27.59% were Fg species. Other species (including *F. meridionale*, *F. avenaceum*, *F. tricinctum*, *F. flocciferum,* and *F. proliferatum*) account for only 3.83% of the total isolates (Fig. 1c). The Fa isolates were the major pathogenic strains in the year of 2021. The Fg isolates were the major pathogenic strains in the year of 2022 (Fig. 1c). Therefore, Fa and Fg are the pathogens with a high frequency of FHB in Sichuan province.

Pathogenicity of *Fusarium* species

Our results showed that all isolates tested were able to infect the wheat spikelet (Fig. 2 and Additional file 1: Table S1). However, significant differences in infectivity were observed between different Fa or Fg isolates, where *F. meridionale*, *F. avenaceum*, *F. tricinctum*, *F. lateritium*, *F. flocciferum*, and *F. proliferatum* showed lower infectivity when compared with Fa and Fg isolates (Fig. 2 and Additional file 1: Table S1). These results indicate that Fa and Fg are highly pathogenic to wheat, despite there is a virulence diversity among different Fa or Fg isolates.

Fungicide resistance in Fa and Fg isolates

Due to the reduced effectiveness of MBC and TEC on controlling the FHB in Sichuan province, all Fa and Fg isolates were analyzed using a higher concentration of MBC (50 μg/mL) and TEC (36 μg/mL) than previously reported (Yin et al. 2009; Qian et al. 2018; Chen et al. 2021). The results showed that 50 μg/mL of MBC completely lost the ability to inhibit the growth of Fa Lz189, Fa Lz263, Fa Lz503, and Fg Lz114 isolates, although the reference Fg isolate PH-1 could not grow. However, the growth of Fa Lz136, Fa Lz167, Fa Lz201, and Fg Lz179

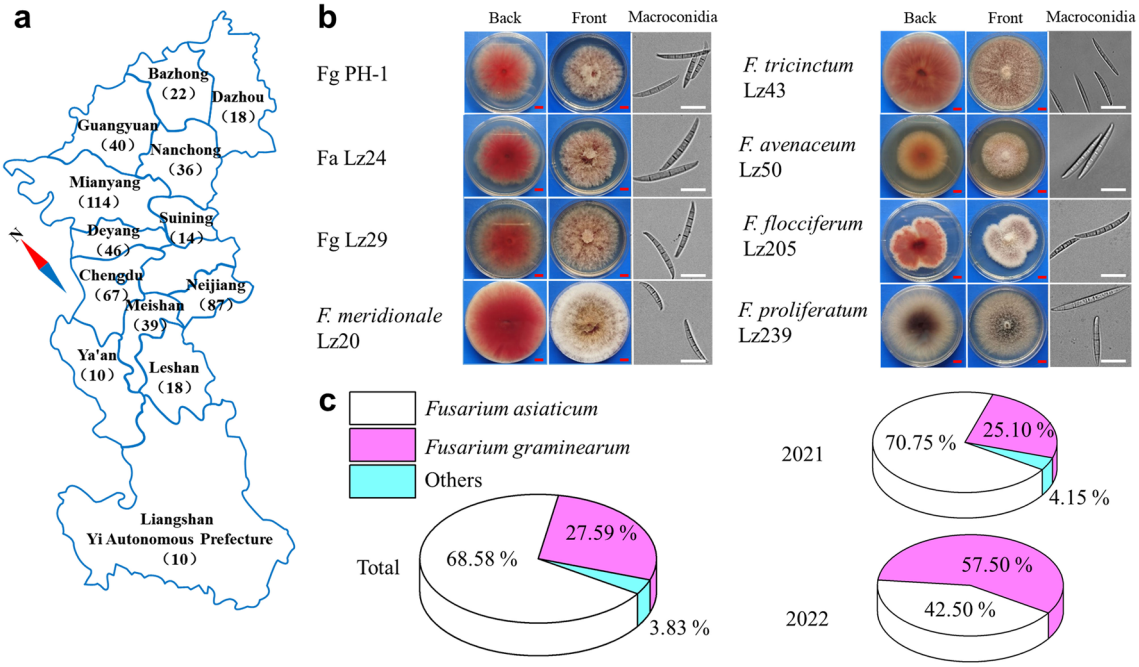

Fig. 1 Identification of FHB in Sichuan province of China. **a** Distribution of the obtained *Fusarium* isolates in Sichuan province. **b** Morphological analyses of *Fusarium* species on PDA plates at the 7th day after inoculation (Scale bars = 1 cm). **c** Frequency of each *Fusarium* species. The detail information of each isolate can be found in Additional file 1: Table S1

