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Disease Resistance and Genes in 146 Wheat Cultivars (Lines) from the Huang-Huai-Hai Region of China

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Abstract: Wheat stripe rust, powdery mildew, and Fusarium head blight (FHB) are the three most important diseases in wheat worldwide. Growing resistant cultivars is the most economic and effective method to control these diseases. To assess the disease resistance of commercial wheat cultivars and regional trial wheat lines in the Huang-Huai-Hai region of China, 146 wheat entries were inoculated with the Chinese prevalent *Puccinia striiformis* f. sp. *tritici* (*Pst*) races CYR32, CYR33, CYR34, and *Blumeria graminis* f. sp. *tritici* (*Bgt*) isolate E09 under controlled greenhouse conditions, respectively; these entries were also tested with the mixed *Pst* races, *Bgt* and FHB isolates at adult-plant stage in the field, respectively. The results showed that 108 (73.97%), 83 (56.85%), 99 (67.81%), and 22 (15.07%) entries were resistant to CYR32, CYR33, CYR34, and E09 at the seedling stage, respectively; 102 (69.86%), 24 (16.44%), and 2 (1.37%) entries were resistant to stripe rust, powdery mildew, and Fusarium head blight at the adult-plant stage, respectively. Additionally, the possible resistance gene(s) in these entries were postulated by the closely linked markers of stripe rust resistance genes *Yr5*, *Yr9*, *Yr10*, *Yr15*, *Yr17*, *Yr18*, *Yr26*, powdery mildew resistance gene *Pm21*, and Fusarium head blight resistance gene *Fhb1*. Combined with disease resistance and molecular markers tests, 62, nine, and three wheat entries were postulated to carry the *Yr9*, *Yr17*, *Yr26* gene, respectively, and no entries contained *Yr5*, *Yr10*, *Yr15*, *Yr18*, *Pm21*, and *Fhb1* gene. This study laid a theoretical foundation for rational utilization of these entries and gene in wheat breeding programs and disease control.

Keywords: wheat stripe rust; powdery mildew; fusarium head blight; resistance identification; resistance gene; molecular detection

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1. Introduction

Wheat (*Triticum aestivum* L.) is one of the most important food crops in the world. China's wheat production occupies a large proportion of the world, and its total output is third only to rice and corn (available online: http://www.fao.org/faostat/zh/#data/QC (accessed on 24 April 2021)). However, wheat yield is constantly challenged by diseases such as stripe rust, powdery mildew and Fusarium head blight (FHB).

Wheat stripe rust, also known as yellow rust, caused by *Puccinia striiformis* f. sp. *tritici* (*Pst*), is a major threat to most wheat growing areas in the world, especially in China. Severe national-wide stripe rust epidemics in 1950, 1964, 1990, 2002, and 2017 caused production losses of 6.0, 3.2, 1.8, 1.3, and 1.5 million metric tons, respectively [1–3]. In addition, the pandemic of wheat stripe rust in 2020 was the highest in the past decade, which has once again sounded an alarm for us [4]. Powdery mildew, caused by the pathogen *Blumeria graminis* (DC.) Speer f. sp. *tritici* emend. É. J. Marchal (*Bgt*), can cause 30–40% yield losses in epidemic years [5,6]. In recent years, the area affected by powdery mildew in China is about 6–8 million hectares each year [7]. Fusarium head blight, also

known as wheat scab, mainly caused by *Fusarium graminearum* Schwabe, occurs directly on wheat spikes and cause both grain yield losses and food toxins, which is imposing health threats to humans and livestock. Chemical controls have been proven to be an effective way for these three wheat diseases, but usually with additional cost and detrimental impact on the environment. In contrast, host resistance has been proposed as an effective and environmentally friendly measure to control the diseases. Therefore, it is necessary to explore wheat germplasm resources to identify genes resistant to the three important wheat diseases [8].

Up to now, 83 *Yr* genes have been officially named [9]. Most of these genes are race-specific and confer resistance at all stages. However, due to the rapid changes in the virulence of pathogen populations, the resistance provided by these genes can be easily triumphed over by the new virulence *Pst* races. For example, *Yr9* and *Yr26* have been widely used in wheat breeding programs in China since the early 1970s and in recent years [10,11], respectively. However, the resistance of *Yr9* and *Yr26* have been overcome by the *Pst* race CYR29 in 1990 and V26 in 2009, respectively [12,13]. Except for *Yr9* and *Yr26*, *Yr17*, which was also widely used in wheat breeding both in China and worldwide [14], has become ineffective in China. So far, only a few *Yr* genes such as *Yr5* and *Yr15* are effectively resistant to all known *Pst* races in China and around the world [15]. Additionally, there are a few *Yr* genes that confer non-race-specific resistance, acting at the adult plant stage such as *Yr18*, which is a multi-pathogen resistance gene and confers part field resistance against stripe rust, leaf rust, stem rust, and powdery mildew have been used in breeding programs for a century and so far, no pathogen adaptability has been found [16].

