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Identification of New Sources of Resistance to Crown Rot and Fusarium Head Blight in Wheat

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Abstract

Crown rot (CR) and Fusarium head blight (FHB) are two serious wheat diseases caused by *Fusarium* pathogens in China. To identify new resistant sources for CR and FHB, 205 Chinese wheat cultivars collected from Huang-Huai wheat-growing region in China were screened for resistance. Cunmai633, LS4607, Pubing01, and Hongyun2 showed seedling resistance to CR with disease index (DI) less than 0.25. Sixteen cultivars showed adult-plant resistance to CR with DI lower than 0.10. Twenty-six cultivars showed moderate resistance to CR at seedling stage with DI from 0.26 to 0.35, and 63 cultivars showed moderate adult-plant resistance with DI from 0.11 to 0.20. Among them, Cunmai633, LS4607, Pubing01, Xinong916, Zhengda161, Xumai14017, Zhengpinmai30, Bainong8822, Jimai216, Huacheng865, Fengyumai5, and Tianmin319 showed resistance or moderate resistance to CR at both seedling and adult plant stages, with Cunmai633 showing the best resistance. Most of the cultivars (>76%) were susceptible to FHB in both the 2017 and 2018 experiments with DI > 0.40. However, some cultivars demonstrated excellent FHB resistance. For example, Zhongyu1526, Tianminxiaoyan369, and Yangao168 were resistant (DI ≤ 0.25) in 2017 and moderately resistant (0.26 ≤ DI ≤ 0.40) in 2018; Zhongwo9 was moderately resistant in 2017 (DI = 0.38) and resistant in 2018 (DI = 0.25). Eight cultivars (Cunmai608, Zhengmai162, Minfeng266, Junda159, LS4607, Deyan1603, Pumai1165, and Fengmai12) showed moderate FHB resistance with DI lower than 0.40 in both experiments. LS4607 showed moderate resistance to both diseases. The resistant cultivars identified in this study can be used for mapping the resistance genes and improving resistance to CR and/or FHB.

Keywords: crown rot, Fusarium head blight, resistance, wheat, *Fusarium graminearum*, *Fusarium pseudograminearum*

Crown rot (CR) and Fusarium head blight (FHB), both caused by *Fusarium* spp., are two serious diseases in most cereal crop producing regions in the world (Backhouse et al. 2004; Goswami and Kistler 2004; Xu and Nicholson 2009). CR usually shows necrosis and browning on the leaf sheath or stem base and white heads, and it causes severe yield losses (Chakraborty et al. 2006; Matny 2015). FHB, with typical symptoms of brown, dark purple to black necrotic lesions on the exterior surface of the floret and glume, can cause severe yield losses, and the pathogen produces mycotoxins such as deoxynivalenol, nivalenol, zearalenone, and so on (Matny 2015; Xu and Nicholson 2009). The mycotoxins accumulated in cereal grains give a potential risk to human and animal health (Mudge et al. 2006; Obanor and Chakraborty 2014; Pestka 2010). CR was an epidemic first in Australia (Burgess et al. 1975) and has gained prominence gradually in Australia (Murray and Brennan 2009), Europe (Pettitt et al. 1996; Rossi et al. 1995), South America (Chakraborty et al. 2006), North America (Fernandez and Zentner 2005; Smiley et al. 2005, 2013), West Asia, North Africa (Mitter et al. 2006), South Africa (Lamprecht et al. 2006), and China (Zhang et al. 2015). FHB was first described in 1884 in England and has become a major threat to wheat production worldwide, especially in Canada, China, Europe, South America, and the United States (Bai and Shaner 1994; Goswami and Kistler 2004). Hot and dry weather during crop anthesis and maturation promotes CR infection in Australia, but this weather prevents FHB epidemics because FHB development requires humid and warm climatic conditions during anthesis (Obanor et al. 2013). Additionally, more severe and frequent endemics of the two diseases in recent years are likely due to conservation tillage involving crop stubble retention, reduced tillage, and single crop rotation, which lead to *Fusarium* pathogens surviving in stubble to increase the inoculum loads (Matny 2015).

Many *Fusarium* species (including *F. pseudograminearum*, *F. culmorum*, *F. graminearum*, *F. avenaceum*, *F. crookwellense*, and *F. poae*) can cause both diseases in wheat, and the symptoms caused by different species are similar. *F. graminearum* and *F. pseudograminearum* are considered to be the predominant pathogens causing FHB and CR, respectively (Akinsanmi et al. 2004; Backhouse et al. 2004; Bai and Shaner 1994; Burgess et al. 1975; Chakraborty et al. 2006; Lenc 2015; Obanor and Chakraborty 2014; Southwell et al. 2003; Xu and Nicholson 2009). Moreover, both *F. graminearum* and *F. pseudograminearum* can cause CR and FHB under artificial inoculation (Akinsanmi et al. 2004; Tunali et al. 2006), and many *Fusarium* species associated with FHB have also be isolated from CR wheat plants (Smiley and Patterson 1996; Williams et al. 2002). It was reported that the development and geographical distribution of *Fusarium* species mainly depend on their temperature requirements, genetic and environmental adaptations, and cropping systems (Parry et al. 1995; Paterson and Lima 2010; Poole et al. 2013; Talas et al. 2011). For example, the principal cause of FHB is *F. graminearum* in moist, warm regions of the United States, Canada, Australia, and central Europe, whereas *F. culmorum* and *F. avenaceum* predominate in maritime and cooler European countries (Akinsanmi et al. 2004; Parry et al. 1995). In Australia, *F. pseudograminearum*, *F. graminearum*, *F. cerealis*, *F. culmorum*, and *F.*