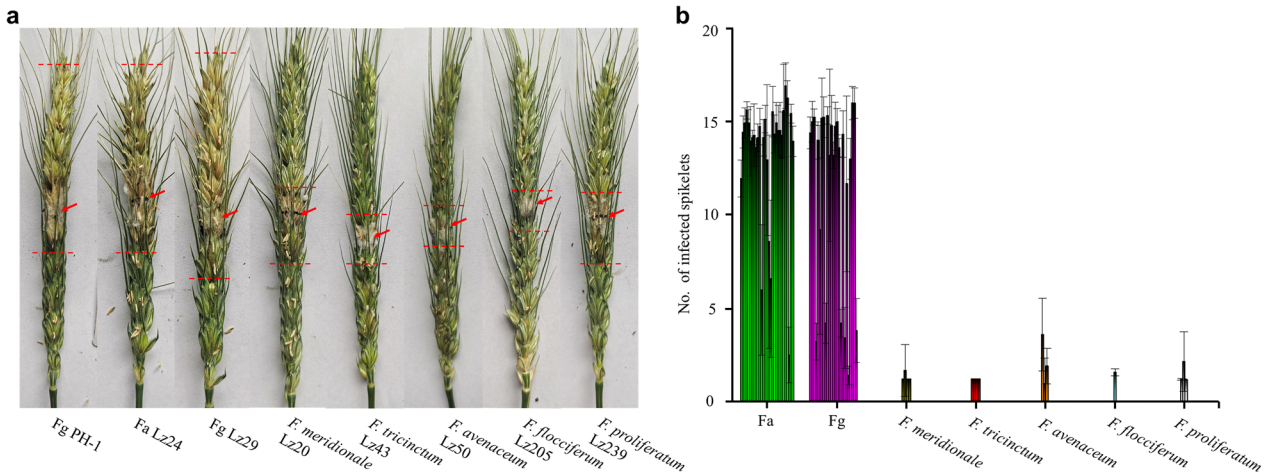

Fig. 2 Virulence assays on wheat. **a** Wheat heads were inoculated with conidial suspensions of *Fusarium* isolates. Infected wheat heads were photographed at 7th day after inoculation. Red arrows indicated the inoculation sites. Red dashed line marked the area of disease. **b** The numbers of infected and bleached spikelets at 7th day after inoculation. Values are means ± standard deviation of ten biological replicates per isolate. The isolate information was listed in Additional file 1: Table S1

isolates was partially inhibited in 36 μg/mL TEC compared with Fg PH-1 which could not grow either (Fig. 3a and Additional file 1: Table S1). Thus, Fa Lz189, Fa Lz263, Fa Lz503, and Fg Lz114 have evolved resistance to MBC, and Fa Lz136, Fa Lz167, Fa Lz201, and Fg Lz179 have evolved resistance to TEC.

Mutation of *β2-tubulin* is associated with MBC resistance

Point mutations in the *β1-tubulin* and *β2-tubulin* genes have been reported to be associated with resistance to MBC in *Fusarium* species (Chen et al. 2015). The open reading frames of the *β1-tubulin* and *β2-tubulin* genes were identified by PCR and sequencing. The results showed that *β1-tubulin* gene had no changes when compared with the reference gene sequence. For the open reading frame of the *β2-tubulin* gene, point mutations at codons 198 (GAG → GCG, E198A) and 200 (TTC → TAC, F200Y) in Fa Lz189, Fa Lz263, and Fa Lz503, and a point mutation at codon 198 (GAG → GCG, E198A) were found in Fg Lz114 (Fig. 3b). Other sensitive strains were further checked, but they did not carry these mutations (data not shown). Thus, the mutations of *β2-tubulin* at codons 198 (GAG → GCG, E198A) and 200 (TTC → TAC, F200Y) are related to resistance to MBC in Fa and Fg.

Mutations of *CYP51-B* is associated with TEC resistance

The point mutations or overexpression of *CYP51-A* and *CYP51-B* genes are associated with resistance to TEC in *Fusarium* species (Ma et al. 2006; Qian et al. 2018). Therefore, the open reading frame of *CYP51-A* and *CYP51-B* genes were identified by PCR and sequencing. The results showed that *CYP51-A* gene had no changes compared with the reference gene sequence. For the *CYP51-B* gene, Fa Lz136, Fa Lz167, Fa Lz201, and Fg strain Lz179 showed no changes in *CYP51-B* gene compared with the Fa reference sequence and those Fa isolates that could not grow at 36 μg/mL TEC (Fig. 3c). We then investigated the expression of *CYP51-A* and *CYP51-B* genes by real-time PCR in Fa strains Lz136, Fa Lz167, Fa Lz201, and Fg strain Lz179. The results showed that *CYP51-A* exhibited a significantly higher expression level than Fg PH-1, but *CYP51-B* showed a significantly lower expression level than Fg PH-1 in Fg strain Lz179 (Fig. 3d).

Genetic diversity of the Fa and Fg isolates

To determine the sequence diversity in the two species, we sequenced the Fa and Fg isolates collected from Sichuan province. The whole-genome sequencing results showed that Fg had 99.31% genome coverage (≥4×) to the Fg reference genome, and Fa had 99.18% genome coverage (≥4×) by alignment with the Fa reference genome. These results show that the Fa and Fg isolates are secured, and can be used for further genetic variation analysis. By genetic variation analysis, a total of 165,888 SNPs, 10,714 Indels, and 6209 SVs variants were discovered in Fa isolates, respectively. The details information is in Additional file 1: Table S2. SNPs were evenly distributed in all four chromosomes, and mainly distributed at the ends of the chromosomes (Fig. 4a).

Among Fg isolates, a total of 379,318 SNPs, 25,356 Indels, and 8059 SVs were found. The detail information is in Additional file 1: Table S3. Compared with the density distribution of all SNPs in the genome, SNPs were evenly distributed in all four chromosomes, and