Similar to *Yr* genes, although more than 100 *Pm* genes/alleles have been reported, few can provide resistance to the predominate *Bgt* isolates [17]. Generally speaking, *Pm* genes derived from wild relatives of wheat are often resistant to most *Bgt* isolates. For example, *Pm21*, derived from *Haynaldia villosa* (2n = 2x = 14, VV) [18], confers high resistance to *Bgt* throughout all growth stages. The commercial varieties harboring *Pm21* have been widely used in wheat production with more than four million hectares in China [19]. In addition, although more than 50 quantitative trait loci (QTLs) for FHB resistance have been documented [20], only *Fhb1* has a consistently significant effect on resistance to a broad spectrum of *Fusarium* specie [21]. *Fhb1*, derived from Chinese wheat cultivar Sumai3, is the most important quantitative trait locus (QTL) and has been reported to provide a high level of resistance against FHB. *Fhb1* and those carrying *Fhb1* have been considered the ideal sources of resistance all over the world for half a century [22].

The Huang-Huai-Hai wheat region is the most important wheat production base in China. Identifying new resistance sources and understanding the distribution of wheat stripe rust, powdery mildew, and FHB resistance genes in wheat entries in this region may provide valuable resistance germplasms and molecular basis for predictive resistance breeding programs. A total of 146 wheat entries collected from this region were inoculated with *Pst* or *Bgt* races (isolate), both in the seedling and adult-plant stage, and the Fusarium head blight resistance were tested in the field. Additionally, the stripe rust resistance genes *Yr5*, *Yr9*, *Yr10*, *Yr15*, *Yr17*, *Yr18*, *Yr26*, powdery mildew resistance gene *Pm21* and Fusarium head blight resistance gene *Fhb1* were selected to test these wheat entries.

2. Materials and Methods

2.1. Plant Materials

A total of 146 wheat entries (Table S1) including 47 commercial wheat varieties, which were the most cultivated, and 98 regional trial wheat lines collected from the Huang-Huai-Hai region of China, were tested in this study. Wheat cultivars Mingxian169 and Avocet S were used as the susceptible controls in the resistance identification both at the seedling and adult-plant stages. *Yr* gene near-isogenic lines (NIL), Avocet S*6/Yr5, Avocet S*6/Yr9, Avocet S*6/Yr10, Avocet S*6/Yr15, Avocet S*6/Yr17, Avocet S*6/Yr18, and Avocet S*6/Yr26 were used as a positive controls in *Yr* gene detection, and Mingxian169 and Avocet

S as the negative controls. Yangmai5 and Sumai3 were also used as the *Pm21* and *Fhb1* positive controls and Jingshuang16 and Mingxian169 as the negative controls, respectively.

2.2. Seedling Tests

Seedling tests of stripe rust resistance were conducted in controlled greenhouse conditions. For each wheat entry, about 6–8 seeds were planted in 7-cm-diameter plastic pots. When the first leaves were fully expanded (about 10 days after planting), seedlings were inoculated with the predominant Chinese *Pst* races, CYR32, CYR33, and CYR34, by dusting with a mixture of fresh urediniospores and talcum powder at a 1:20 ratio, respectively. After inoculation, the seedlings were incubated in a dark box at 8–10 °C and 100% relative humidity for 24 h, and then transferred to a controlled condition greenhouse for 16 h/light and 8 h/darkness, and a temperature of 15–17 °C. The infection types (ITs) were scored about 15–17 days after inoculation according to the 0–9 level described by Line and Qayoum [23]. Entries with IT 0–5 were considered as the all-stage resistant (ASR) group, and those with ITs 6–9 as the susceptible group.

In the meantime, seeds of the 146 cultivars (lines) and control cultivar Jingshuang16 were planted in plastic trays. When the first leaf fully expanded, inoculation was conducted with the prevalent *Bgt* isolate E09 by sprinkling the conidia from the spore-susceptible seedlings of Jingshuang16 onto the tested seedlings. After inoculation, the seedlings were kept in an incubator with a 12 h light/12 h dark cycle and a temperature of about 18 °C. The infection type (IT) was scored based on 0–4 levels at ten days post inoculation (dpi) [24]; the phenotypes of plants were considered as resistant (ITs 0–2) or susceptible (ITs 3–4) to powdery mildew.

The seedling tests of wheat stripe rust and powdery mildew were conducted three times after susceptible controls fully onset, and the highest IT was selected as the final investigation result.

2.3. Adult-Plant Stage Tests in Field

The adult-plant resistance (APR) evaluations were performed during the 2018–2019, and 2019–2020 crop seasons in a field of the Northwest A&F University experimental station. About 20–30 seeds of each cultivar were planted in a 1-m line with 25-cm space between rows in each disease nursery. A total of three square nurseries were designed for stripe rust, powdery mildew, and head blight, separated by a protective row in the middle. Susceptible cultivars Mingxian169 (stripe rust), Jingshuang16 (powdery mildew), and the resistance cultivar Sumai3 (Fusarium head blight) were planted every 20 rows throughout each disease nursery. Stripe rust and powdery mildew inoculations with the mixture *Pst* races or *Bgt* isolates were carried out at the beginning of stem extension stage, respectively. Type II FHB resistance, defined as a disease that inhibits the spread of point infection, was employed to inoculate with *F. graminearum* spores with single basal florets on spikelets as previously described [25]. A total of 146 entries were identified by artificial inoculation, 20 spikes of each entry, and the rest of the spikes were sprayed with water as a control. Before heading and blooming of wheat, inoculating the middle spikelet of the wheat ear with single basal florets, and the inoculation amount of each spikelet was one millet mycelium. Water was sprayed on the inoculated ear and covered with a transparent plastic bag to keep it moisturized. After the inoculated spikelet turned brown, we removed the plastic bag immediately to allow it to expand under natural conditions. Disease severity (DS), scored as the percentage leaf area with disease symptoms, was evaluated three times between the early and late dough stages. According to Bariana and McIntosh [26], recording IT of strip rust on a scale of 0–4 as "0", "0;", "1", "2", "3", "4". Accession with IT 0–2 was categorized into the resistant and those with IT 3–4 into susceptible.

