Postprint: Identification and Genetic Analysis of Powdery Mildew Resistance at the Seedling Stage in Wheat Landrace 'Hongyouzi'

Authors: Cao Yanwei, Xu Hongxing, Li Xiuquan, has been installed and debugged

Date: 2017-11-08T00:00:00+00:00

Abstract

Powdery mildew is one of the major diseases of wheat, and identification and utilization of elite disease-resistant germplasm constitute effective measures for controlling this disease. Currently, most cultivated varieties and lines in wheat production in China exhibit susceptibility to powdery mildew, with few elite resistance genes available for utilization; therefore, it is essential to explore novel effective resistance sources and resistance genes. The Chinese wheat landrace 'Hongyouzi' (Jing 2350) displays high resistance to immunity against 34 of 38 domestic wheat powdery mildew pathogen strains at the seedling stage, and exhibits immunity or near-immunity against field mixed powdery mildew pathogens in the Shijiazhuang area at the adult plant stage. To further investigate the genetic characteristics of powdery mildew resistance in 'Hongyouzi' and to discover and utilize elite powdery mildew resistance genes therein, this study employed powdery mildew isolate E09 to conduct seedling resistance evaluation and genetic analysis on F1 and F2 generation plants and F2:3 lines from crosses of 'Hongyouzi' with susceptible parents 'Mingxian 169' and 'Huixianhong', respectively. The results demonstrated that all F1 plants from both 'Hongyouzi' × 'Mingxian 169' and 'Hongyouzi' × 'Huixianhong' crosses were susceptible, the ratio of resistant to susceptible plants in each F2 generation conformed to a 1:3 segregation ratio, and the ratio of homozygous resistant:heterozygous:homozygous susceptible lines in each F2:3 generation conformed to a 1:2:1 ratio. Therefore, resistance in 'Hongyouzi' to the E09 isolate is controlled by a single recessive gene, tentatively designated as PmHYZ. 'Hongyouzi' represents an elite powdery mildew resistance source among Chinese wheat landraces, and investigation and discovery of resistance genes therein will lay the foundation for its effective utilization in disease resistance breeding.

Full Text

Identification and Genetic Analysis of Powdery Mildew Resistance at Seedling Stage in 'Hongyouzi' Chinese Wheat Landrace

CAO Yanwei[1,3], **XU Hongxing**[1], LI Xiuquan[2], AN Diaoguo[1]
[1]Center for Agricultural Resources Research, Institute of Genetics and Developmental Biology, Chinese Academy of Sciences, Shijiazhuang 050022, China
[2]Institute of Crop Sciences, Chinese Academy of Agricultural Sciences, Beijing 100081, China
[3]University of Chinese Academy of Sciences, Beijing 100049, China

Abstract

Powdery mildew, caused by *Blumeria graminis* f. sp. *tritici* (Bgt), is a devastating foliar disease of wheat. The identification and utilization of elite resistant germplasm is an effective control measure for this disease. At present, most wheat cultivars/lines in China' s wheat production are susceptible to powdery mildew, and few powdery mildew resistance genes are utilized in China. Therefore, it is essential to identify more effective and elite resistance sources and genes.

The 'Hongyouzi' Chinese wheat landrace ('Jing 2350') has shown immune to highly resistant responses to 34 out of 38 Bgt isolates at the seedling stage, and immune or nearly immune responses to a composite of Bgt isolates at the adult stage in Shijiazhuang, China. To further study the resistance characteristics and identify the resistance gene(s) of 'Hongyouzi' , genetic analysis of F_1 plants, F_2 populations, and their derived F_2:$_3$ families from crosses of 'Huixianhong' × 'Hongyouzi' and 'Hongyouzi' × 'Mingxian169' were conducted at the seedling stage using Bgt isolate E09. The results showed that all F_1 plants derived from both combinations were susceptible to Bgt isolate E09. The ratios of resistant to susceptible F_2 plants conformed to 1:3, and those of homozygous resistant F_2:$_3$ lines:heterozygous lines:homozygous susceptible lines conformed to 1:2:1. These results indicated that the resistance of 'Hongyouzi' to Bgt isolate E09 was conferred by a single recessive resistance gene, temporarily named *PmHYZ*. 'Hongyouzi' represents an elite powdery mildew resistance source among Chinese wheat landraces, and studying and exploiting its resistance gene will lay the foundation for its effective utilization in disease resistance breeding.