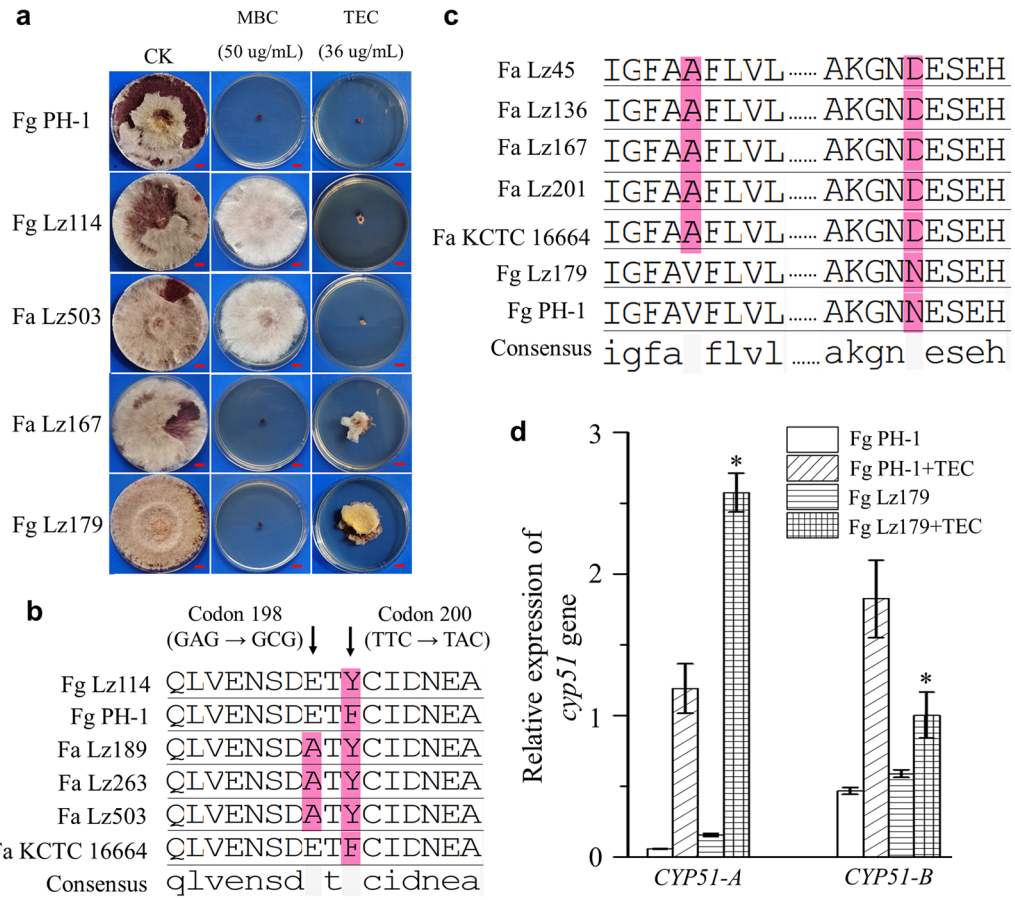

Fig. 3 Fa and Fg isolates showed resistance to MBC and TEC. **a** Mycelial growth of Fg Lz114, Fa Lz503, Fa Lz167, and Fg Lz179 on PDA plates with the treatments of MBC and TEC at the 7th day after inoculation (Scale bars = 1 cm). Fg PH-1 was used as a control. CK means no addition of fungicide. Three biological replicates for each experiment. **b** Multiple protein sequence alignment of β2-tubulin protein sequence. Black arrows indicate the mutation sites. **c** Multiple protein sequence alignment of CYP51-B protein sequence. Fa Lz45, a control with same protein sequence, could not grow in 36 μg/mL TEC. **d** Relative expression levels of *CYP51-A* and *CYP51-B* in Fg Lz179. Mycelia were collected at 7th day after incubation on PDA plates supplied with 36 μg/mL TEC. There were three biological replicates. Asterisk indicates significance at $P < 0.05$

mainly distributed in chromosome 1 and 2 (Fig. 4b). Besides, there are 15.22% potential alterations in gene function in Fg isolates due to SNPs (58,674), InDels (2432), and SVs (4450) by nonsynonymous SNPs, early stop codon, loss of stop codon, frameshift mutation, and SV in exons (Additional file 1: Table S3).

Among all variants, Fa isolates had a lower number of variants compared with Fg isolates. There was no significant difference in InDels length between Fa and Fg isolates (Fig. 4c, d). The chromosomal inversions (27.94%) were frequent discovered in the Fg isolates, and chromosomal insertions of large fragments frequently occurred in Fa (11.97%) (Fig. 4e, f). Notably, Fg isolates may have more variants that potentially affected genetic differentiation compared with Fa isolates.

Discussion

FHB is caused by several *Fusarium* species. The determination of the distribution of *Fusarium* species could allow effective monitoring of the occurrence of FHB (Dweba et al. 2017; Xu et al. 2021). In this study, seven species of *Fusarium* involved in FHB were isolated from diseased wheat spikelet (Additional file 1: Table S1), including Fa, Fg, *F. meridionale*, *F. avenaceum*, *F. lateritium*, *F. flocciferum*, and *F. proliferatum*. Fa and Fg were the dominant species, which is consistent with the previous studies in Asia (Huang and Ye 2005; Van der Lee et al. 2015; Xu et al. 2021). *F. meridionale*, *F. flocciferum*, and *F. proliferatum* were new isolates. Similarly, *F. meridionale* and *F. proliferatum* have been reported to be the major pathogens of maize and soybean in Sichuan province (Chang et al. 2018; Liu et al. 2020; Wang et al. 2021).