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avenaceum are the causal species responsible for FHB outbreaks (Obanor et al. 2013), and *F. pseudograminearum*, *F. culmorum*, and *F. acuminatum* are pathogens for CR in the Victorian high-rainfall region and the south-east region of South Australia (Backhouse et al. 2004; Williams et al. 2002). In the United States, *F. pseudograminearum* and *F. culmorum* are associated with CR throughout the rain-fed dryland wheat production region of the Pacific Northwest; however, *F. culmorum* is more frequently isolated from cooler, higher elevations, whereas *F. pseudograminearum* occurs with significantly greater frequency in warmer, lower elevations (Poole et al. 2013). In China, *F. asiaticum* is the predominant species causing FHB in the warmer regions of southern China, whereas *F. graminearum* is mainly distributed in the cooler regions in northern China (Qu et al. 2008; Shen et al. 2012). Zhang et al. (2015) investigated the phylogenetic structure of *Fusarium* species that cause CR in major winter wheat-growing regions including Jiangsu, Anhui, Henan, Hebei, and Shandong provinces in China from 2009 to 2013 and revealed that the most predominant species was *F. asiaticum*, followed by *F. graminearum*, and smaller numbers of isolates consisted of *F. acuminatum*, *F. pseudograminearum*, and *F. avenaceum*. These results indicate that different *Fusarium* species causing CR and FHB may be prevalent in different geographic areas.

Shaanxi province, situated in a semihumid to semiarid transitional area, is one of the most important winter wheat-producing regions in northwestern China. In recent years, epidemics of both FHB and CR have become more severe and frequent, threatening wheat production in Shaanxi province. Based on the investigation of both diseases in different wheat-growing areas in Shaanxi province, we found that the spread of these diseases was likely due to wide adoption of some tillage practices, such as reduced tillage, single crop rotation between corn and wheat, and stubble return, leading to *Fusarium* pathogens surviving in stubble to increase the inoculum loads. It is well known that growing resistant cultivars would be the most effective and environmentally friendly way to manage the diseases. To date, very limited FHB and CR resistant sources have been used in wheat breeding. Although a considerable effort has been made to search for FHB resistance, only a few resistant accessions have been identified to be FHB resistant, such as Arina, Fundulea 201R, and Renan from Europe; Wangshuibai and Sumai 3 from China; Frontana and Encruzilhada from Brazil; Shinchunaga and Nobeokabouzu from Japan; and Ernie and Freedom from the United States (Badea et al. 2008; Bai and Shaner 2004; Buerstmayr et al. 2012; Rudd et al. 2001; Xue et al. 2010, 2011). Also, more than 250 FHB resistance quantitative trait loci (QTLs), including more than 50 unique QTLs, were found on all wheat chromosomes (Buerstmayr et al. 2009; Liu et al. 2009; Ren et al. 2019; Su et al. 2019), but *Fhb1* (originally identified in Sumai 3) is the most consistent QTL for FHB resistance and is widely used in breeding programs (Cuthbert et al. 2006; Rawat et al. 2016; Su et al. 2019). Although some resistant accessions have been successfully used to improve FHB resistance in wheat-breeding programs worldwide, most of them were unsuccessful because of their undesirable agronomic traits or because the resistance was difficult incorporate into elite lines (Bai and Shaner 2004). For CR, only limited efforts have been made in searching for novel sources of resistance, and few resistant cultivars are available (Liu and Ogbonnaya 2015; Liu et al. 2012). In the present study, 205 Chinese cultivars collected from the Huang-Huai wheat-growing region were evaluated for their reactions to CR and FHB in both greenhouse and field experiments to identify new sources of resistance to both diseases.

Materials and Methods

Pathogen strains and wheat cultivars. *F. graminearum* strain PH-1 and *F. pseudograminearum* strain SX4-6 were used to screen the 205 Chinese wheat cultivars from the Huang-Huai wheat-growing region for resistance to FHB and CR, respectively (Supplementary Tables S1 and S2). Due to seed limitation and/or emergence problems of a few cultivars, not all of the 205 cultivars were evaluated in every test. A total of 203 and 196 cultivars were evaluated for FHB resistance in the field in 2017 and 2018, respectively. Wheat cultivars Sumai 3 and Chinese Spring were used as the resistant and susceptible controls, respectively. A total of 194 and 205 cultivars including Sumai 3 and Chinese Spring were evaluated for resistance to CR in seedling and adult-plant tests, respectively.