Keywords: Wheat; Powdery mildew; Wheat landrace; Hongyouzi; Genetic analysis

Wheat (*Triticum aestivum*) is one of the most important food crops in China, and its yield is critical to national food security. Powdery mildew, caused by *Blumeria graminis* f. sp. *tritici* (Bgt), is a major disease of wheat and ranks among the three most important diseases affecting wheat production. The identification and utilization of elite resistant germplasm remain the most economical, effective, and environmentally friendly measures for controlling this disease [?]. Due to the co-evolution of Bgt virulence and host resistance, resistance genes widely deployed in production often gradually lose their effectiveness [?]. Therefore, wheat breeders must continuously work to discover and utilize new, effective, and broad-spectrum powdery mildew resistance genes [?].

To date, more than 70 powdery mildew resistance genes have been formally designated at 53 loci (*Pm1-Pm58*, with *Pm8* being allelic to *Pm17*, *Pm18=Pm1c*, *Pm22=Pm1e*, *Pm2=Pm4c*, *Pm31=Pm21*) [?]. In addition, over 20 temporarily designated powdery mildew resistance genes have been reported. These genes are distributed across all wheat chromosomes [?]. In terms of origin, they are derived from common wheat, wheat relatives, and distant relatives. At least eight powdery mildew resistance genes have been identified in Chinese wheat landraces, including *Pm2c* [?], *PmX* [?], *Pm5e* [?], *mlxbd* [?], *Pm24a* [?], *Pm24b* [?], *Pm45* [?], and *Pm47* [?], originating from landraces 'Niaomai' , 'Xiaohongpi' , 'Fuzhuang 30' , 'Xiaobaidongmai' , 'Chiyacao' , 'Baihulu' , 'D57' , and 'Hongyanglazi' , respectively. Thus far, these genes have maintained good resistance against Bgt isolates. Therefore, landraces represent an important genetic source of powdery mildew resistance and valuable germplasm resources for genetic improvement of wheat cultivars [?].

The Chinese wheat landrace 'Hongyouzi'(Jing 2350), obtained from the National Germplasm Bank and originating from Xichuan County, Nanyang City, Henan Province, showed immune or nearly immune responses to a composite of Bgt isolates in the field in Shijiazhuang. Preliminary screening identified 'Hongyouzi' as an excellent powdery mildew resistance source. This study conducted seedling resistance screening using 38 Bgt isolates from different regions with varying virulence profiles, and performed genetic analysis of segregating populations derived from crosses with susceptible parents 'Huixianhong' and 'Mingxian169' using isolate E09 to elucidate the genetic characteristics of its powdery mildew resistance.

1.1 Plant Materials

The wheat landrace 'Hongyouzi' (Jing 2350) used in this study was obtained from the National Germplasm Bank and selected based on preliminary powdery mildew resistance screening. The susceptible parents 'Huixianhong' and 'Mingxian169' , along with 38 Bgt isolates (E03, E05, E06, E09, E11, E13, E16, E17, E18, E21, E22, E23-1, E23-2, E26, E30-2, E31, E32, E49, E50, B13, B14, B41, B15, B16, B17, B42, B43, B44, B45, B46, B47, B48, B49, B54, B55, B56, B57, B58), were maintained at the Wheat Germplasm Innovation Laboratory, Center for Agricultural Resources Research, Institute of Genetics and Developmental Biology, Chinese Academy of Sciences.