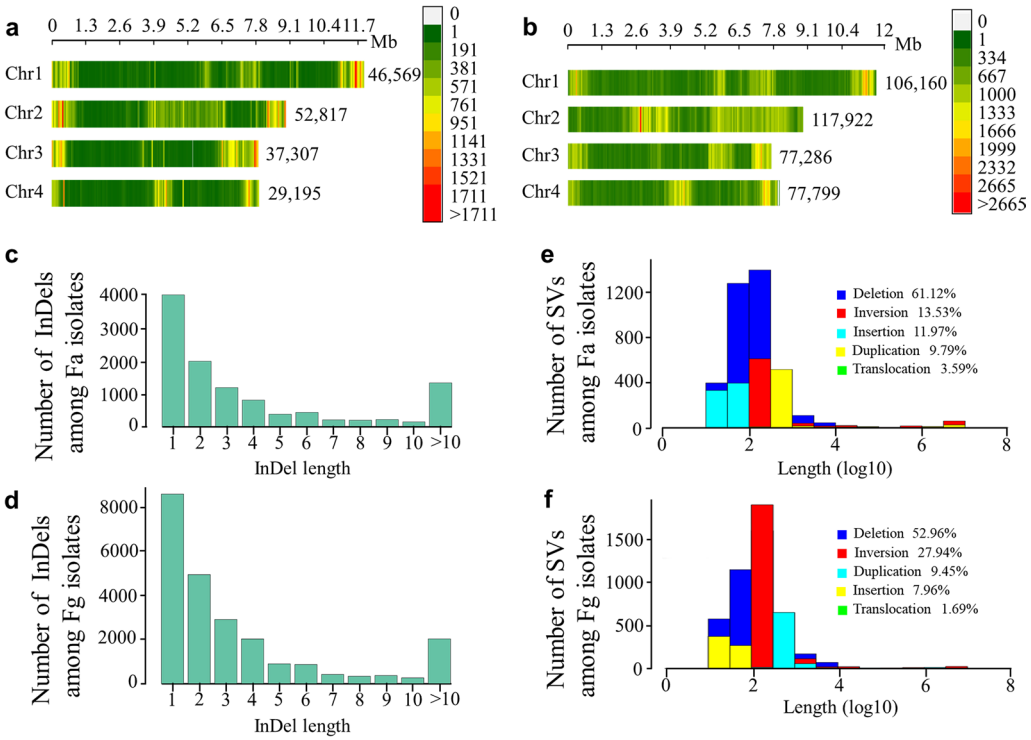

Fig. 4 Genetic variation analysis in Fa and Fg isolates. **a** Distribution of SNPs within 0.05 Mb window size in chromosome among Fa isolates. **b** Distribution of SNPs within 0.05 Mb window size in chromosome among Fg isolates. **c** Distribution of InDels among Fa isolates. **d** Distribution of InDels among Fg isolates. **e** Distribution of SVs. Different color columns represented different type of SVs among Fa isolates. **f** Distribution of SVs length. Different color columns represented different type of SVs among Fg isolates. All detail information can be found in Additional file 1: Tables S2 and S3

All *Fusarium* isolates showed less pathogenicity towards wheat, except for Fa and Fg (Fig. 2a, b). These strains were isolated from spikelet of wheat which were intercropped with maize or rotated in rice fields in the year of 2021, where the soil and diseased crop residues could serve as the initial infection source of *Fusarium* (Parry et al. 1995). Because the straw returning is encouraged in China when applying intercropping or rotation (Yan et al. 2020), these isolates might come from the straw residues of maize, soybean, or rice that had been infected by *Fusarium*.

Because of the climatic changes, the distribution of *Fusarium* species has been altered (Zhang et al. 2012). In this study, the major pathogenic strains are the Fa isolates in 2021, and there is a change for Fg in 2022 (Fig. 1c). The drier conditions during the winter and warmer conditions during the infection and grain-colonization period occurred in 2022 compared to the year of 2021. A previous study showed that Fg isolates are associated with drier and cooler conditions during the winter, as well as warmer conditions during the infection period (Xu et al. 2017). Thus, it suggests that climatic conditions are related to the distribution of Fa and Fg.

The demethylation inhibitor type of fungicides, such as TEC and prochloraz, are highly effective against *Fusarium* (Pasquali et al. 2020). Yin et al. (2009) showed that the 50% mycelial growth inhibition (EC_{50}) values of TEC-resistant isolates ranged from 0.034 to 6.235 μg/mL in 159 isolates of Fa and Fg. Talas et al. (2015b) also showed that the EC_{50} values of TEC-resistant isolates ranged from 5.4 to 62.2 μg/mL in 231 isolates. These values exhibited a normal distribution with a mean value of 22.2 μg/mL. So, different isolates have different EC_{50} values for TEC (Yin et al. 2009; Talas et al. 2015b). In this study, we observed that TEC at a concentration of 36 μg/mL was unable to completely inhibit the mycelial growth of Fa and Fg isolates (Fig. 3a). This concentration was higher than the previously reported concentration (Yin et al. 2009; Qian et al. 2018; Chen et al. 2021). There is no doubt that TEC-resistant isolates are emerging in Sichuan province, which increases the risk of fungicide application. Fa and Fg share similar morphological and molecular characteristics, toxicology, and genome sequences (Lee et al. 2014; Walkowiak et al. 2016; Yang et al. 2020). Lee et al. (2014) have revealed more than 80% nucleotide similarity between Fa and Fg in trichothecene

biosynthetic genes. Walkowiak et al. (2016) showed that Fa is more closely related to Fg, with 93.1% sequence similarity of the genome. In this study, our results also showed that Fa and Fg isolates have similar morphology and pathogenesis (Figs. 1, 2). However, Fg isolates had more variants compared with Fa isolates (Additional file 1: Table S2 and Table S3), and the distribution of SNPs and types of SVs were significantly different (Fig. 3). Thus, Fa may have a different direction of evolution compared with Fg, and can adapt to different conditions.

The isolates that used to determine the sequence diversity among Fa and Fg were isolated from different places and environments. A large number of variants may be linked to the polymorphism of biological functions, and may aid *Fusarium* in adapting to different stresses, including fungicides. The following two examples confirmed this assumption. Previous reports have shown that genetic variants in codon 198 aa and 200 aa in the open reading frame of *β2-tubulin* gene were related to resistance to MBC (Chen et al. 2015; Duan et al. 2015). The same variants were found in Fa Lz189, Fa Lz263, Fa Lz503, and Fg Lz114, and were confirmed by fungicide treatments on PDA plates (Fig. 3a). Over-expression of *CYP51-A* and specific point mutations in *CYP51-B* contributed to the TEC resistance (Ma et al. 2006; Yin et al. 2009; Qian et al. 2018). However, the *CYP51-A* and *CYP51-B* did not have sequence changes compared with the reference gene (Fig. 3c). This finding suggests that genes other than the *CYP51* family may be the most important contributors to TEC resistance (Talas et al. 2015b). In the current study, *CYP51-A* exhibited a significantly higher expression level than Fg PH-1 (Fig. 3d). Compared with genome sequencing results for Fg PH-1, exons of the *CYP51-A* gene showed six non-synonymous SNPs for Fa, and three non-synonymous SNPs for Fg. Twenty SNPs of Fa and one SNP variant occurred upstream of the *CYP51-A* gene. The relationship between these SNP variants and over-expression of *CYP51-A* needs to be further verified in strain Fg Lz179.

Conclusions

In this study, Fa and Fg isolates have developed resistance to MBC and TEC in Sichuan province (Fig. 3a and Additional file 1: Table S1). Because of the extensive use of MBC and TEC, the frequency of emerging fungicide-resistant isolates will gradually increase (Chen et al. 2015, 2021). The increased resistant strains will undoubtedly erode the control effect of FHB. Among the genetic variants we discovered, 15.22% of them could potentially affect gene function among Fg isolates, which may change the biological functions (Additional file 1: Table S3). Thus, genetic diversity can aid *Fusarium* in adapting to different environmental stresses and counteracting FHB management approaches, and increases the risk of FHB outbreaks. The fungicide resistance assays confirmed that the landscape of genomic assortment linked to the biological functions (Laurent et al. 2017). Importantly, these 521 isolates can provide an isolate library for quick search of the resistance genes in *Fusarium* species. The genetic diversity data can also be used to develop special molecular markers to assist in FHB risk assessment.