ITs of wheat powdery mildew were scored according to the 0–9 scale as Saari and Prescott described [27]. Plants with ITs 0–6 were classified as resistant, and ITs 7–9 as susceptible. For evaluation of the type II FHB resistance of wheat, the total number of diseased spikelets and rachises were calculated as disease severity indices at 1–3 weeks

after inoculation with *F. graminearum*, and disease index was recorded as Liu et al. [28]. Disease severity was calculated as the average percentage of infected spikelets. FHB severity of 0 was considered immune (I), those with severity greater than 0 and less than 2.0 were considered resistant (R), severity greater than or equal to 2.0 and less than 3.0 were considered moderately resistant (MR), those greater than or equal to 3.0 and less than 3.5 were considered moderately susceptible (MS), and 3.5 or more were considered as susceptible (S) (Table S1).

2.4. DNA Extraction

After seedling tests, the healthy leaves of 146 wheat entries and the control lines were collected for DNA extraction. Genomic DNA was isolated using the cetyltrimethylammonium bromide (CTAB) protocol with slight modifications [29]. The DNA was quantified using electrophoresis and spectrophotometry with NanoDrop (ND-1000, Thermo Scientific, Wilmington, DE, USA) and the concentration were adjusted to 80 ng/μL with sterilized ddH_2O for use as a polymerase chain reaction (PCR) template.

2.5. PCR Amplification and Electrophoresis Analysis

Two pairs of closely linked markers for each of the resistance genes *Yr5*, *Yr9*, *Yr10*, *Yr15*, *Yr17*, *Yr18*, *Yr26*, *Pm21*, and *Fhb1* were selected for molecular detection (replaced by the follow Table 1), which were synthesized by Sangon Biotech Co. Ltd. (Shanghai, China). The specific amplification procedures were in accordance with the corresponding references (Tables 2 and 3). The test was repeated for each sample three to four times, all three repetitions were detected as present. The products of DNA amplification were separated by 2% agarose gel. A total of four pairs of KASP primers were used to detect the presence of *Yr5*, *Yr15*, *Yr26*, and *Fhb1*. Each KASP reaction was carried out using a 5.07 μL reaction mixture consisting of 2.5 μL DNA, 2.5 μL 2 × KASP master mix (LGC, Hoddesdon, Herts, UK), and 0.07 μL KBD Assay mix primer (12 mM of each allele-specific primer and 30 mM of the common primer). The KASP thermal protocol was run as outlined in Table 4. The FLUOstar Omega microplate reader (BMG Labtech, Durham, NC, USA) was used to read the different genotypes, FAM homozygotes, and HEX homozygotes using the KlusterCaller^{TM} software (available online: http://www.lgcgroup.com/ (accessed on 25 April 2021).

Table 1. Primers used to detect *Yr, Pm*, and *Fhb* resistance genes in the Huang-Huai-Hai region of China wheat cultivars (lines).

Selected Gene(s)	Primer Name	Primer Sequence (5′-3′)	Annealing Temp (°C)	Product Size (bp)	Reference
Yr5	*Yr5-M1*	FAM:GAAGGTGAC CAAGTTCATGC TATATCAC TGCTGCCTGTAG TGGA HEX:GAAGGTCGGAG TCAACGGAT TATCAC TGCTGCCTGTAG TGGG Reverse:ACGAGTAGCTG TAATTAAAC CAACAATGAA			[30]

Table 1. *Cont.*

Selected Gene(s)	Primer Name	Primer Sequence (5′-3′)	Annealing Temp (°C)	Product Size (bp)	Reference
	Yr5-candidate	FAM:GAAGGTGAC CAAGTTCATGCT CAGGAGATCTT GAAGGACAT HEX:GAAGGTCGGAG TCAACGGAT TCAGGA GATCTTAAAGGA ATA Reverse:AAACTCTTT GACTGGTACTCG			[30]
Yr9	*H20*	F:GTT GGAAGGGAGCT CGAGCTG R:GTTGGG CAGAAAGGTCG ACATC	60	+1598	[31]
	AF1/AF4	F:GGAGA CATCATGAAAC ATTTG R:CTGTTGTTGGG CAGAAAG	60	+1500	[32]
Yr10	*Yr10F/Yr10R*	F:TCAAAGA CATCAA GAGCCGC R:TGGCC TACATGAACTCT GGAT	64	540	[33]
	Yr10F1/Yr10R1	F:TTGGAATT GGCGACAA GCGT R:GTGATGAT TACCCAC TTCCTC	64	755	[33]
Yr15	*uhw300*	F:CCGTGTCAGCCA CCTACAAT R:GCACTCTAC CACCGAACACA	58	936	[34]
	Yr15-R8	RAM:GAAGGTGAC CAAGTTCATGCT CAGATCCCCGGT TTCTCTCAAG HEX:GAAGGTCGGAG TCAACGGAT TCAGATCCCCGG TTCTCTCAAA Reverse:CCCCCAAAT GATCGAGAATA			[35]

