Genetic, Physical and Comparative Mapping of the Powdery Mildew Resistance Gene Pm21 Originating from Dasypyrum villosum

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Pm21, originating from wheat wild relative Dasypyrum villosum, confers immunity to all known races of Blumeria graminis f. sp. tritici (Bgt) and has been widely utilized in wheat breeding. However, little is known on the genetic basis of the Pm21 locus. In the present study, four seedling-susceptible D. villosum lines (DvSus-1 ∼ DvSus-4) were identified from different natural populations. Based on the collinearity among genomes of Brachypodium distachyon, Oryza, and Triticeae, a set of 25 gene-derived markers were developed declaring the polymorphisms between DvRes-1 carrying Pm21 and DvSus-1. Fine genetic mapping of Pm21 was conducted by using an extremely large F2 segregation population derived from the cross DvSus-1/DvRes-1. Then Pm21 was narrowed to a 0.01-cM genetic interval defined by the markers 6VS-08.4b and 6VS-10b. Three DNA markers, including a resistance gene analog marker, were confirmed to co-segregate with Pm21. Moreover, based on the susceptible deletion line Y18-S6 induced by ethyl methanesulfonate treatment conducted on Yangmai 18, Pm21 was physically mapped into a similar interval. Comparative analysis revealed that the orthologous regions of the interval carrying Pm21 were narrowed to a 112.5 kb genomic region harboring 18 genes in Brachypodium, and a 23.2 kb region harboring two genes in rice, respectively. This study provides a high-density integrated map of the Pm21 locus, which will contribute to map-based cloning of Pm21.

Keywords: Dasypyrum villosum, Pm21, powdery mildew resistance, genetic mapping, physical mapping, comparative mapping

INTRODUCTION

Common wheat (Triticum aestivum L.) is the most widely grown cereal crop occupying ∼17% of all cultivated land of the world and provides ∼20% of the calories consumed by humankind (Fu et al., 2009). However, wheat production is seriously threatened by various diseases, such as head scab, rusts, and powdery mildew. Wheat powdery mildew caused by the obligate biotrophic fungal pathogen Blumeria graminis f. sp. tritici (Bgt) is one of the most important factors leading to yield losses. Development of resistant varieties using powdery mildew resistance (Pm) genes is an effective, economical and environmental-friendly way to reduce yield losses caused by Bgt. Up to now, 58 resistant genes have been formally designated (Pm1 ∼ Pm58). Among them, some Pm genes were identified from the species in the tertiary gene pool, including Pm7,
**Genetic, Physical and Comparative Mapping of Thinopyrum intermedium**

**D. villosum**

A collection of 110 accessions of *D. villosum* were kindly provided by Germplasm Resources Information Network (GRIN) (51), GRIN Czech (16), Genebank Information System of the IPK Gatersleben (GBIS-IPK) (35), Nordic Genetic Resource Center (NordGen) (7), and the Cytogenetics Institute, Nanjing Agricultural University (CI-NAU) (1). The susceptible *D. villosum* line DvSus-1 was crossed with the resistant line DvRes-1 carrying *Pm21* (Table 1), and the generated *F₂* population containing 10,536 individuals was used for genetic analysis. The powdery mildew resistant wheat variety Yangmai 18, carrying a pair of translocated chromosomes T6AL.6VS, and the susceptible variety Yangmai 9 were both developed in Yangzhou Academy of Agricultural Sciences (YAAS). About 2,000 seeds of Yangmai 18 were treated with 0.8% ethyl methanesulfonate (EMS), and 1,216 *M₂* families were generated to use for screening of mutants susceptible to powdery mildew.

### Evaluation of Powdery Mildew Resistance

The *D. villosum* accessions, *F₁* and *F₂* individuals of the cross DvSus-1/DvRes-1, and *M₂* individuals of EMS-induced Yangmai 18 at one-leaf stage were inoculated with *Bgt* isolate YZ01, a predominant race collected from Yangzhou (He et al., 2016), by dusting from sporulating susceptible variety Yangmai 9 and powdery mildew responses were assessed at 7 days.