Methods

Fungal isolates

Wheat spikelet with visible FHB signs and symptoms was selected from Sichuan province in the year of 2021 (FHB outbreak) and 2022. All wheat spikelet with visible FHB signs were collected from forty-three fields that were evenly distributed in wheat-producing areas of Sichuan province (Fig. 1a). Five or six wheat spikelet with visible FHB signs were collected from each field. The collected wheat spikelet with visible FHB signs were washed with tap water, and were cut into small pieces (approximately 1 mm^3), and surface-sterilized with 75% ethanol (v/v) for 30 s, 1% NaClO (w/v) for 15 s, then rinsed three times with sterile distilled water. These pieces were placed on Petri dishes containing potato dextrose agar medium (PDA, Aoboxing Biotechnology, Beijing, China), then incubated at 25 ℃ for 7 days in the dark. Pure cultures were obtained by a single macroconidia isolation method described by Chang et al. (2018). Two or three single isolates were randomly selected from each wheat spikelet with visible FHB signs for further research. All isolates were stored at −80℃ with 20% glycerol at Sichuan Agriculture University.

Molecular markers analysis

For molecular identification, total genomic DNA was extracted from fungal mycelia that were collected at the 7th day from the PDA plates by the cetyl trimethyl ammonium bromide (CTAB) method (Lodhi et al. 1994). Partial gene sequences of internal transcribed spacer (*ITS*), β-tublin, translation elongation factor 1-alpha (*EF-1α*), and RNA polymerase beta large subunit II (*RPB2*) were amplified as molecular markers by PCR. Primers, PCR conditions, and product size are listed in Additional file 1: Table S4. PCR products were sequenced in BGI (https://en.genomics.cn/), and classified by blaste in the FUSARIUM-ID (http://www.fusariumdb.org) and *Fusarium* MLST (http://www.cbs.knaw.nl/Fusarium/) database (Geiser et al. 2004).

Morphological analysis

Morphological characteristics of fungal species were identified from isolates and macroconidia based on previous studies (Leslie and Summerell 2006). The

color and morphology of isolates were observed at the 7th day from the PDA plates. Image Java (National Institutes of Health, Bethesda, MD, USA) was used to calculate the growth rate of the hyphal area on PDA plates. Three biological replicates of each isolate were used, and *F. graminearum* PH-1 (Fg PH-1) was as used as the control (CK). Macroconidia were produced in carboxymethyl cellulose liquid medium at 28 °C, with shaking (180 rpm) for 7 days, and collected at the 7th day (Capellini and Peterson 1965). The shape and septum of the macroconidia were recorded using at least 1000 conidia per isolate under a compound microscope (Nikon-80i, Japan).

Virulence assay

To determine if these *Fusarium* strains caused disease, 41 Fa isolates, 23 Fg isolates, 3 *F. meridionale* isolates, 4 *F. avenaceum* isolates, 3 *F. tricinctum* isolates, 2 *F. lateritium* isolates, 3 *F. flocciferum* isolates, and 2 *F. proliferatum* isolates were used for inoculation on wheat. The common wheat cultivar '*shumai482*', which is susceptible to *Fusarium* infection, were grown in a greenhouse under a 16 h/8 h (day/night) cycle at 23 °C/18 °C. Plants were watered as necessary and fertilized before planting with 15-15-15 (N-P-K) compound fertilizer. After inoculation, the plants were maintained in a growth chamber at 25 ℃ under 16 h/8 h (day/night) cycle, with 90% moisture for 7 days. Two florets of a single central wheat (*Triticum aestivum* cv. '*shumai482*') spikelet were inoculated at each point using a micropipette at the mid-anthesis stage with 1×10^3 macroconidia. The average value of infected spikelet was used to represent the infectivity of the *Fusarium* isolates. Ten wheat spikelets were used per isolate, and Fg PH-1 was used as the control.

Fungicide-resistance assay for Fa and Fg isolates

To determine *Fusarium* resistance to fungicides, all Fg and Fa isolates were cultured by transferring 5 mm diameter plugs from the edge of a 3-day-old active colony to PDA medium. The concentrations of MBC (1.25 μg/mL) and TEC (36 μg/mL) that were determined to completely inhibit Fg PH-1 growth in PDA medium were used to select as resistant isolates (Additional file 2: Figure S1). Chen et al. (2015) reported that a concentration of 50 μg/mL of MBC can be used to select MBC-resistant isolates. We then used 50 μg/mL of MBC to further confirm the isolates with MBC resistance. All isolates were incubated at 25 °C for 7 days in the dark. Three biological replicates were used for each strain. Resistant isolates were further studied.

Cloning and sequencing of *β1-tubulin* and *β2-tubulin* gene in Fa and Fg isolates

Total RNA was extracted from the mycelia of resistant isolates that were grown on PDA plates for 7 days at 25 °C, using the E.Z.N.A.® Total RNA Kit I (Omega Bio-Tek, Norcross, GA, USA) according to the manufacturer's instructions. The RNA was reverse transcribed using the PrimeScript™ RT Reagent Kit with genomic DNA Eraser (Takara, Dalian, China) following the manufacturer's protocol. The cDNA sequences of *β1-tubulin* (Fa: *CP088260.1*_6189185 to 6,190,813; Fg: *FGSG_09530*), *β2-tubulin* (Fa: *CP088260.1*_643341 to 645053; Fg: *FGSG_06611*), *CYP51-A* (Fa: *CP088258.1*_6962848 to 69644; Fg: *FGSG_04092*), and *CYP51-B* (Fa: *CP088257.1*_3457782 to 3459530; Fg: *FGSG_01000*) genes were amplified by PCR. Primers, PCR conditions, and product size were shown in Additional file 1: Table S4. PCR products were sequenced in BGI (https://en.genomics.cn/). The Fg isolates sequencing results were aligned with the Fg PH-1 reference gene using DNAman 7.0 software (Lynnon Biosoft, USA). The Fa isolates sequencing results were aligned with the Fa KCTC 16664 reference gene using DNAman 7.0 software (Lynnon Biosoft, USA).