Table 1. *Cont.*

Selected Gene(s)	Primer Name	Primer Sequence (5′-3′)	Annealing Temp (°C)	Product Size (bp)	Reference
Yr17	*VENTRIUP/LN2*	F:AGGGGC TACTGAC CAAGGCT R:TGCAGCTACAG CAG TATGTACACAAA A	65	262	[36]
	SC385	F:CTGAATACAAA CAGCAAACCAG R:ACAGAAAGTGA TCATTTCCATC	54	400	[36]
Yr18	*CSlV34*	F:GTTGGTTAA GACTGGTGATGG R:TGCTTGC TATTGCTGAA TAGT	58		[37]
	Cssfrs3	TTGATGAAAC CAG TTTTTTTTCTA GCCATTTAACAT AATCATGATGGA GTTGGTTAA GACTGGTGATGG TGCTTGC TATTGCTGAA TAGT	58		[38]
Yr26	*WE173*	F:GGGACAAGGG GAGTTGAAGC R:GAGAGTTCCAA GCAGAACAC	55	510 −750	[39]
	WRS435	FAM:GAAGGTGAC CAAGTTCATGCT GCACATATCC TACGCCTCTGT HEX:GAAGGTCGGAG TCAAC GGATTGCACAT ATCCTAC GCCTCTGG Reverse:CCG CAATCATTTATT TGAGCCTCAG			[40]
Pm21	*ws-1*	F:TTGGTGTTTT GCTTCTGGA R:CTGA TATTGCGGTGAA TGTT	55	949	[41,42]

Table 1. *Cont.*

Selected Gene(s)	Primer Name	Primer Sequence (5′-3′)	Annealing Temp (°C)	Product Size (bp)	Reference
	SCAR1400	F:CAC TCTCCTCAAAC CTTGCAAG R:CAC TCTCCTCCAC TAACAGAGG	55	1400	[43]
Fhb1	*His-InDel*	F:ATGCGTGCGCTG TACTTG R:CGTCACAGAG TCCAGTGAAA	65	1309 −2061	[44,45]
	Fhb1-TaHRC-KASP	FAM:GAAGGTGAC CAAGTTCATGCT TTGGGCTCAC GTCGTG CAAATGGT HEX:GAAGGTCGGAG TCAACGGATTT GTCTGTTTCGCT GGGATG Reverse:CTTCCAG TTTCTGCTGCCA T			[46]

Table 2. The PCR reactions of different selected markers.

Gene	Primer	DNA (μL)	Taq/U (μL)	dNTP/mM (μL)	$MgCl_2$/mM (μL)	10× buffer (μL)	ddH_2O (μL)	2× ES Taq Master Mix (μL)	F/R-Primer (μL)
Yr9	*AF1/AF4*	0.6	0.75	0.4	1	1	6.1	7.5	0.4
	H20	1	0.75	0.2	2	1	4.9	7.5	0.8
Yr10	*Yr10F/Yr10R*	1	0.75	0.2	2	1	4.5	7.5	1
	Yr10F1/Yr10R1	1	0.75	0.2	2	1	4.5	7.5	1
Yr15	*Uhw300*	2.1	1	2.5	0.16	1	2.4	7.5	1.5
Yr17	*VENTRIU-LN2*	1	1	0.2	1.5	1	6.1	7.5	0.2
	SC-385	2.1	1	0.2	1.5	1	3.4	7.5	1
Yr18	*Cslv34*	2.1	1	2.5	0.16	1	2.4	7.5	1.5
	Cssfrs3	3	1	2.5	0.16	1	5.5	10	0.5:0.25
Yr26	*WE173*	1	0.75	0.2	2	1	5.3	7.5	0.6
Pm21	*WS-1*	2.1	1	0.5	1.2	1	2.4	7.5	1.5
	SCAR1400	1	1	0.2	1	1	8	10	0.5
Fhb1	*His-InDel*	1	1	0.2	2.5	1	7	10	1

Table 3. The PCR protocols of different molecular markers.

Gene	Primer	Pre-Degeneration (94 °C, min)	Generation (94 °C, S)	Annealing (°C, S)	Extension (72 °C, S)	Number of Cycles	Final Extension
Yr9	*AF1/AF4*	3	15	55,60	120	45	7
	H20	5	60	60,60	120	45	10
Yr10	*Yr10F/Yr10R*	4	30	64,30	60	35	10
	Yr10F1/Yr10R1	4	30	64,30	60	35	10
Yr15	*Uhw300*	4	60	64,30	120	32	10
Yr17	*VENTRIUP-LN2*	4	45	65,30	60	35	10
	SC-385	3	45	54,30	60	30	10
Yr18	*Cslv34*	4	45	58,45	60	34	10
	Cssfrs3	4	45	58,45	50	35	5
Yr26	*WE173*	4	45	55,45	60	34	10
Pm21	*WS-1*	4	30	55,60	60	35	10
	SCAR1400	5	50	55,50	120	36	10
Fhb1	*His-InDel*	3	30	65,30	150	35	7

Table 4. Thermal cycle conditions for KASP genotyping reactions.