Fungal isolation. Wheat samples with typical symptoms of CR were collected from five fields in Yangling, Sanyuan, Pucheng, Fuping, and Huayin, which are located in the Guanzhong wheat-growing region in Shaanxi province. The ~2-mm sections from the margin between healthy and diseased tissue of symptomatic samples were surface sterilized with 75% ethyl alcohol for 10 s, 1% chlorine solution for 3 min, rinsed with sterile distilled water three times, plated on potato dextrose agar (PDA) modified by adding 10 μg/ml of tetracycline hydrochloride and 100 μg/ml of streptomycin sulfate, and incubated at 25°C for about 3 days. Colonies were subcultured on PDA and incubated at 25°C for 5 to 7 days. A single-conidium isolate from each sample was obtained by spreading a spore suspension onto a water agar plate, cultivating it overnight at 25°C, and then transferring a single colony with a sterile needle onto a new PDA plate. Each isolate was kept in a PDA slant at 4°C until use. *F. graminearum* strain PH-1 was provided by the Wheat Head Blight Research Group of Northwest A&F University.

DNA isolation and polymerase chain reaction (PCR) sequencing of *Fusarium* isolates. Genomic DNA was extracted from freeze-dried mycelia using a cetyltrimethylammonium bromide protocol as described in Kim et al. (1992) and purified using a Wizard Genomic DNA Purification Kit (Promega, Madison, WI). The internal transcribed spacer (ITS) regions of the nuclear ribosomal DNA and translation elongation factor 1α (EF-1α) gene were amplified using PCR primers ITS1/ITS4 (White et al. 1990) and EF1-728F/EF1-986R (Carbone and Kohn 1999), respectively. The nuclear ribosomal DNA intergenic spacer region (IGS) was amplified using PCR primers NL11/CNS1 (O'Donnell et al. 2009). The RNA polymerase II gene (RPB2) was amplified using PCR primers RPB2-5F/RPB2-7cR and RPB2-7cF/RPB2-11aR (Liu et al. 1999) (Table 1). PCR amplifications were done with Platinum *Taq* DNA polymerase (Invitrogen Life Technologies, Carlsbad, CA) in an Applied Biosystems 9700 thermocycler (Applied Biosystems, Foster City, CA) using the following program: 1 cycle of 2 min at 94°C; 35 cycles of 30 s at 94°C, 30 s at 55°C, and 2 min at 72°C; followed by 1 cycle of 10 min at 72°C and a 4°C hold. All PCR products were separated in a 1.5% agarose gel, stained with ethidium bromide, and photographed over a UV transilluminator. PCR products were purified using the Agarose Gel Extraction Kit (Roche, Mannheim, Germany), cloned into pDM18-T vector (TaKaRa, Tokyo, Japan), and transformed into *Escherichia coli* JM109 using an electroporation method (Dower et al. 1988). After cloning, 10 positive colonies from each transformation were randomly selected, and each colony was recovered in a LB liquid medium supplemented with 100 μg/ml of ampicillin following overnight incubation at 37°C. The recombinant plasmid was extracted using a Plasmid Miniprep Kit (Biomiga, San Diego, CA). The confirmation of positive colonies was confirmed through restriction digestion with *Kpn*I and *Hind*III. The plasmid was sequenced by Shanghai Biotech Engineering Company in China. Sequence analysis was performed to identify the type of pathogen isolate.

Disease assessment. Seedling reactions of the wheat cultivars to CR were evaluated in the greenhouse of Northwest A&F University as follows: seed surface was disinfested with 75% ethyl alcohol for 30 s, rinsed with sterile distilled water two times, and then pregerminated on moist sterile blotting paper in plastic Petri dishes for 3 to 4 days at 25°C. Eight germinated seeds per cultivar were planted into each well in 32-well plastic trays containing moist, aerated, and sterile potting mix. Plants were grown in a greenhouse under a day/night photoperiod of 14/10 h, temperatures of 25/15°C, and a relative humidity of 60/80% (±5%). Five healthy seedlings at the two-leaf stage in each well were inoculated with the pathogen. The inocula were prepared by transferring a 1-cm^2 disk from a 7-day-old PDA plate of aggressive *F. pseudograminearum* isolate SX4-6 into a 500-ml flask containing wheat grains that was boiled for 30 min and autoclaved at a temperature of 121°C and 103 kPa pressure for 20 min,