### Materials and Methods

**Plant Materials**

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post-inoculation in greenhouse. Due to that \textit{Pm21} confers immunity to \textit{Bgt} isolate YZ01, powdery mildew responses can be simply divided into two types, resistant and susceptible.

**Development of DNA Markers**

Polymorphic DNA markers between the resistant line DvRes-1 and the susceptible line DvSus-1 were developed using the CISP-IS (conserved intron scanning primer combined with intron sequencing) strategy based on the collinearity relationship between \textit{Brachypodium}, rice and \textit{Triticaceae} species (He et al., 2013). The common genes between \textit{Brachypodium} and rice were used to search their homologous full-length cDNAs or ESTs of wheat, barley, \textit{Aegilops} or other \textit{Triticaceae} crops deposited in GenBank database. After alignment of these sequences by the Clustalw tool, primers were designed according to the conserved exon sequences and used to amplify the corresponding intronic regions of the parents DvRes-1 and DvSus-1. After T/A cloning and sequencing of the target fragments, the flanking conserved sequences of the variant introns were further used to design primers and screen polymorphic markers. DNA markers used for physical mapping were also developed using the same strategy. The details of polymorphic markers used in this study were listed in Supplementary Tables S1, S2.

**Genetic and Deletion Mapping of \textit{Pm21}**

Genetic analysis of \textit{Pm21} was performed on an F\textsubscript{2} population derived from the cross between the resistant line DvRes-1 carrying \textit{Pm21} and the seedling-susceptible line DvSus-1 of \textit{D. villosum}. Chi-squared ($\chi^2$) test was used to determine the goodness-of-fit of the observed segregation ratio to theoretical Mendelian ratio. Chromosomal deletion analysis was carried out using the susceptible mutant line Y18-S6 obtained from an EMS-induced Yangmai 18 population.

**Comparative Genomics Analysis**

The genome sequences of \textit{Brachypodium}, rice and wheat were obtained from the \textit{Brachypodium distachyon} genome assemblies v2.0\textsuperscript{1}, the rice genome pseudomolecule release 7\textsuperscript{2}, and the IWGSC Sequence Repository\textsuperscript{3}, respectively. Genes were predicted using the FGENESH tool\textsuperscript{4} and re-annotated using the BLAST program\textsuperscript{5} and the SMART program\textsuperscript{6}.

**RESULTS**

**Reactions of \textit{D. villosum} Accessions to \textit{Bgt} Isolate YZ01**

A total of 110 \textit{D. villosum} accessions were collected and inoculated with \textit{Bgt} YZ01 at one-leaf stage. Fortunately,
susceptible individuals were identified from four different accessions, and all the other accessions were immune. The susceptible lines obtained were designated as DvSus-1, DvSus-2, DvSus-3, and DvSus-4, respectively (Table 1). At different growth stages, powdery mildew responses of the four susceptible D. villosum lines were further investigated. All individuals were susceptible at one-leaf stage, but interestingly, an unknown complex resistance gradually increased at two-leaf stage, and fully expressed from three-leaf stage to adult stage (Figures 1A–C).

**Development of Polymorphic Markers at Different Ploidy Levels**

To develop polymorphic markers between the resistant line DvRes-1 and the susceptible line DvSus-1, a total of 54 susceptible individuals were identified from four different accessions, and all the other accessions were immune. The susceptible lines obtained were designated as DvSus-1, DvSus-2, DvSus-3, and DvSus-4, respectively (Table 1). At different growth stages, powdery mildew responses of the four susceptible D. villosum lines were further investigated. All individuals were susceptible at one-leaf stage, but interestingly, an unknown complex resistance gradually increased at two-leaf stage, and fully expressed from three-leaf stage to adult stage (Figures 1A–C).