Step	Description	Temperature	Time	Number of Cycles Per Step
1	Activation	94 °C	15 min	1 cycle
2	Denaturation	94 °C	20 s	10 cycles
	Annealing/Elongation	55–61 °C	60 s (drop 0.6 °C per cycle)	
3	Denaturation	94 °C	20 s	26 cycles
	Annealing/Elongation	55 °C	60 s	

2.6. Data Analysis

Phenotype and genotype data analysis were performed using Microsoft® Excel® 2016 (MS, Redmond, WA, USA). The distribution of resistance genes in the Huang-Huai wheat region was determined by screening varieties that showed resistance in both the genotype and phenotype according to Sections 2.2 and 2.3.

3. Results

3.1. Seedling Resistance Evaluation

Among the 146 entries, 108 were resistant to *Pst* race CYR32, 83 resistant to CYR33, and 99 resistant to CYR34, accounting for 73.97%, 56.85%, and 67.81% (Figure 1, Table S1), respectively; 68 entries, accounting for 46.58%, were resistant all the tested *Pst* races. However, only 22 entries showed resistance to *Bgt* isolate E09 at the seedling stage, accounting for 15.07%. In addition, 11 (7.53%) wheat varieties showed resistance to both stripe rust and powdery mildew. In total, the stripe rust resistance of these entries was better than that of powdery mildew and few entries were resistant to both stripe rust and powdery mildew.

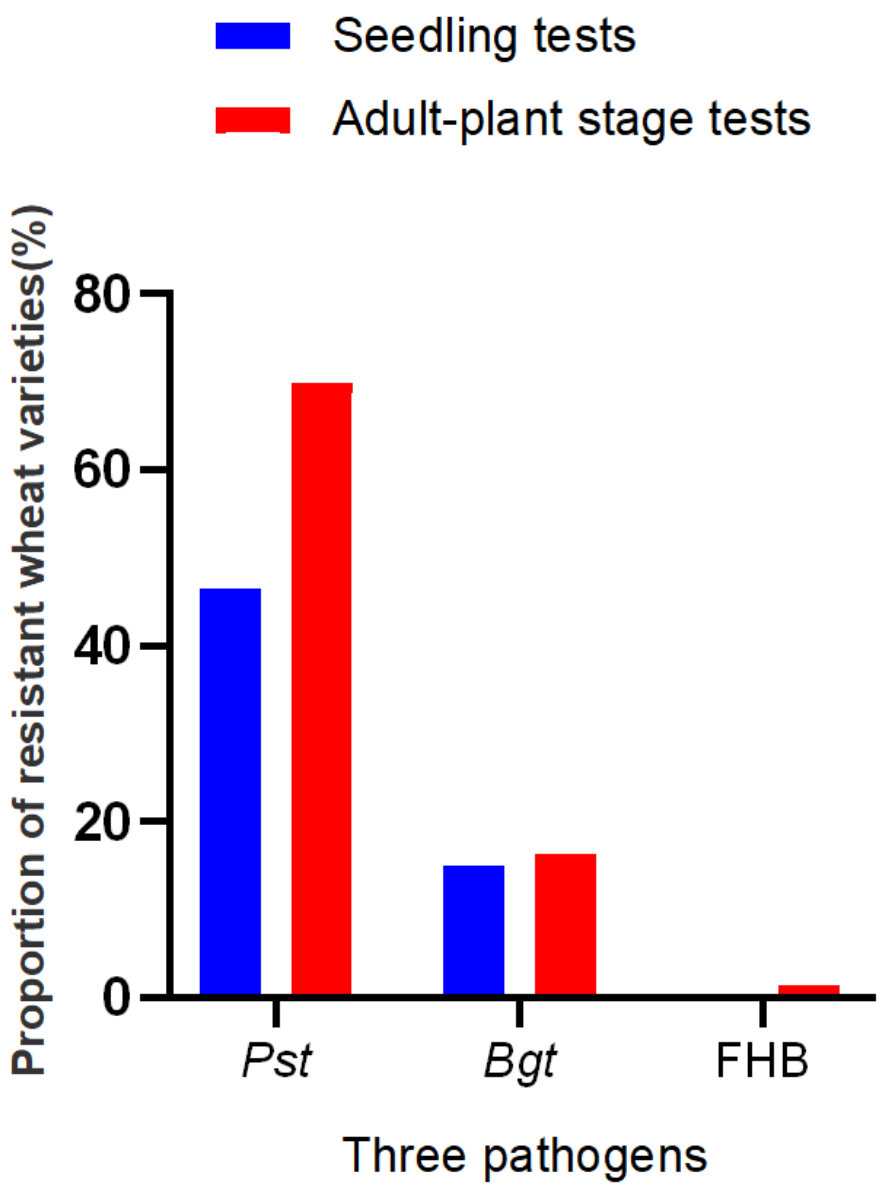

Figure 1. The proportion of wheat entries with different types of resistance to stripe rust, powdery mildew, and FHB.

3.2. Adult-Plant Resistance Evaluation

The results of adult-plant resistance evaluation indicated that 102 entries showed resistance to stripe rust, accounting for 69.86%; 24 entries showed resistance to powdery mildew, accounting for 16.44%; and two entries showed resistance to head blight, accounting for 1.37% (Figure 1, Table S1). It was worth mentioning that among these 102 stripe rust resistance entries and 24 powdery mildew resistant entries, 48 and 10 entries were susceptible in the seedling evaluation, respectively, which indicated that these entries had adult-plant stage resistance (APR). Only two cultivars, Xinong650 and Fugao2, showed resistant to head blight, accounting for 1.37%. In addition, 17(11.64%) wheat entries showed resistance to both stripe rust and powdery mildew and only one accession, Xinong650, showed resistance to both strip rust and head blight. No variety was resistant to all three wheat diseases.