Measure the expression levels of *CYP51-A* and *CYP51-B* in Fg isolates

The primers CYP51AF/CYP51AR and CYP51BF/CYP51BR were used to amplify *CYP51-A* and *CYP51-B*, respectively. The relative expression levels of *CYP51-A* and *CYP51-B* were analyzed using the $2^{-\Delta\Delta Ct}$ method. *Actin* (*FGSG_07335*) and *β-tubulin* (*FGSG_09530*) were used as the references to normalize the expression data. The Fg strain PH-1 was used as a calibrator. The qPCRs were performed using a MyiQ Real-Time PCR Detection System (Bio-Rad, Hercules, CA, USA). All the primers mentioned above are listed in Additional file 1: Table S4.

Sequencing and data processing

Genomic DNAs were extracted from the fungal mycelia of 140 Fa isolates (Additional file 1: Table S5) that were cultured on PDA plates for 7 days at 25 °C, and the extracted DNA were equally premixed to build a DNA library at the Annoroad Genome company (http://genome.annoroad.com/). The same process was used to build a DNA library from 140 Fg isolates (Additional file 1: Table S5). Whole-genome sequencing was performed in BGI company (https://en.genomics.cn/). High quality sequences (clean reads) were filtered as follows: (1) remove the adaptor-polluted reads (reads containing > 5 adapter-polluted bases); (2) remove the low-quality reads (Phred quality value < 19); (3) remove reads

with the number of N bases accounting for more than 5%. The obtained clean reads after filtering were used for further statistical analyses. The Fg sequences were aligned to the reference genome Fg PH-1 (Submitted NCBI sequence: GCA_000240135.3), and Fa sequences were aligned to the reference genome Fa KCTC 16664 (GCA_025258505.1) using the BWA software (Li and Durbin 2009) and the BWA-MEM mode (Li 2013). Based on the alignment to the reference genome sequence, the software GATK (McKenna et al. 2010) was used to call SNPs and Indels present in the whole genome. The filtering settings were as follows: SNP: QD < 2.0, Read Pos Rank Sum < −8.0, FS > 60.0, QUAL < 30.0, DP < 4.0, MQ < 40.0, Mapping quality rank sum < −12.5, and INDEL: QD < 2.0, Read pos rank sum < −20.0, FS > 200.0, QUAL < 30.0, DP < 4.0. Finally, SNP and InDel data sets of high reliability were obtained. All potential chromosome SVs were detected by chromosomal structural variation analysis using the DELLY software (Rausch et al. 2012).

Statistical analyses

Student's *t*-test (implemented in the DPS (Data Procession System) version 12.01 software (Zhejiang University, Hangzhou, China) was used to examine the significance of differences among average values of isolates grown rate on PDA plates, percent of infected spikelets and the relative expression levels of *CYP51-A* and *CYP51-B* genes. Statistical differences were analyzed using the Least Significant Difference test at $P \leq 0.05$. In order to minimize errors, two independent tests were performed, and the average value of the two tests was taken as the final result.

Abbreviations

aa	Amino acid
bp	Base pair
CYP51	Cytochrome P450 family 51
Fa	*Fusarium asiaticum*
Fg	*Fusarium graminearum*
FHB	Fusarium head blight
Indel	Insertion and deletion
MBC	Methyl benzimidazol-2-ylcarbamate
PCR	Polymerase chain reaction
PDA	Potato dextrose agar
qPCR	Quantitative PCR
RT-PCR	Reverse transcription PCR
SNP	Single nucleotide polymorphisms
SV	Structure variation
TEC	Tebuconazole

Supplementary Information

The online version contains supplementary material available at https://doi.org/10.1186/s42483-023-00206-9.

Additional file 1: Table S1. Detail information of *Fusarium* species, virulence, and fungicide resistance. **Table S2.** Variations calling statistics and detail information among Fa isolates. **Table S3.** Variations calling statistics and detail information among Fg isolates. **Table S4.** Primer sequences, PCR settings, and amplified fragments obtained from different *Fusarium*. **Table S5.** Strain name of the sequenced Fa and Fg isolates.

Additional file 2 Fig. S1. Mycelial growth of Fg Lz114, Fa Lz503, Fa Lz167, and Fg Lz179 on PDA plates was observed under different concentrations of a MBC and b TEC at the 7th day after inoculation, Scale bars = 1 cm.

Acknowledgements

The authors thank local agricultural science and technology institutes in Sichuan province for providing wheat spikelet with visible FHB symptoms.

Author contributions

YZZ and YMW designed the experiments. YZZ, ZL, and YMW wrote the manuscript and analyzed the data. YZZ, ZL, and DX prepared the figures. YZZ, ZL, DX, JM, and LW performed the experiments. QX, QTJ, GYC, YLP, KZ, and MD provided key reagents and advice. All authors reviewed the results and approved the final version of the manuscript.

Funding

This research was supported by the National Natural Science Foundation of China (3210170116), the Science and Technology Department of Sichuan Province (2022YFSY0035), the Science and Technology Department of Sichuan Province (2020YFH0150), and the Applied Basic Research Programs of Science and Technology Department of Sichuan Province, China (2021YJ0298).

Availability of data and materials

Not applicable.

Declarations

Ethics approval and consent to participate

Not applicable.

Consent for publication

Not applicable.

Competing interests

The authors declare that they have no competing interests.

Received: 26 May 2023 Accepted: 26 September 2023
Published online: 07 November 2023

References

Al-Jaal BA, Jaganjac M, Barcaru A, Horvatovich P, Latiff A. Aflatoxin, fumonisin, ochratoxin, zearalenone and deoxynivalenol biomarkers in human biological fluids: a systematic literature review, 2001–2018. Food Chem Toxicol. 2019;129:211–28. https://doi.org/10.1016/j.fct.2019.04.047.