**Development of Polymorphic Markers at Different Ploidy Levels**

To develop polymorphic markers between the resistant line DvRes-1 and the susceptible line DvSus-1, a total of 54
6VS-specific markers reported previously (Qi et al., 2010; Chen et al., 2013; He et al., 2016) were tested. Among them, however, only four markers, Xcfe164, 6VS-11, 6VS-25, and 6VS-30, showed polymorphisms between DvRes-1 and DvSus-1. To get more markers for mapping Pm21, the CISP-IS strategy based on comparative genomics were applied and 21 polymorphic markers were further developed (Supplementary Table S1), among which, six were single nucleotide polymorphism (SNP) markers. Taken together, a total of 25 markers were recruited for genetic mapping of Pm21 in diploid D. villosum. Using the CISP-IS strategy, another seven 6VS-specific markers were newly developed here and used for physical mapping of Pm21 in hexaploid wheat (Supplementary Table S2).

**Fine Genetic Mapping of Pm21**

For genetic mapping of Pm21 gene, a total of 10,536 F₂ individuals derived from the cross between the resistant DvRes-1 and the susceptible DvSus-1 were evaluated for their resistance to powdery mildew at one-leaf stage. The result showed that 8,147 were resistant while 2,389 were susceptible, resulting in a resistance-to-susceptible ratio as 3.41:1 that is significantly higher than 3:1, the theoretical Mendelian segregation ratio ($\chi^2 = 30.500$, $P < 0.01$). Genotyping of F₂ individuals using the co-segregated marker 6VS-09.4b indicated that the ratio of 2,492 resistant homozygotes, 5,655 heterozygotes, and 2,389 susceptible homozygotes is 1.04:2.37:1, not in accordance with a 1:2:1 ratio ($\chi^2 = 59.066$, $P < 0.01$).

To accelerate screening for recombinants, a double-PCR system was conducted using two markers 6VS-00.1 and Xcfe164, respectively, located on the distal and paracentric regions of 6VS (Figure 2). Sixty-four recombinants were identified from the F₂ population, indicating that the total genetic distance between the two markers was approximately 0.30 cM. Subsequently, a set of 25 markers were used to genotyping these recombinants. In combination with powdery mildew resistance evaluation, Pm21 was finely mapped into a 0.01-cM interval defined by the markers 6VS-08.4b and 6VS-10b (Figure 3A). Furthermore, three genic markers (6VS-08.8b, 6VS-09b and 6VS-09.4b) were found to co-segregate with Pm21 (Figure 4).

**Physical Mapping of Pm21**

From 1,216 EMS-induced M₂ families of Yangmai 18 carrying Pm21, a total of 12 independent mutant lines highly susceptible to powdery mildew were screened out (Y18-S1 ~ Y18-S12) (Figure 1D). Among them, the susceptible mutant line Y18-S6
was characterized as a chromosomal deletion involving the Pm21 locus. Molecular analysis revealed the chromosomal breakpoints b1 and b2 in Y18-S6 were closely flanked by the markers 6VS-03 and 6VS-04, 6VS-10 and 6VS-10.2, respectively (Figures 3B, 5). Interestingly, the breakpoint b1 in Y18-S6 was close to that in the deletion line del.6VS-1 (FL0.58) reported previously (He et al., 2016). Physical mapping also proved that the deleted chromosomal segment in Y18-S6 spanned the genetic interval carrying Pm21.

Comparative Mapping of Pm21

The orthologs of the corresponding genes of the flanking markers 6VS-08.4b and 6VS-10b were Bradi3g03840 (2574344–2579921) and Bradi3g03970 (2692381–2693197) in Brachypodium, LOC_Os02g05610 (2728848–2734454) and LOC_Os02g05640 (2757693–2758714) in rice, respectively. Hence, the orthologous regions of the Pm21 locus were narrowed to a 112.5 kb genomic region harboring 18 predicted genes in Brachypodium, and a 23.2 kb region harboring two predicted genes in rice. In the orthologous regions, two genes were shared by Brachypodium and rice (Figure 6 and Table 2) and used to develop the DNA markers 6VS-08.8b and 6VS-09b. Both of the markers were tested to co-segregate with Pm21 in the F2 population.