1.2 Seedling Resistance Screening

Wheat materials for seedling resistance screening were planted in 72-cell trays (4 cm × 4 cm), with 6-8 seeds per cell. 'Mingxian169' was randomly planted in each tray as a susceptible control. For genetic analysis, F_1, F_2, and F_2:$_3$ progeny from the crosses, in addition to the resistant and susceptible parent controls, were evaluated. F_1 and F_2 progeny were planted with one seed per cell to facilitate transplanting after evaluation. Each F_2:$_3$ family was planted in five cells (30-40 seeds total) with 6-8 seeds per cell. All wheat materials were grown in a greenhouse with controlled temperature, humidity, and light conditions. When the first leaf was fully expanded, seedlings were inoculated with Bgt isolates. After inoculation, seedlings were incubated in darkness at 18°C with high humidity for 24 h, then transferred to a controlled greenhouse with 14 h photoperiod, 22°C day/18°C night temperature, and 80-90% relative humidity. Using 'Mingxian169' as the susceptible control, disease reactions were recorded 8-10 days post-inoculation when 'Mingxian169' showed full susceptibility. Resistance was evaluated using the 6-class system proposed by Sheng [?], where infection type (IT) 0 = immune, IT 0; = nearly immune, and IT 1-4 = highly resistant, moderately resistant, moderately susceptible, and highly susceptible, respectively.

1.3 Statistical Analysis

Based on the recorded data, the ratio of resistant to susceptible plants in the F_2 populations was calculated, and F_2:$_3$ families were used to verify the genotypes of individual F_2 plants. Chi-square tests were used to analyze the goodness-of-fit of segregation ratios. The evaluation results were analyzed according to the theoretical segregation patterns for wheat resistance genes described by He [?], combined with the resistance reactions of F_1 plants and segregation ratios in F_2 populations.

2.1 Resistance Evaluation of Hongyouzi

At the seedling stage, 'Hongyouzi' was evaluated for resistance to 38 single-spore Bgt isolates with different virulence profiles. The results showed that 'Hongyouzi' was immune (IT=0) to 15 isolates (E11, E13, E16, E23-1, E23-2, E30-2, E32, B41, B42, B44, B54, B55, B56, B57, B58), nearly immune (IT=0;) to 15 isolates (E03, E05, E09, E17, E18, E21, E22, E26, E50, B13, B15, B16, B17, B43, B49), highly resistant (IT=1) to four isolates (E31, B14, B45, B48), and highly susceptible (IT=4) to four isolates (E06, E49, B46, B47). [Figure 1: see original paper] shows the seedling reactions of 'Hongyouzi' to some Bgt isolates. The susceptible controls 'Huixianhong' and 'Mingxian169' were highly susceptible to all 38 tested isolates. At the adult stage, 'Hongyouzi' showed immune or nearly immune responses to the composite of Bgt isolates from the field in Shijiazhuang.

2.2 Genetic Analysis of Resistance in Hongyouzi

'Hongyouzi' showed an IT=0; reaction to Bgt isolate E09, while the susceptible parents 'Huixianhong' and 'Mingxian169' showed IT=4 reactions. The F$_1$ plants from both 'Huixianhong' × 'Hongyouzi' and 'Hongyouzi' × 'Mingxian169' crosses were also IT=4, indicating that resistance in 'Hongyouzi' to E09 is controlled by recessive gene(s). In the F$_2$ population of 234 plants from the 'Huixianhong' × 'Hongyouzi' cross, 57 plants were resistant (18 with IT=0, 13 with IT=1, and 16 with IT=2) and 177 were susceptible (21 with IT=3 and 156 with IT=4). The resistant:susceptible ratio fit a 1:3 segregation pattern (^{2}=0.023, P=0.88,). For the 291 F$_2$:$_3$ families derived from this cross, at least 24 plants per family were evaluated, revealing 54 homozygous resistant families, 98 heterozygous segregating families, and 39 homozygous susceptible families. The ratio of homozygous resistant:heterozygous:homozygous susceptible families fit a 1:2:1 segregation pattern (^{2}=2.49, P=0.29,).