mixed thoroughly, and incubated for 14 to 21 days at 22 ± 1°C by shaking it daily to provide uniform colonization of grains. The colonized grains were used for inoculation as described by Erginbas-Orakci et al. (2016). Briefly, approximately five to seven colonized grains were placed around the base of the seedling stem above the soil surface, covered with sterile soil mix, and watered immediately to stimulate germination of the conidia on the grains. Control treatments were inoculated with sterilized grains without conidia. Each treatment had three replications. All the plants were grown in the same environment and watered as needed to prevent water stress. About 35 days after inoculation, plants were rated for CR severity using a 0 to 6 scale (Fig. 1A), where 0 = no disease, 1 = partially necrotic lesions on the first leaf sheath, 2 = completely necrotic lesions on the first leaf sheath and the below subcrown internode, 3 = partially necrotic lesions on the second leaf sheath, 4 = completely necrotic lesions on the second leaf sheath and the below subcrown internode, 5 = partially necrotic lesions on the third leaf sheath, and 6 = completely necrotic lesions on the third leaf and developed on all subcrown internodes.

Adult-plant reactions of the wheat cultivars to CR and their FHB reactions were evaluated in a field experiment using a complete randomized block design with two replicates at the Experimental Station of Northwest A&F University, Yangling, Shaanxi, China. Thirty seeds per cultivar were planted in a 1.2-m row spaced 25 cm apart between rows. When the plants reached the early jointing stage, a small piece of sterilized absorbent cotton was wrapped around the base of the main stem of each plant just 1.0 cm above the soil surface and fixed by a plastic sleeve. Each plant was inoculated by adding 0.5 ml of conidial suspension of the isolate SX4-6 at the concentration of 1×10^6 conidia/ml into the cotton. Inoculated plants were incubated under high relative humidity condition in a wet black plastic bag for 48 h. At the dough stage, plants were assessed for CR severity using a 0 to 4 scale (Fig. 1B), where 0 = no disease, 1 = browning or necrotic lesions on the first stem node, 2 = complete necrotic lesions of the first stem node, browning or necrotic lesions on the second stem node, 3 = complete necrotic lesions of the second stem node, browning or necrotic lesions extending to the third stem node, and 4 = necrotic lesions on the forth stem node, formation of white heads or failure of heading.

Table 1. Primers used in this study

Primer name	Primer sequence 5′-3′	Loci amplified	Annealing temperature (°C)	Primer sequence origin
ITS1	TCCGTAGGTGAACCTGCGG	ITS	55	White et al. (1990)
ITS4	TCCTCCGCTTATTGATATGC			
EF1-728F	CATCGAGAAGTTCGAGAAGG	*EF-1α*	55	Carbone and Kohn (1999)
EF1-968R	TACTTGAAGGAACCCTTACC			
NL11	CTGAACGCCTCTAAGTCAG	IGS	**55**	O'Donnell et al. (2009)
CNS1	GAGACAAGCATATGACTACTG			
RPB2-5F	GA(T/C)GA(T/C)(A/C)G(T/A)GATCA(T/C)TT(T/C)GG	*RPB2*	55	Liu et al. (1999)
RPB2-7cR	CCCAT(A/G)GCTTG(T/C)TT(A/G)CCCAT			
RPB2-7cF	ATGGG(T/C)AA(A/G)CAAGC(T/C)ATGGG	*RPB2*	55	Liu et al. (1999)
RPB2-11aR	GC(A/G)TGGATCTTRTC(A/G)TC(C/G)ACC			
M13F(-47)	CGCCAGGGTTTTCCCAGTCACGAC	…	55	pMD18-T, TaKaRa
M13R(-48)	AGCGGATAACAATTTCACACAGGA			
FLR.W1F	TGGCGGATCTGACACTGTCG	…	51	Primer walking sequence
FLR.W1R	CACGCCAGAACTGCTTCGTG			
G013.W1F	CCACCAATGCCGCCATTCTT	…	49	Primer walking sequence
G013.W1R	GCATCCTCAAGGCACCAACA			