Comparative analysis also revealed a conserved resistance gene analog (RGA) locus between Brachypodium and wheat orthologous regions (Figure 6). In Brachypodium, four highly homologous RGAs, Bradi3g03874, Bradi3g03878, Bradi3g03882,
Table 2: Gene annotation in Brachypodium, rice and wheat orthologous regions of the Pm21 locus.

| Brachypodium | Rice | Wheat | Gene annotation |
|--------------|------|-------|-----------------|
| Bradi3g03845 | 6AS_contigs_4399884 | Retrotransposable element protein |
| Bradi3g03850 | 6BS_contigs_2953799 | Eukaryotic translation initiation factor |
| Bradi3g03860 | 6AS_contigs_4431592 | Exocyst complex subunit |
| | 6BS_contigs_2953283 | EXO70-like protein |
| Bradi3g03870 | 6AS_contigs_4383243 | Serine/threonine protein phosphatase 2C |
| | 6BS_contigs_2962596 | |
| | 6DS_contigs_2093935 | |
| Bradi3g03874a | 6AS_contigs_4428294 | Disease resistance protein |
| Bradi3g03878a | 6BS_contigs_2926507 | Photosystem II protein J |
| Bradi3g03882a | 6DS_contigs_2114667 | |
| Bradi3g03886 | Unknown protein |
| Bradi3g03890 | Unknown protein |
| Bradi3g03900 | Unknown protein |
| Bradi3g03910 | Cytochrome P450 |
| Bradi3g03920 | Unknown protein |
| Bradi3g03930 | Poly(A) polymerase |
| Bradi3g03935a | Six contigs6 | Disease resistance protein |
| Bradi3g03940 | 6AS_contigs_4428294 | Photosystem II |
| | 6BS_contigs_2926507 | cytochrome b559 alpha subunit |
| Bradi3g03945 | 6AS_contigs_4428294 | Unknown protein |
| | 6BS_contigs_2926507 | Polyubiquitin |
| Bradi3g03950 | 6AS_contigs_4431958 | |
| | 6BS_contigs_2953283 | |
| Bradi3g03957 | 6DS_contigs_2081863 | |

Four RGAs (Bradi3g03874, Bradi3g03878, Bradi3g03882, and Bradi3g03935) in Brachypodium are highly homologous with six contigs (6AS_contigs_4399884, 6AS_contigs_4431592, 6BS_contigs_2953283, 6BS_contigs_2953799, 6DS_contigs_2111757, and 6DS_contigs_2179378) on the short arms of wheat homologous group 6.

and Bradi3g03935, were identified. Among them, Bradi3g03874, Bradi3g03878, and Bradi3g03882 were tandemly aligned as a cluster, and Bradi3g03935 were segregated by other six genes. In wheat, the orthologous RGA loci were also located in the contigs on 6AS, 6BS, and 6DS, and each locus harbored three RGAs at least. According to the conserved locus, the RGA markers 6VS-09.4 and 6VS-09.4b was developed. Physical mapping showed that 6VS-09.4 was lost in the susceptible deletion line Y18-S6, whereas genetic analysis demonstrated that 6VS-09.4b co-segregated with Pm21 in the F2 population.