Similarly, in the F$_2$ population of 156 plants from the 'Hongyouzi' × 'Mingxian169' cross, 42 plants were resistant and 114 were susceptible, with the resistant:susceptible ratio also fitting 1:3 (^{2}=0.21, P=0.88,). Among 257 F$_2$:$_3$ families from this cross, there were 67 homozygous resistant families, 121 heterozygous families, and 69 homozygous susceptible families, fitting a 1:2:1 ratio (^{2}=1.10, P=0.58,). Collectively, these genetic analyses demonstrate that resistance in 'Hongyouzi' to Bgt isolate E09 is controlled by a single pair of recessive genes, temporarily designated *PmHYZ*.

2.3 Comparison of Resistance Spectra Between PmHYZ and Known Recessive Resistance Genes

To differentiate the recessive resistance gene *PmHYZ* from previously reported recessive resistance genes, four known gene donor varieties– 'CI14125' , 'Aquila' , 'Fuzhuang 30' , and 'Xiaobaidongmai' –were evaluated at the seedling stage using the same 38 Bgt isolates for resistance spectrum comparison. The results showed that *Pm5a* and *Pm5b* carriers were susceptible to 33 of the 38 isolates, while *Pm5e* donor 'Fuzhuang 30' was susceptible to five isolates (E43, E50, B13, B15, B47), showing different reactions to four isolates (E50, B13, B15, B47) compared with *PmHYZ*. The *mlxbd* donor 'Xiaobaidongmai' was susceptible to only three isolates (E43, B15, B47). Therefore, *PmHYZ* is distinct from *Pm5a*, *Pm5b*, *Pm5e*, and *mlxbd* ().

Discussion

The Chinese wheat landrace 'Hongyouzi' is a broad-spectrum, elite powdery mildew resistance source, showing immune to highly resistant responses to 34 of the 38 tested Bgt isolates. Preliminary genetic analysis indicates that 'Hongyouzi' carries a single recessive powdery mildew resistance gene. Further molecular marker mapping and development of breeder-friendly linked markers will facilitate the effective utilization of this gene in wheat breeding programs.

Landraces possess rich genetic variation and exhibit excellent stress tolerance and wide adaptability, making them important genetic resources for broadening the genetic base of cultivated wheat varieties and for cultivar improvement [?]. China is rich in wheat landrace resources, and many studies have reported resistance gene discovery through genetic analysis. For example, Dai et al. [?] and Wang et al. [?] reported that landraces ‘Dazicao’ and ‘Xiaohongmang’ each contain a pair of recessive stripe rust resistance genes. Zhai et al. [?] found that four landraces– ‘Mazhamai’ , ‘Xiaobaidongmai’ , ‘Youbailan’ , and ‘Hongjuanmang’ –each carry a pair of recessive powdery mildew resistance genes. To date, multiple powdery mildew resistance genes have been reported in Chinese landraces, including *Pm2c* [?], *PmX* [?], *Pm5e* [?], *mlxbd* [?], *Pm24a* [?], *Pm24b* [?], *Pm45* [?], and *Pm47* [?].

Through multi-isolate resistance spectrum evaluation and genetic analysis, this study identified ‘Hongyouzi’ as an elite powdery mildew resistance source among Chinese wheat landraces and determined that it carries a single recessive powdery mildew resistance gene. This finding not only enriches the pool of powdery mildew resistance genes from Chinese wheat landraces but also provides a foundation for further research and utilization of this gene.