3.3. Identification of the Resistance Genes Using Molecular Markers

3.3.1. Identification of Yr Genes

The known stripe rust resistance genes *Yr5* [30], *Yr9* [31,32], *Yr10* [33], *Yr15* [34,35], *Yr17* [36], *Yr18* [37,38], *Yr26* [39,40], closely linked markers or functional markers, were used for molecular detection of 146 wheat varieties in the Huang-Huai-Hai wheat region, and these seven stripe rust resistance gene donors were used as positive controls (Figure 2A–G). It was concluded that among the tested wheat varieties in the Huang-Huai-Hai wheat region, only two entries (1.37%), Baojingmai186 and Baojingmai166, could produce *Yr5* alleles; a total of 62 wheat entries (43.15%) produced *Yr9* specific bands in both markers; nine entries (6.16%), Haomai18, Xinong328, WeifengT03, Xi'an139, Xichun919, Hangmai9, Huaikemai8, Xinong369, and Xinong528 amplified *Yr17* target bands; three entries, Xinong161, Shaanza10, and Shaanmai139, may carry *Yr26*. Among these, Baojingmai186 may carry *Yr5* and *Yr9* at the same time and Shaanza10 may carry *Yr9* and *Yr26* at the same time; three accessions, WeifengT03, Xichun919, and Xinong528 may carry *Yr9* and *Yr17* at the same time. No entries that may carry *Yr10*, *Yr15*, and *Yr18* were detected in the test materials (Table S1).

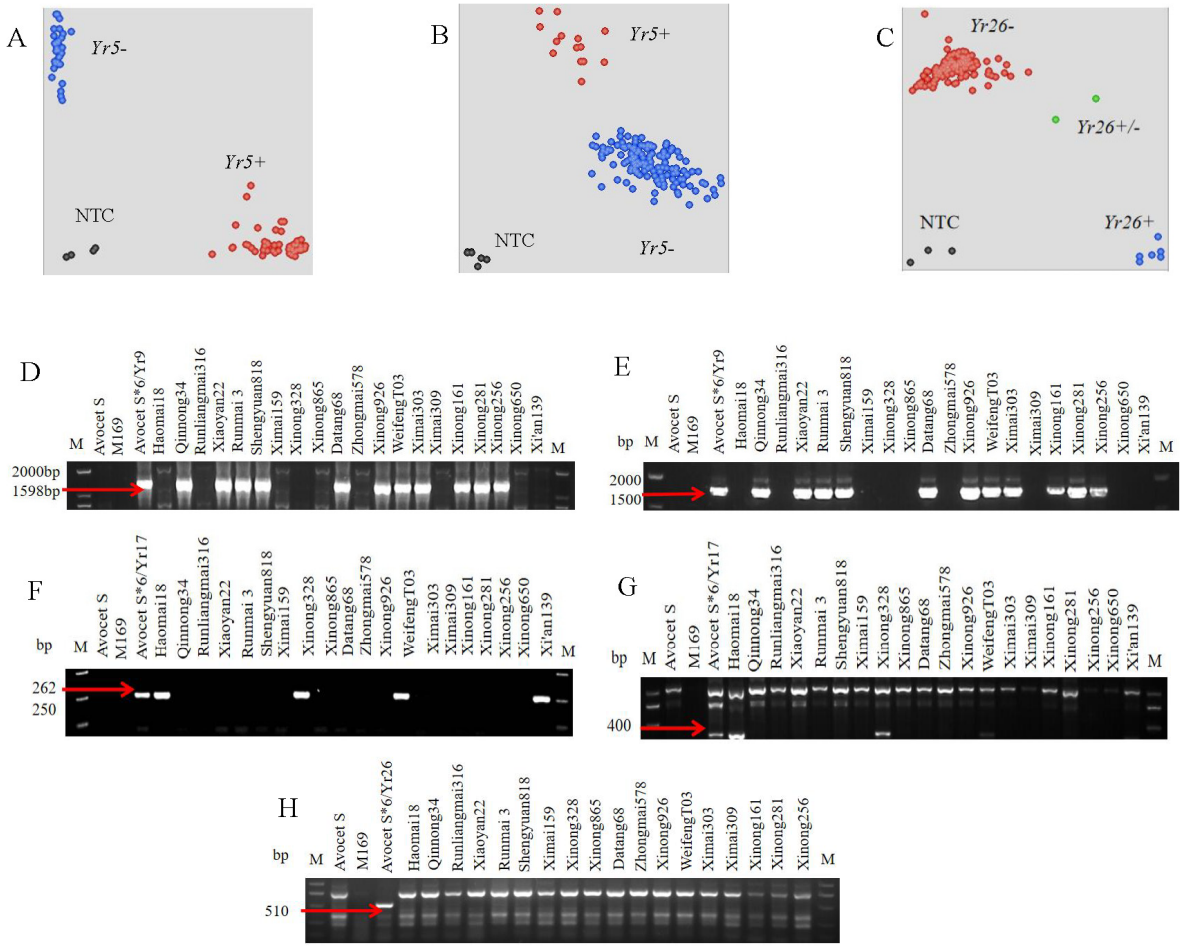

Figure 2. Electrophoretogram and genotyping data of different markers. (**A**,**B**), identification of *Yr5* resistance gene using KASP makers; (**D**,**E**), identification of *Yr9*; (**F**,**G**), identification of *Yr17*; (**C**,**H**), identification of *Yr26*. *Yr5+/Yr26+*, *Yr5−/Yr26−*, indicating presence/absence of *Yr5/Yr26* in the tested entries for the horizontal and vertical axis, respectively. NTC, non-template control.