Discussion

The powdery mildew resistance gene Pm21 was previously mapped to the physical bin FL0.45–0.58 of the chromosome 6VS (Cao et al., 2011; Chen et al., 2013). In our previous study, the orthologous regions of the bin FL0.45–0.58 in Brachypodium and rice were comparatively mapped (He et al., 2016). In the present study, we carried out physical mapping of Pm21 by using the susceptible deletion line Y18-S6 obtained from the Yangmai 18 population induced by EMS. We also successfully performed genetic mapping of Pm21 in diploid D. villosum, which was mainly attributed to the finding of D. villosum lines seedling-susceptible to wheat powdery mildew. Qi et al. (1998) observed that wheat-D. villosum disomic addition line DA6V#1 (Sears, 1953) was susceptible to powdery mildew. However, due to the utilization of colchicine in the incorporation of alien genome by Sears, it is difficult to explain whether the variation came from colchicine treatment or natural variation of the alien parent D. villosum. Nevertheless, it remains the possibility to mine susceptible D. villosum from natural populations. Thereafter, Qi et al. (1998) screened 46 D. villosum accessions but none was susceptible. We also did not find any D. villosum accession susceptible to powdery mildew through the whole growth period. In this study, all the four susceptible D. villosum lines displayed complex resistance to wheat powdery mildew, which fully expressed since...
resistance of *D. villosum* comes from multi-gene effects, in which, the gene on 6VS carry out an immunologic mechanism different from the others, such as *Pm5S*, an adult plant resistance (APR) gene newly found (Zhang et al., 2016). Therefore, to eliminate the background noise, we have to evaluate the *Pm21*-mediated resistance reaction at a very early stage (one-leaf stage). Furthermore, because *Pm21* is immune to all Bgt races (Chen et al., 1995), problems probably resulted from race-specific resistance conferred by other genes in background should be avoided in resistance evaluation. Hence, the isolate *B. graminis* av. *avenae* HC is suggested that these genes play roles in complex resistance pathways but none of them is the *Pm21* gene itself. It’s now known to us that powdery mildew *Pm21* varieties containing *Pm21* or *PmV* (Li et al., 2005; Zeng et al., 2005) were immune. Additionally, *D. villosum* is a cross-pollinated species, which is adverse to forming homozygote of the recessive mutant gene (Hartfield and Glémín, 2014). So, the susceptible homozygotes account for a very small proportion naturally (2 ~ 5%) as our observation. As a result, susceptible *D. villosum* is difficult to obtain. But fortunately, in this study, we successfully found susceptible individuals from 4 of 110 *D. villosum* accessions.

Based on the discovery of susceptible *D. villosum* resources, genetic mapping of *Pm21* was fulfilled. By double-PCR program, 64 recombinants were identified from 10,536 F2 plants, indicating that the total genetic distance of the interval flanked by the markers 6VS-00.1 and Xcef164 was only about 0.30 cM. It suggested that there exists recombination suppression between two 6VS chromosomes with different origins. The observation given by Qi et al. (1998) also showed that, in wheat background, the two alien 6V chromosomes could not pair normally in pollen mother cells of hybrids between the resistant DA6V#2 (Hyde, 1953) and the susceptible DA6V#1 (Sears, 1953). It is not clear whether recombination suppression observed here is related to low pairing of two different 6V chromosomes in *D. villosum*. In addition, the ratio of resistant and susceptible homozygotes (1.04:1) in the F2 population was fit for the ratio 1:1 ($\chi^2 = 2.174$, $P > 0.01$), suggesting that the male and female gametes have no obvious difference in viability and transmission. However, unexpectedly, the ratio of heterozygotes (53.6%) was higher than the theoretic ratio (50%), indicating that heterozygotes could survive more easily, which might be due to the heterosis in cross-pollinated *D. villosum*.

In the previous studies, eight genes, including *Stpk-V, DvMPK1, DvMLPK, DvUPK, DvPSYR1, DvPP2C, DvGATA*, and *DvWHY*, have been mapped to the bin FL0.45–0.58 carrying *Pm21* and confirmed to be required by the *Pm21* resistance. However, silencing of these genes mediated by barley stripe mosaic virus (BSMV) could not lead to macroscopic symptom of powdery mildew (Cao et al., 2011; He et al., 2016). It is suggested that these genes play roles in complex *Pm21* resistance pathways but none of them is the *Pm21* gene itself. Interestingly, overexpression of *Stpk-V* can confer high resistance to powdery mildew in transgenic wheat (Cao et al., 2011). Hence, these genes might have potential to be used to transgenic breeding for control of wheat powdery mildew. Furthermore, revealing their functions and mechanisms could contribute to understanding disease resistance pathways mediated by *Pm21*. Among the above genes, only *DvPP2C* (6VS-09b), encoding a serine/threonine protein phosphatase 2C, was confirmed to be located in the genetic interval carrying *Pm21* by physical, genetic and comparative analyses in this study. In addition, another gene *DvEXO70* (the corresponding markers 6VS-08.8 and 6VS-08.8b), encoding an exocyst complex subunit EXO70-like protein, appears in the *Pm21* involved interval; however, whether it plays a role in the *Pm21* resistance remains unclear.