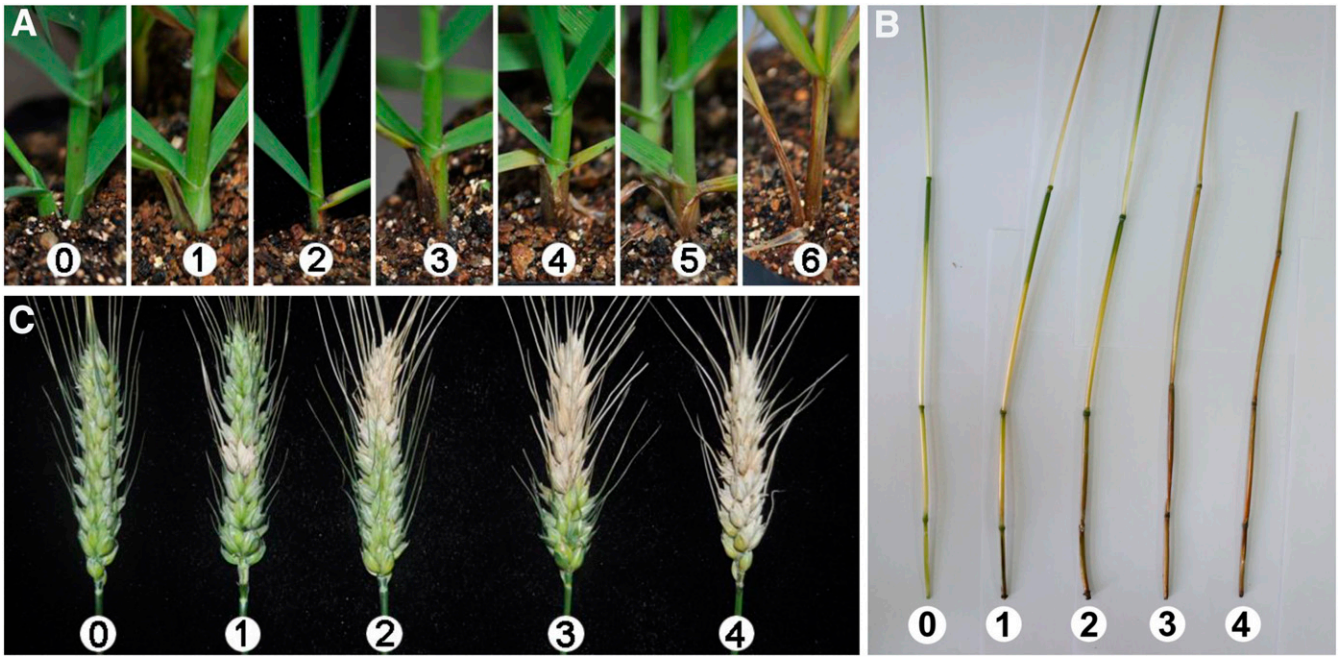

Fig. 1. The rating system for crown rot (CR) and *Fusarium* head blight (FHB) severity. **A,** The 0 to 6 rating system for CR seedling severity. **B,** The 0 to 4 rating system for CR adult-plant severity. **C,** The 0 to 4 rating system for FHB severity.

The single-floret inoculation method was used to evaluate the FHB reactions of the wheat cultivars. At anthesis, 10 plants per cultivar in each replicate were marked and inoculated by injecting 20 μl of PH-1 spore suspension at 10^6 macroconidia/ml into one floret of a central spikelet per spike. High relative humidity was maintained by covering the inoculated spikes with a premisted wet plastic bag for 48 h. Disease was assessed by visually counting the number of diseased spikelets per spike at 21 days postinoculation. Disease rating was recorded according to the 0 to 4 scale (Fig. 1C), where 0 = no disease, 1 = less than 1/4 of the spike infected, 2 = 1/4 to 1/2 of the spike infected, 3 = 1/2 to 3/4 of the spike infected, and 4 = more than 3/4 of the spike infected.

Statistical analysis of virulence data. Average disease rating (ADR) and median disease rating (MDR) were calculated using the disease severity data. Disease index (DI) was calculated using the following formula: $DI = \sum(c \times f)/(n \times N)$, where c is the disease rating value, f is the frequency of the disease rating, n is the total number of tested plants, and N is the greatest value of disease rating adopted in the experiment. Analysis of variance (ANOVA) was performed separately for CR severity in seedlings and adult plants and for FHB severity in 2017 and 2018 using ANOVA PROC of Statistical Analysis System (SAS) version 8.01 (SAS Institute, Cary, NC).

Table 2. *Fusarium* species isolated from crown rot samples in Guanzhong wheat-growing region in Shaanxi province

Location	Year	Number of samples	*Fusarium pseudograminearum*	*Fusarium graminearum*
Yangling	2016	16	16	0
	2017	8	8	0
Sanyuan	2016	12	12	0
	2017	21	21	0
Fuping	2016	14	14	0
	2017	12	12	0
Pucheng	2016	20	19	1
	2017	16	16	0
Huayin	2016	17	17	0
	2017	6	6	0
Total	2016	79	78	1
	2017	63	63	0

Table 3. Analysis of variance for wheat seedling reactions (SR) and adult-plant reactions (APR) to crown rot (CR) and field reactions to Fusarium head blight (FHB) in 2017 and 2018

Trait	Source	DF[a]	MS[b]	*F* value	*P* (> *F*)
CR-SR	Cultivar	195	0.0222	1.9556	<0.0001
	Replication	2	0.0324	2.8444	0.05938
	Error	390	0.0114		
CR-APR	Cultivar	206	0.0311	2.4045	<0.0001
	Replication	1	0.0000	0.0029	0.9569
	Error	206	0.0129		
FHB-2017	Cultivar	204	0.0776	5.5965	<0.0001
	Replication	1	0.0017	0.1230	0.7262
	Error	204	0.0139		
FHB-2018	Cultivar	197	0.0871	21.8582	<0.0001
	Replication	1	0.0267	6.6975	0.01038
	Error	197	0.0040		

[a] Degrees of freedom.
[b] Mean square.