Among the reported wheat powdery mildew resistance genes, most are dominantly inherited, with only a few showing recessive inheritance, including *Pm5a*–*Pm5e*, *Pm9*, *Pm26*, *Pm42*, *Pm47*, *mlxbd*, and *pm2026* [?, ?]. The landrace ‘Hongyouzi’ identified in this study also carries a recessive resistance gene. *Pm5a* and *Pm5b* have largely lost their effectiveness in China [?], the *Pm5b* carrier variety ‘Kormoran’ is susceptible to Bgt isolate E09 [?], and the *Pm5e* carrier ‘Fuzhuang 30’ is susceptible to isolates E18 and E23, whereas ‘Hongyouzi’ in this study is resistant to E09. This study also differentiated *PmHYZ* from known resistance genes, demonstrating that the powdery mildew resistance gene in ‘Hongyouzi’ is distinct from *Pm5a*, *Pm5b*, *Pm5e*, and *mlxbd*. Additionally, *Pm26* [?] and *Pm42* [?] were derived from wild emmer wheat, and *pm2026* from einkorn wheat, whereas the resistance gene in ‘Hongyouzi’ originates from a landrace, suggesting it may differ from *Pm26*, *Pm42*, and *pm2026*. However, *PmHYZ* cannot yet be distinguished from *Pm47*, *Pm5c*, and *Pm5d*. True differentiation of the resistance gene in ‘Hongyouzi’ from these reported genes will require molecular marker mapping, more comprehensive resistance spectrum comparisons, and allelism tests.

Acknowledgments

We thank Dr. Zhou Yilin and Dr. Li Hongjie from the Chinese Academy of Agricultural Sciences for providing the powdery mildew isolates, and all teachers who contributed to this study.

References

[1] Wang Z Z, Li H W, Zhang D Y, et al. Genetic and physical mapping of powdery mildew resistance gene *MlHLT* in Chinese wheat landrace Hulutou[J]. *Theoretical and Applied Genetics*, 2015, 128(2): 365-373

[2] Xiao M G, Song F J, Jiao J F, et al. Identification of the gene *Pm47* on chromosome 7BS conferring resistance to powdery mildew in the Chinese wheat landrace Hongyanglazi[J]. *Theoretical and Applied Genetics*, 2013, 126(5):

[3] He Z H, Lan C X, Chen X M, et al. Progress and perspective in research of adult-plant resistance to stripe rust and powdery mildew in wheat[J]. *Scientia Agricultura Sinica*, 2011, 44(11): 2193-2215

[4] Petersen S, Lyerly J H, Worthington M L, et al. Mapping of powdery mildew resistance gene *Pm53* introgressed from *Aegilops speltoides* into soft red winter wheat[J]. *Theoretical and Applied Genetics*, 2015, 128(2): 303-312

[5] Zhang R Q, Sun B X, Chen J, et al. *Pm55*, a developmental-stage and tissue-specific powdery mildew resistance gene introgressed from *Dasypyrum villosum* into common wheat[J]. *Theoretical and Applied Genetics*, 2016, 129(10): 1975-1984

[6] Li H H, Jiang B, Wang J C, et al. Mapping of novel powdery mildew resistance gene(s) from *Agropyron cristatum* chromosome 2P[J]. *Theoretical and Applied Genetics*, 2017, 130(1): 109-121

[7] Xu H X, Yi Y J, Ma P T, et al. Molecular tagging of a new broad-spectrum powdery mildew resistance allele *Pm2c* in Chinese wheat landrace Niaomai[J]. *Theoretical and Applied Genetics*, 2015, 128(10): 2077-2084

[8] McIntosh R A, Dubcovsky J, Rogers W J, et al. Catalogue of gene symbols for wheat: 2017 supplement[EB/OL]. https://shigen.nig.ac.jp/wheat/komugi/genes/macgene/supplement2017.pdf

[9] Fu B S, Chen Y, Li N, et al. *pmX*: A recessive powdery mildew resistance gene at the *Pm4* locus identified in wheat landrace Xiaohongpi[J]. *Theoretical and Applied Genetics*, 2013, 126(4): 913-921