Although disease resistance genes evolve rapidly and have limited collinearity in cereals (Keller et al., 2005), a conserved RGA locus was observed in *Brachypodium* and wheat orthologous regions of the genetic interval of *Pm21*. The members in this RGA locus encode typical coiled-coil, nucleotide-binding site, leucine-rich repeat (CC-NBS-LRR) proteins that are the major class of plant disease resistance proteins (Gururani et al., 2012). Both physical mapping and genetic mapping demonstrated that the corresponding RGA markers 6VS-09.4 and 6VS-09.4b were located in the *Pm21* locus. It suggested that this co-regulated RGA locus could be considered as an important candidate of the *Pm21* locus. However, due to large genome size, it would be still a great challenge to directly perform map-based cloning of *Pm21* in *D. villosum*. Recently, a fast cloning method using mutagenesis and next-generation sequencing (NGS) has been successfully utilized to identify the stem rust resistance genes Sr22 and Sr45 (Steuerang et al., 2016) and the powdery mildew resistance gene *Pm2* (Sánchez-Martín et al., 2016) in wheat. In this study, 11 independent susceptible wheat mutants, not involving in chromosomal deletion, could be used to find the exact sequence of *Pm21*, and further researches are in progress.

### AUTHOR CONTRIBUTIONS

HH and TB conceived and designed the experiments. HH screened the resource of *D. villosum*. YJ and SZ developed DNA markers. YJ, BL, RZ, and ZJ performed genetic mapping. HH and TB performed physical mapping. HH and SZ analyzed the data and wrote the paper. HH and TB revised the paper.

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**REFERENCES**

Bie, T., Zhao, R., Jiang, Z., Gao, D., Zhang, B., and He, H. (2015). Efficient marker-assisted screening of structural changes involving *Haynaldia villoosa* chromosome 6V using a double-distal-marker strategy. *Mol. Breed.* 35, 34. doi: 10.1007/s11032-015-0211-y

Cao, A., Xing, L., Wang, X., Yang, X., Wang, W., Sun, Y., et al. (2011). Serine/threonine kinase gene Spk-V, a key member of powdery mildew resistance gene Pm21, confers powdery mildew resistance in wheat. *Proc. Natl. Acad. Sci. U.S.A.* 108, 7727–7732. doi: 10.1073/pnas.1016981108

Chen, P. D., Qi, L. L., Zhou, B., Zhang, S. Z., and Liu, D. J. (1995). Development of *Dasypyrum villosum* chromosome 6V-specific DNA markers using a CISP-IS strategy. *Plant Breed.* 132, 290–294. doi: 10.1111/pbr.12035

Hyde, B. B. (1932). Development of individual *Haynaldia villoosa* chromosomes to hexaploid wheat. *Am. J. Bot.* 40, 174–182. doi: 10.2307/2438775

He et al. Genetic, Physical and Comparative Mapping of Pm21. The Supplementary Material for this article can be found online at: https://www.frontiersin.org/articles/10.3389/fpls.2017.01914/full#supplementary-material

McIntosh, R. A., Dubcovsky, J., Rogers, J., Morris, C., Appels, R., and Xia, X. C. (2013). *Catalogue of Gene Symbols for Wheat: Komugi Integrated Wheat Science Database*. Available at: http://www.shigen.nig.ac.jp/wheat/komugi/genes/download.jsp