Bottalico A, Perrone G. Toxigenic *Fusarium* species and mycotoxins associated with head blight in small-grain cereals in Europe. Eur J Plant Pathol. 2002;108:611–24. https://doi.org/10.1023/a:1020635214971.

Capellini RA, Peterson JL. Macroconidium formation in submerged cultures by a non-sporulating strains of *Gibberella zeae*. Mycologia. 1965;57:962–6. https://doi.org/10.2307/3756895.

Chang XL, Dai H, Wang DQ, Zhou HH, He WQ, Fu Y, et al. Identification of *Fusarium* species associated with *soybean* root rot in Sichuan province. China Eur J Plant Pathol. 2018;151:563–77. https://doi.org/10.1007/s10658-017-1410-7.

Cheat S, Gerez JR, Cognié J, Alassane-Kpembi I, Bracarense APFL, Raymond-Letron I, et al. Nivalenol has a greater impact than deoxynivalenol on pig jejunum mucosa in vitro on explants and in vivo on intestinal loops. Toxins. 2015;7:1945–61. https://doi.org/10.3390/toxins7061945.

Chen Y, Yang X, Gu CY, Zhang AF, Gao TC, Zhou MG. Genotypes and phenotypiccharacterization of field *Fusarium asiaticum* isolates resistant to carbendazim in Anhui province of China. Plant Dis. 2015;99:342–6. https://doi.org/10.1094/PDIS-04-14-0381-RE.