Table 4. Phenotypic correlation values among wheat seedling reactions (SR) and adult-plant reactions (APR) to crown rot (CR) and field reactions to *Fusarium* head blight (FHB) in 2017 and 2018[a]

Traits	CR-SR	CR-APR	FHB-2017	FHB-2018
CR-SR	1			
CR-APR	–0.0882	1		
FHB-2017	0.0481	–0.0180	1	
FHB-2018	0.0689	0.0941	0.3743*	1

[a] Asterisk (*) indicates significant at $P = 0.05$.

Results

CR pathogen isolation and identification. From 36 CR-infected samples collected from Shaanxi province in 2016 and 2017, 142 *Fusarium* isolates were identified. All the CR isolates were *F. pseudograminearum* based on morphology and ITS, EF-1α, IGS, and RPB2 gene sequences except one isolate from Pucheng in 2016 that was identified as *F. graminearum* (Table 2). Results indicated that *F. pseudograminearum* was the predominant species causing CR in the Guanzhong wheat-growing region in Shaanxi province. The strain SX4-6 had the strongest pathogenicity to cultivar Xiaoyan22, one of the major wheat cultivars grown in the region, among nine *F. pseudograminearum* isolates randomly selected from 141 isolates and tested (data not shown).

Phenotypic analyses. ANOVA revealed significant differences ($P < 0.0001$) in CR reaction and also in FHB reaction among the tested cultivars (Table 3). Phenotypic correlations were conducted among all tests, indicating that there were no correlations between seedling and adult-plant reactions to CR, and between CR reaction and FHB reaction, but a significant positive correlation was found between FHB reaction between the 2017 and 2018 experiments (Table 4).

Evaluation of wheat cultivars for reactions to CR. The results of disease assessment (Supplementary Table S1) showed that ADR ranged from 1.25 to 4.07, with MDR from 1.00 to 4.00 and DI from 0.21 to 0.68. In the adult-plant test, ADR, MDR, and DI ranged from 0.20 to 2.90, from 0.00 to 4.00, and from 0.05 to 0.73, respectively. The tested cultivars can be assigned into four categories based on DI (Fig. 2): resistant (R), moderately resistant (MR), susceptible (S), and highly susceptible (HS). In the seedling test, four cultivars (Cunmai633, LS4607, Pubing01, and Hongyun2) with DI lower than 0.25 were classified as R; 26 cultivars with DI from 0.26 to 0.35 were classified as MR, accounting for 13.3% of the tested cultivars. Most of the tested cultivars (84.7%) with DI from 0.36 to 0.45 and ≥0.46 were grouped into S (38.3%) and HS (46.4%), respectively. In the adult-plant test, 16 cultivars (7.7%), including Cunmai633, Yanfeng168, Lemai185, Jimai216, Xinmai38, Xinong733, Ximai45, Luofeng2419, Fengyunmai5, Huaihe15076, Zhengmai22, Zhoumai38, Zhoumai37, Xinong625, Guohemai12, and Zhengmai162, had high

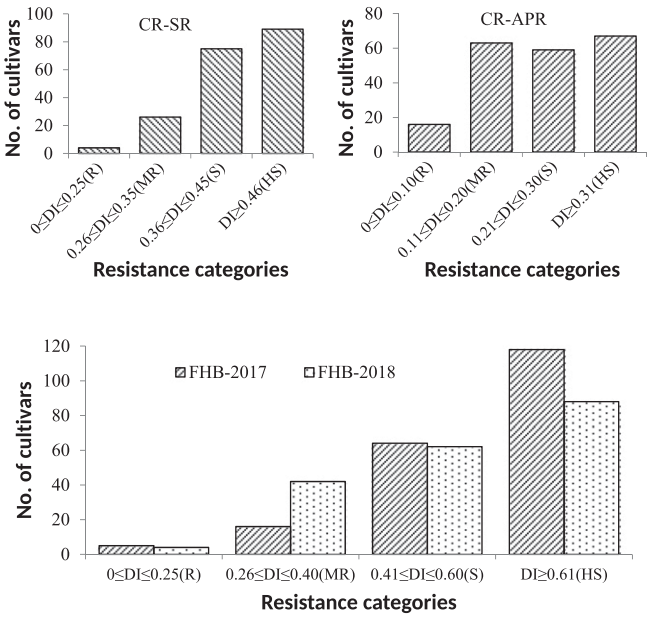

Fig. 2. The number of cultivars assigned to four categories based on disease index (DI). CR = crown rot; FHB = Fusarium head blight; SR = seedling reactions; APR = adult-plant reactions; R = resistant; MR = moderately resistant; S = susceptible; and HS = highly susceptible.