3.3.2. Identification of Pm Gene

Pm21, located on the short arm of chromosome 6V (6VS) of *Dasypyrum villosum*, confers immunity to all known *Bgt* isolates and has been widely used in wheat breeding [41].

The presence of *Pm21* was identified with markers *WS-1* [42] and specific SCAR marker *SCAR1400* [43], which can produce 949 bp and 1400 bp target bands in wheat carrying *Pm21*, respectively. No entry can amplify the *Pm21* target band of 949 bp when using the primer of *WS-1*. Interestingly, only Xinong136 and Gaoke1128 can amplify the target band of 1400 bp when using the primer of *SCAR1400*. However, both varieties showed susceptibility to powdery mildew in the identification of seedling and adult-plant stage, which means that these two entries may not carry the *Pm21* gene **(**Table S1).

3.3.3. Identification of Fhb Gene

Fhb1, originating from Sumai 3, is one of the most important *Fhb* resistance genes in hexaploid wheat [44]. When detected with diagnostic marker *His-InDel* [45], a 1309 bp band can be amplified in wheat entries containing the *Fhb1* gene, if not, a band of 2061 bp can be amplified. Among all 146 wheat varieties, no target band of 1309 bp could be amplified. When using the *Fhb1-TaHRC-KASP* marker [46], susceptible genotypes will cluster along the vertical axis and resistant genotypes will cluster along the horizontal axis. The test results based on the two types of markers showed that no entries may contain the resistance gene *Fhb1* **(**Table S1).

4. Discussion

In the disease identification of 146 wheat varieties, 51 (34.93%) entries showed resistance to stripe rust both at the seedling and adult-plant stages. That is to say, these 51 entries had ASR and the remaining 51 (34.93%) had APR to stripe rust. Twenty-four (16.44%) showed resistance to *Bgt* mixture isolates at the adult stage and 14 wheat varieties were susceptible to powdery mildew at the seedling stage, indicating that 14 (9.59%) entries had APR to powdery mildew, and 10 (6.85%) entries showed moderate resistance to powdery mildew at all stages. Two (1.37%) entries showed resistance to head blight. It could be seen that whether it was to stripe rust or to powdery mildew, the resistance at the adult-plant stage was stronger than that at the seedling stage (Figure 1). There were still few wheat varieties that were resistant to FHB. In the molecular detection of 146 wheat varieties, the detection rates from high to low were *Yr9*, *Yr17*, *Yr26*, and *Yr5* genes. Surprisingly, none of the entries were detected to contain *Yr10*, *Yr15*, *Yr18*, *Pm21*, and *Fhb1*. Two cultivars that have been detected to contain *Yr5* were not resistant to stripe rust at the seedling stage, which indicated that they may not have the *Yr5* gene and might be due to the false positives in molecular testing. *Yr9* was susceptible to these tested *Pst* races, which means those that have been detected to contain *Yr9* and were resistant at the seedling stage may also contain other effective ASR *Yr* genes. *Yr17* was also susceptible to CYR32, CYR33, and CYR34, however, all nine wheat entries showed resistance to one or more *Pst* races in the seedling stage and were resistant to the mixture *Pst* race in the adult-plant stage, therefore, these entries may also carry some other effective *Yr* gene(s). 51 wheat entries had APR to stripe rust in this study, whereas no entries were detected to carry *Yr18*, which indicated that these entries may carry other effective APR gene(s). *Yr26* was resistant to *Pst* races CYR32 and CYR33 but susceptible to CYR34 in the seedling stage. In fact, all three entries were resistant to CYR34 in the seedling tests, which suggested that these entries may also carry other *Yr* gene(s) besides *Yr26*.

When testing 494 Chinese wheat entries for stripe rust resistance in both seedling and adult-plant stages, Zeng et al. [47] found that 16 (3.24%) entries had ASR in all race tests, and 99 (20.04%) had APR. The frequencies of ASR and APR in this study were all higher than that of Zeng et al., which maybe resulted by different *Pst* races. Zhu et al. [48] reported that 29.08% and 11.41% of wheat lines were resistant to *Bgt* and FHB in the field, respectively, which is similar with that in this study. Therefore, more attention should be paid to wheat resistance to stripe rust, powdery mildew, and Fusarium head blight in wheat breeding programs, especially to powdery mildew and Fusarium head blight. According to the virulence and avirulence formula of *Yr* genes [49], *Yr5* is known to have all-stage resistance to all tested *Pst* races, and *Yr9* has become susceptible to most Chinese *Pst*