Chen JP, Wei JQ, Fu LY, Wang S, Liu JL, Guo QW, et al. Tebuconazole resistance of *Fusarium graminearum* field populations from wheat in Henan province. J Phytopathol. 2021;169:525–32. https://doi.org/10.1111/jph.13021.
Cuomo CA, Güldener U, Xu JR, Trail F, Turgeon BG, Pietro AD, et al. The *Fusarium graminearum* genome reveals a link between localized polymorphism and pathogen specialization. Science. 2007;317:1400–2. https://doi.org/10.1126/science.1143708.
de Chaves MA, Reginatto P, da Costa BS, de Paschoal RI, Teixeira ML, Fuentefria AM. Fungicide resistance in *Fusarium graminearum* species complex. Curr Microbiol. 2022;79:62. https://doi.org/10.1007/s00284-021-02759-4.
Duan YB, Yang Y, Wang JX, Liu CC, He LL, Zhou MG. Development and application of loop-mediated isothermal amplification for detecting the highly benzimidazole-resistant isolates in *Sclerotinia sclerotiorum*. Sci Rep. 2015;5:17278. https://doi.org/10.1038/srep17278.
Dweba CC, Figlan S, Shimelis HA, Motaung TE, Sydenham S, Mwadzingeni L, et al. Fusarium head blight of wheat: Pathogenesis and control strategies. Crop Prot. 2017;91:114–22. https://doi.org/10.1016/j.cropro.2016.10.002.
Geiser DM, Jiménez-Gasco MM, Kang S, Makalowska I, Veeraraghavan N, Ward TJ, et al. FUSARIUM-ID v. 1.0: A DNA sequence database for identifying Fusarium. Eur J Plant Pathol. 2004;110:473–9. https://doi.org/10.1023/B:EJPP.0000032386.75915.a0.
Huang XH, Ye HZ. The population structure of *Fusarium* spp. from wheat in Sichuan Southweat China. J Agri Sci. 2005;18:281–5.
Laurent B, Moinard M, Spataro C, Ponts N, Barreau C, Foulongne-Oriol M. Landscape of genomic diversity and host adaptation in *Fusarium graminearum*. BMC Genomics. 2017;18:203. https://doi.org/10.1186/s12864-017-3524-x.
Lee T, Lee SH, Shin JY, Kim HK, Yun SH, Kim HY, et al. Comparison of trichothecene biosynthetic gene expression between *Fusarium graminearum* and *Fusarium asiaticum*. Plant Pathol J. 2014;30:33–42. https://doi.org/10.5423/PPJ.OA.11.2013.0107.
Leslie JF, Summerell BA. *Fusarium* laboratory workshops—A recent history. Mycotoxin Res. 2006;22(2):73–4. https://doi.org/10.1002/9780470278376.
Li H, Durbin R. Fast and accurate short read alignment with Burrows–wheeler transform. Bioinformatics. 2009;25:1754–60. https://doi.org/10.1093/bioinformatics/btp324.
Li H. Aligning sequence reads, clone sequences and assembly contigs with BWA-MEM. Cambridge MA: Genomics; 2013. p. 1–3. https://doi.org/10.48550/arXiv.1303.3997.
Liu JX, Cai YN, Jiang WY, Li YG, Zhang QF, Pan HY. Population structure and genetic diversity of fungi causing rice seedling blight in northeast China based on microsatellite markers. Plant Dis. 2020;104:868–74. https://doi.org/10.1094/PDIS-08-19-1620-RE.
Lodhi MA, Ye GN, Weeden NF, Reisch BI. A simple and efficient method for DNA extraction from grapevine cultivars and *Vitis* species. Plant Mol Biol Rep. 1994;12:6–13. https://doi.org/10.1007/BF02668658.
Ma ZH, Proffer TJ, Jacobs JL, Sundin GW. Overexpression of the 14α-demethylase target gene (*CYP51*) mediates fungicide resistance in *Blumerie llajaapii*. Appl Environ Microbiol. 2006;72:2581–5. https://doi.org/10.1128/AEM.72.4.2581-5.2006.
Marin S, Ramos AJ, Cano-Sancho G, Sanchis V. Mycotoxins: occurrence, toxicology, and exposure assessment. Food Chem Toxicol. 2013;60:218–37. https://doi.org/10.1016/j.fct.2013.07.047.
Mckenna A, Hanna M, Banks E, Sivachenko A, Cibulskis K, Kernytsky A, et al. The genome analysis toolkit: a MapReduce framework for analyzing next-generation DNA sequencing data. Genome Res. 2010;20:1297–303. https://doi.org/10.1101/gr.107524.110.
Oghenekaro AO, Oviedo-Ludena MA, Serajazari M, Wang X, Henriquez MA, Wenner NG, et al. Population genetic structure and chemotype diversity of *Fusarium graminearum* populations from wheat in Canada and North Eastern United States. Toxins. 2021;13:180. https://doi.org/10.3390/toxins13030180.
Parry DW, Jenkinson P, Mcleod L. Fusarium ear blight (scab) in small grain cereals-a review. Plant Pathol. 1995;44:207–38. https://doi.org/10.1111/j.1365-3059.1995.tb02773.x.
Pasquali M, Pallez-Barthel M, Beyer M. Searching molecular determinants of sensitivity differences towards four demethylase inhibitors in *Fusarium graminearum* field strains. Pestic Biochem Physiol. 2020;164:209–20. https://doi.org/10.1016/j.pestbp.2020.02.006.
Qian HW, Du J, Chi MY, Sun XM, Liang WX, Huang JG, et al. The *Y137H* mutation in the cytochrome P450 *FgCYP51B* protein confers reduced sensitivity to tebuconazole in *Fusarium graminearum*. Pest Manag Sci. 2018;74:1472–7. https://doi.org/10.1002/ps.4837.
Rausch T, Zichner T, Schlattl A, Stütz AM, Benes V, Korbel JO. DELLY: structural variant discovery by integrated paired-end and split-read analysis. Bioinformatics. 2012;28:i333–9. https://doi.org/10.1093/bioinformatics/bts378.
Salgado JD, Madden LV, Paul PA. Quantifying the effects of *Fusarium* head blight on grain yield and test weight in soft red winter wheat. Phytopathology. 2015;105:295–306. https://doi.org/10.1094/PHYTO-08-14-0215-R.
Spolti P, Del Ponte EM, Dong YH, Cummings JA, Bergstrom GC. Triazole sensitivity in a contemporary population of *Fusarium graminearum* from New York wheat and competitiveness of a tebuconazole-resistant isolate. Plant Dis. 2014;98:607–13. https://doi.org/10.1094/PDIS-10-13-1051-RE.
Talas F, McDonald BA. Genome-wide analysis of *Fusarium graminearum* field populations reveals hotspots of recombination. BMC Genomics. 2015;16:996. https://doi.org/10.1186/s12864-015-2166-0.
Talas F, McDonald BA. Significant variation in sensitivity to a DMI fungicide in field populations of *Fusarium graminearum*. Plant Pathol. 2015;64:664–70. https://doi.org/10.1111/ppa.12280.
Thapa A, Horgan KA, White B, Walls D. Deoxynivalenol and zearalenone-synergistic or antagonistic agri-food chain Co-contaminants? Toxins. 2021;13:561. https://doi.org/10.3390/toxins13080561.
Van der Lee T, Zhang H, Van Diepeningen A, Waalwijk C. Biogeography of *Fusarium graminearum* species complex and chemotypes: a review. Food Addit Contam a. 2015;32:453–60. https://doi.org/10.1080/19440049.2014.984244.
Vaughan M, Backhouse D, Del Ponte EM. Climate change impacts on the ecology of *Fusarium graminearum* species complex and susceptibility of wheat to *Fusarium* head blight: a review. World Mycotoxin J. 2016;9:685–700. https://doi.org/10.3920/WMJ2016.2053.
Walkowiak S, Rowland O, Rodrigue N, Subramaniam R. Whole genome sequencing and comparative genomics of closely related *Fusarium* Head Blight fungi: *Fusarium graminearum*, *F. meridionale* and *F. asiaticum*. BMC Genomics. 2016;17:1014. https://doi.org/10.1186/s12864-016-3371-1.
Wang W, Wang B, Sun X, Qi X, Zhao C, Chang X, et al. Symptoms and pathogens diversity of corn *Fusarium* sheath rot in Sichuan province. China Sci Rep. 2021;11:2835. https://doi.org/10.1039/C8EE02656D.
Wang Q, Song R, Fan SH, Coleman JJ, Xu XM, Hu XP. Diversity of *Fusarium* community assembly shapes mycotoxin accumulation of diseased wheat heads. Mol Ecol. 2022;32:2504–18. https://doi.org/10.1111/mec.16618.
Xu F, Liu W, Song YL, Zhou YL, Xu XM, Yang GQ, et al. The distribution of *Fusarium graminearum* and *Fusarium asiaticum* causing *Fusarium* head blight of wheat in relation to climate and cropping system. Plant Dis. 2021;105:2830–5. https://doi.org/10.1094/PDIS-01-21-0013-RE.
Yan SS, Song JM, Fan JS, Yan C, Dong SK, Ma CM, et al. Changes in soil organic carbon fractions and microbial community under rice straw return in Northeast China. Glob Ecol Conserv. 2020;22:2351–9894. https://doi.org/10.1016/j.gecco.2020.e00962.
Yang MX, Zhang H, Van der Lee TAJ, Waalwijk C, Van Diepeningen AD, Feng J, et al. Population genomic analysis reveals a highly conserved mitochondrial genome in *Fusarium asiaticum*. Front Microbiol. 2020;11:839. https://doi.org/10.3389/fmicb.2020.00839.
Yin Y, Liu X, Li B, Ma Z. Characterization of sterol demethylation inhibitor-resistant isolates of *Fusarium asiaticum* and *F. graminearum* collected from wheat in China. Phytopathology. 2009;99:487–97. https://doi.org/10.1094/PHYTO-99-5-0487.
Zeller KA, Bowden RL, Leslie JF. Population differentiation and recombination in wheat scab populations of *Gibberella zeae* from the United States. Mol Ecol. 2004;13:563–71. https://doi.org/10.1046/j.1365-294X.2004.02098.x.
Zhang H, Van der Lee TAJ, Waalwijk C, Chen WQ, Xu J, Xu JS, et al. Population analysis of the *Fusarium graminearum* species complex from wheat in China show a shift to more aggressive isolates. PLoS ONE. 2012;7:e31722. https://doi.org/10.1371/journal.pone.0031722.