resistance to CR with DI lower than 0.10. Sixty-three cultivars (30.4%) showed MR with DI of 0.11 to 0.20, suggesting that these cultivars have adult-plant resistance to CR. Sixty cultivars (29.0%) with DI of 0.21 to 0.30 and 68 cultivars (32.9%) with DI ≥ 0.31 were classified as S and HS, respectively. On the basis of these data, Cunmai633 has the best resistance to CR at both seedling and adult-plant stages. LS4607 and Pubing01 were classified as R in seedlings and MR in adult plants, and Jimai216 and Fengyunmai5 were MR in seedlings and R in adult plants. Xinong916, Zhengda161, Xumai14017, Zhengpinmai30, Bainong8822, Jimai216, Huacheng865, Fengyumai5, and Tianmin319 showed MR to CR at both seedling and adult-plant stages. These cultivars are useful sources for improvement of resistance to CR. However, most of cultivars (61.9%) tested in this study were susceptible to CR, suggesting that it is an urgent task to improve wheat cultivar resistance to CR in the Huang-Huai wheat-growing region.

Evaluation of wheat cultivars for resistance to FHB. The ADR, MDR, and DI values ranged from 0.83 to 4.00, from 1.00 to 4.00, and from 0.21 to 1.00 in 2017 and from 0.60 to 4.00, from 1.00 to 4.00, and from 0.15 to 1.00 in 2018, respectively. The resistant control Sumai 3 had the lowest DI in both 2017 (DI = 0.21) and 2018 (DI = 0.15). The susceptible control Chinese Spring had high DI of 0.50 in 2017 and 0.43 in 2018 (Supplementary Table S2). The tested cultivars can be assigned into four categories based on DI (Fig. 2): R, MR, S, and HS. Five cultivars (Zhongyu1526, Tianminxiaoyan369, Luomai906, Yangao168, and Taihemai6) in 2017 and four cultivars (Zhongwo9, Huaimai608, Luomai36, and Yunong188) in 2018 were classified as R to FHB with DI lower than 0.25. Sixteen cultivars (7.8%) in 2017 and 42 cultivars (21.2%) in 2018 were classified as MR to FHB with DI from 0.26 to 0.40. Most of the cultivars (over 76%) were classified as S and HS to FHB in both years with DI from 0.41 to 0.60 and higher than 0.60, respectively. In general, Zhongyu1526, Tianminxiaoyan369, and Yangao168 were classified as R in 2017 and MR in 2018; Zhongwo9 was classified as MR in 2017 and R in 2018. Another eight cultivars (Cunmai608, Zhengmai162, Minfeng266, Junda159, LS4607, Deyan1603, Pumai1165, and Fengmai12) were classified as MR to FHB with DI lower than 0.40 in both years. These cultivars could be used as potential sources for further confirmation of the FHB resistance and used in wheat-breeding programs.

Discussion

Besides wheat, other cereal crops such as rice, corn, barley, oat, rye, and triticale also suffer diseases caused by *Fusarium* spp. (Backhouse and Burgess 2002; Backhouse et al. 2004; Parry et al. 1995). Both FHB and CR have become more prevalent due in large part to conservation farming practices involving cereal crop rotation and stubble retention, because *Fusarium* pathogens survive in crop residues (Chakraborty et al. 2006). In China, these two diseases have occurred seriously in the Huang-Huai wheat-growing region in recent years (Qu et al. 2008; Yang et al. 2019). The Guanzhong wheat-growing region of Shaanxi province is located in this region, where corn and wheat are major crops, cultivated in different seasons every year. This single crop rotation and stubble retention practice allow more *Fusarium* pathogens to survive on host residual stubbles. FHB becomes epidemic when abundant rainfall occurs during wheat anthesis. However, if dry weather occurs in the spring at the wheat seedling stage, a serious CR outbreak may occur. In the present study, we surveyed the pathogens and the incidence of CR at five locations in the Guanzhong wheat-growing region in Shaanxi, China. The results showed that *F. pseudograminearum* is the predominant pathogen causing CR. Burgess et al. (1987) reported that the CR pathogen *F. pseudograminearum* has caused severe FHB epidemics in Australia. Both FHB and CR are found in the same fields in South Africa (Marasas et al. 1988). Zhang et al. (2015) found that all CR isolates were able to cause FHB. This evidence suggested that CR and FHB are linked by etiology, pathogen biology, and epidemiology (Chakraborty et al. 2006). Therefore, there may be a genetic relationship between FHB resistance and CR resistance. However, Li et al. (2010) were not able to colocate QTLs for FHB and CR, suggesting different genes involved in resistance to the two diseases. In the present study, the FHB-resistant control Sumai 3 was susceptible to CR in the adult plant stage with a DI of 0.25 and highly susceptible in the seedling stage with a DI of 0.64. Cunmai633 was resistant to CR with a DI of 0.21 in the seedling stage and a DI of 0.05 in the adult plant stage but was susceptible to FHB with a DI of 0.63 in 2017 and a DI of 0.43 in 2018. The fact that resistance categories to the two diseases were inconsistent in most cultivars in the present study agrees with the findings of Li et al. (2010) that loci conferring resistance to these two diseases are different. R genes to either disease may exist in some germplasm but differently expressed under different conditions, including plant growth stages. Hence, screening wheat germplasm for reactions to these diseases separately seems to be essential for identifying sources of CR and FHB resistance.