races since 1990 [50], and 43.15% of entries were detected to carry *Yr9*, which is consistent with previous studies [51,52]. *Yr5* and *Yr15* were still effectively resistant to almost all the known *Pst* races in China. However, no entries were postulated to carry *Yr5* and *Yr15* in this study. According to the previous studies, *Yr5* has been rarely used in wheat breeding programs in China [50,51], and *Yr15* has not been detected in Chinese cultivars [50–53]. Both *Yr5* and *Yr15* were successfully cloned in 2018 [30,34], which will facilitate their use in wheat breeding programs in the future. In fact, *Yr18/Lr34/Sr57/Pm38* has been successfully used in wheat resistance breeding since the beginning of the last century, and it provides durable, adult-plant, slow-rusting resistance to stripe rust, leaf rust, stem rust, and powdery mildew. Similar to our study, Huang et al. [50] also reported that no entries carried *Yr18* in 66 selected commercial wheat cultivars. However, *Yr18* has a high detection percent in Chinese wheat landraces [53,54]. Moreover, the disease resistance will be improved dramatically if *Yr18* is pyramided with other all-stage resistance gene(s) or adult-plant resistance gene(s) [55]. Therefore, Chinese wheat landraces should be developed as the *Yr18* resource for durable resistance cultivars breeding.

The *Pm21* gene has been proven to be very effective against a broad-spectrum of wheat powdery mildew isolates identified in China [56]. Using molecular marker *WS-1*, Jiang et al. (2014) found that 49 (7.4%) of 662 wheat cultivars (lines) may carry *Pm21* [42]. However, other researchers have shown that in Guizhou and Sichuan Provinces where wheat powdery mildew is severe, there is a high frequency of *Pm21* in wheat varieties [57]. Therefore, *Pm21* is not suggested for use alone in wheat breeding programs although it has low frequency occurrence in some regions. It would be great if it could be pyramided with other effective *Pm* resistance genes such as *Pm12, Pm24* [58], and *Pm68* [17].

Several types of FHB resistance have been identified in wheat, resistance to initial infection (Type I), and resistance to spread within the spike (Type II) following single floret injections. The greatest effect of *Fhb1* imparting stable FHB type II resistance is the main source of resistance in wheat breeding. In North America, *Fhb1* has been widely used in spring wheat areas where wheat scab occurred seriously, for example, the cultivars Alsen [59] and Sabin [60]. In China, *Fhb1* has been successfully used in wheat breeding programs in spring wheat areas such as Jiangsu Province, however, it has not been used widely in winter wheat areas. In this study, there were only two wheat entries resistant to head blight in the field, but *Fhb1* was not detected in both of the entries, which suggested that the FHB resistance may be related to other FHB resistance gene(s). Xu et al. [61] reported that none of the 22 commercial wheat cultivars in the Huang-Huai-Hai contained the *Fhb1* gene and six of nine cultivars in the middle and lower reaches of the Yangtze River carried *Fhb1*. Zhu et al. [62] found that *Fhb1* contained in Chinese wheat entries were mainly derived from Sumai3 and Ningmai9, with the latter being the main one, which can be used as a valid source of resistance for breeding. Recently, another effective FHB resistance gene *Fhb7*, which was derived from *Thinopyrum elongatum*, has been isolated. The resistance to FHB of wheat cultivars can be significantly enhanced after introducing the *Fhb7* gene [8]. Therefore, *Fhb1* and *Fhb7* have been suggested for use together in wheat breeding programs.

Compared to other studies that have only focused on one type of wheat disease at a time, this research is more in line with agricultural practices. It not only lays a theoretical foundation for the balanced application of these genotypes and genes in wheat breeding programs and disease control, but also improves the host resistance to these three dangerous pathogens widespread in nature at the same time.

5. Conclusions

The results revealed that the number of resistant cultivars to Bgt and FHB in Huang-Huai-Hai region is small, and the diversity of resistance genes is also low. Due to *Yr9, Yr10, Yr17*, and *Yr26* have become ineffective to *Pst* prevalent races, these genes are not recommended for use in wheat breeding; *Yr5*, *Yr15*, *Yr18*, *Pm21*, and *Fhb1* should not be used alone, but can be used with other effective gene(s) to get broad-spectrum and durable

resistance cultivars. The use of resistant cultivars with multiple or different resistance genes can reduce the accumulation of inoculum. If the cultivar is susceptible, it should be withdrawn from production as soon as possible.

Supplementary Materials: The following are available online at https://www.mdpi.com/article/10.3390/agronomy11061025/s1, Table S1: Infection types to *Pst* races, *Bgt* and FHB isolates and molecular detection results with the specific markers. Notes: NT, not tested; "+", had the same genotyping to the reference primers, may carry the tested gene(s); "−", indicating that the entry may not carry the resistance gene(s) as the target band could not be amplified.

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Abbreviations

APR	Adult-plant resistance
ASR	All-stage resistance
Bgt	Blumeria *graminis* f.sp. *tritici* Em. Marchal
CYR	Chinese yellow rust
DNA	Deoxyribonucleic acid
DS	Disease severity
EST	Expressed sequence tag
FHB	*Fusarium* head blight
Fhb	Fusarium head blight resistance genes
IT	Infection type
KASP	Kompetitive allele-specific PCR
NIL	near isogenic line
PCR	Polymerase chain reaction
Pm	Powdery mildew resistance genes
Pst	*Puccinia striiformis* Westend. f. sp. *tritici* Erikss
SSR	Simple sequence repeat
STS	Sequence-tagged site
Yr	Yellow rust (stripe rust) resistance genes

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