[10] Huang X Q, Wang L X, Xu M X, et al. Microsatellite mapping of the powdery mildew resistance gene *Pm5e* in common wheat (*Triticum aestivum* L.)[J]. *Theoretical and Applied Genetics*, 2003, 106(5): 858-865

[11] Huang X Q, Hsam S L K, Zeller F J. Chromosomal location of two novel genes for resistance to powdery mildew in Chinese landraces (*Triticum aestivum* L. em. Thell.)[J]. *Journal of Genetics and Breeding*, 2000, 54: 311-317

[12] Huang X Q, Hsam S L K, Zeller F J, et al. Molecular mapping of the wheat powdery mildew resistance gene *Pm24* and marker validation for molecular breeding[J]. *Theoretical and Applied Genetics*, 2000, 101(3): 407-414

[13] Xue F, Wang C Y, Li C, et al. Molecular mapping of a powdery mildew resistance gene in common wheat landrace Baihulu and its allelism with *Pm24*[J].

Theoretical and Applied Genetics, 2012, 125(7): 1425-1432

[14] Ma H Q, Kong Z X, Fu B S, et al. Identification and mapping of a new powdery mildew resistance gene on chromosome 6D of common wheat[J]. *Theoretical and Applied Genetics*, 2011, 123(7): 1099-1106

[15] Li H J, Wang X M, Song F J, et al. Response to powdery mildew and detection of resistance genes in wheat cultivars from China[J]. *Acta Agronomica Sinica*, 2011, 37(6): 943-954

[16] Sheng B X. Score wheat seedlings resistance to powdery mildew by infection type[J]. *Plant Protection*, 1988, 14(1): 49

[17] He J B. *Plant Disease Resistance Genetics*[M]. Beijing: China Agriculture Press, 1994

[18] Cao Y P. Origin, evolution and genetic resources of wheat in China[J]. *Journal of Wheat Research*, 2008, 29(3): 1-10

[19] Dai J L, Niu Y C. Inheritance of stripe rust resistance in the native wheat variety dazicao from China[J]. *Hereditas*, 2003, 25(3): 311-313

[20] Wang J C, Feng J, Wang F T, et al. Genetic analysis on the adult plant resistance of a wheat landrace 'Xiaohongmang' to *Puccinia striiformis*[J]. *Plant Protection*, 2015, 41(1): 154-157

[21] Zhai W W, Duan X Y, Zhou Y L, et al. Inheritance of resistance to powdery mildew in four Chinese landraces[J]. *Plant Protection*, 2008, 34(1): 37-40

[22] Rong J K, Millet E, Manisterski J, et al. A new powdery mildew resistance gene: Introgression from wild emmer into common wheat and RFLP-based mapping[J]. *Euphytica*, 2000, 115(2): 121-126

[23] Xu H X, Yao G Q, Xiong L, et al. Identification and mapping of *pm2026*: A recessive powdery mildew resistance gene in an einkorn (*Triticum monococcum* L.) accession[J]. *Theoretical and Applied Genetics*, 2008, 117(4): 471-

[24] Li G Q, Fang T L, Zhang H T, et al. Molecular identification of a new powdery mildew resistance gene *Pm41* on chromosome 3BL derived from wild emmer (*Triticum turgidum* var. *dicoccoides*)[J]. *Theoretical and Applied Genetics*, 2009, 119(3): 531-539

[25] Nematollahi G, Mohler V, Wenzel G, et al. Microsatellite mapping of powdery mildew resistance allele *Pm5d* from common wheat line IGV1-455[J]. *Euphytica*, 2008, 159(3): 307-313

[26] Hua W, Liu Z J, Zhu J, et al. Identification and genetic mapping of *pm42*, a new recessive wheat powdery mildew resistance gene derived from wild emmer (*Triticum turgidum* var. *dicoccoides*)[J]. *Theoretical and Applied Genetics*, 2009, 119(2): 223-230

Note: Figure translations are in progress. See original paper for figures.