It was found that *F. pseudograminearum* caused seedling death before or after seedling emergence and extensive browning of subcrown internodes and leaf sheaths shortly after infection for the entire plant development stage; thus, different inoculation methods can be used at different developmental stages (Kazan and Gardiner 2018). Although a relatively high correlation was detected between seedling and adult-plant CR resistance in wheat (Wildermuth and McNamara1994), different genes for CR resistance can be detected at different developmental stages of wheat. For example, Martin et al. (2015) identified four genotypes with partial resistance to CR and detected several QTLs for CR resistance, among which some were only effective in seedlings and others in adult plants. In our study, different inoculation methods were used to evaluate seedling and adult-plant reactions to CR. The results showed that CR resistance was not identical between seedlings and adult plants; for example, Hongyun2 was CR resistant in the seedling stage but was susceptible in adult plants, whereas Huaihe15076, Luofeng2419, Zhengmai22, Lemai185, Zhoumai37, Xinmai38, Xinong733, Xinong625, Xinmai45, Guohemai12, and Zhengmai163 were classified as susceptible or highly susceptible as seedlings but were resistant as adult plants. Also, in this study, no significant phenotypic correlation of CR resistance was observed between seedling and adult-plant tests. These pieces of evidence suggested that seedling and adult-plant resistance to CR may be conditioned by different genes or that R genes for seedling and adult-plant resistance are differently expressed at different stages.

It has been proven that growing resistant and tolerant cultivars is an effective measure in managing the diseases. New and diverse sources of resistance genes may facilitate the achievement of high levels of potentially durable resistance in wheat cultivars (Yu et al. 2008). However, because resistance to FHB or CR in wheat is a complex and quantitative trait, there are only a few sources with stable resistance to the two diseases. Although considerable efforts regarding screening of genes or germplasm resistance to FHB have been conducted, reliable FHB resistance is still lacking in the current wheat cultivars (Buerstmayr et al. 2012; Yu et al. 2008). Bai and Shaner (2004) found that only a few wheat lines, such as Sumai 3 and its derivatives, have been extensively used as major FHB-resistance sources in breeding programs worldwide. Another widely used resistance source is the spring wheat cultivar Frontana from Brazil (Badea et al. 2008). Compared with FHB, only limited efforts have been made in germplasm screening to identify sources of resistance to CR due to the lack of reliable and high-throughput assay methods for screening a large number of genotypes (Liu and Ogbonnaya 2015). The genetics of CR resistance have been reported in only three partially resistant genotypes in wheat, and the results suggest that CR resistance is quantitative in nature (Bovill et al. 2006; Collard et al. 2005; Wallwork et al. 2004). Fully CR-resistant or immune cultivars have not been detected in common wheat. Only partial CR resistance was found in commercial cultivars, wild relatives, and landraces, and various QTLs conferring partial CR resistance have been identified in Australian and U.S. wheat cultivars (Kazan and Gardiner 2018; Liu and Ogbonnaya 2015; Ma et al. 2010; Martin et al. 2015; Smiley and Yan 2009). To date, QTLs related to CR resistance were identified on 14 of the 21 wheat chromosomes (Liu and Ogbonnaya 2015; Yang et al. 2019). Yang et al. (2019) recently showed that a major QTL on 6A of Chinese materials was significant for CR resistance.

A cultivar with resistance to both CR and FHB is the most important to reduce losses of both diseases. In the present study, cultivar LS4607 has excellent resistant to both diseases, and Zhongyu1526 has resistance to FHB and moderate resistance to CR at the seedling stage. Thus, these two cultivars are valuable sources for breeding for CR and FHB resistance. In the study, only type II resistance, which is resistance to spread of the fungus to an adjacent spikelet, was used to assess FHB resistance. Bai and Shaner (1996) showed that the ratings for highly resistant and susceptible cultivars using type II resistance in the greenhouse were consistent, and resistance reactions can be clearly distinguished even in a single test. However, the ratings for moderately resistant and moderately susceptible cultivars were variable among trials and isolates in the present study, so different tests using methods to measure different types of resistance may be needed for a more reliable assessment (Badea et al. 2008). Therefore, although cultivars LS4607 and Zhongyu1526 would be excellent sources for CR and FHB resistance, efficient and successful use of these sources requires further confirmation in the practices of breeding and can be accelerated by molecular mapping the resistance QTLs and developing markers that can be used in marker-assisted selection.

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