Murray, M. G., and Thompson, Y. F. (1980). Rapid isolation of high molecular weight plant DNA. *Nucleic Acids Res.* 8, 4321–4325. doi: 10.1093/nar/8.19.4321

Qi, L. L., Wang, S. L., Chen, P. D., Liu, D. J., and Gill, B. S. (1998). Identification and physical mapping of three *Haynaldia villoosa* chromosome-6V deletion lines. *Theor. Appl. Genet.* 97, 1042–1046. doi: 10.1007/s001220050989

Chen, C., You, C., Hu, Y., Chen, S., Zhou, B., Cao, A., et al. (2013). Radiation-induced translocations with reduced *Haynaldia villoosa* chromatin at the Pm21 locus for powdery mildew resistance in wheat. *Mol. Breed.* 31, 477–484. doi: 10.1007/s11032-012-9804-x

Chen, P. D., Qi, L. L., Zhou, B., Zhang, S. Z., and Liu, D. J. (1995). Development and molecular cytogenetic analysis of wheat-*Haynaldia villoosa* 6V/6AL translocation lines specifying resistance to powdery mildew. *Theor. Appl. Genet.* 91, 1125–1128. doi: 10.1007/BF00223930

Fu, D., Uauy, C., Distelfeld, A., Blechl, A., Epstein, L., Chen, X., et al. (2009). A kinase-START gene confers temperature-dependent resistance to wheat stripe rust. *Science* 323, 1357–1360. doi: 10.1126/science.1166289

Gururani, M. A., Venkatesh, J., Upadhyaya, C. P., Nookaraju, A., Pandey, S. K., and Park, S. W. (2012). Plant disease resistance genes: current status and future directions. *Physiol. Mol. Plant Pathol.* 81, 93–100. doi: 10.1016/j.pmpp.2012.01.002

Hartfield, M., and Glémin, S. (2014). Hitchhiking of deleterious alleles and the cost of adaptation in partially selfing species. *Theor. Appl. Genet.* 128, 853–863. doi: 10.1007/s00122-016-2753-8

He, H., Zhu, S., Sun, W., Gao, D., and Bie, T. (2013). Efficient development of *Haynaldia villoosa* chromosome 6V-specific DNA markers using a CISP-IS strategy. *Theor. Appl. Genet.* 129, 819–829. doi: 10.1007/s00122-016-2668-4

Hyde, B. B. (1933). Addition of individual *Haynaldia villoosa* chromosomes to hexaploid wheat. *Am. J. Bot.* 40, 174–182. doi: 10.2307/2438775

Keller, B., Feuillet, C., and Yahiaoui, N. (2005). Map-based isolation of disease resistance genes from bread wheat: cloning in a supersize genome. *Genet. Res.* 85, 93–100. doi: 10.1017/S0016672305007391

Li, H., Chen, X., Xin, Z., Ma, Y., Xu, H., Chen, X., et al. (2005). Development and identification of wheat-*Haynaldia villoosa* Td6DL6V chromosome translocation lines conferring resistance to powdery mildew. *Plant Breed.* 124, 203–205. doi: 10.1111/j.1439-0523.2004.01062.x

Sánchez-Martín, J., Steuernagel, B., Ghosh, S., Herren, G., Hurni, S., Adamski, N., et al. (2016). Rapid gene isolation in barley and wheat by mutant chromosome sequencing. *Genome Biol.* 17, 221. doi: 10.1186/s13059-016-1082-1

Qi, X. L., Cui, F., Yu, L., Ding, A. M., Li, J., Chen, G. L., et al. (2010). Molecular tagging wheat powdery mildew resistance gene Pm21 by EST-SSR and STS markers. *Mol. Plant Breed.* 1, 22–26. doi: 10.5376/mbp.2010.01.0004

He et al. Genetic, Physical and Comparative Mapping of Pm21. The Supplementary Material for this article can be found online at: https://www.frontiersin.org/articles/10.3389/fpls.2017.01914/full#supplementary-material

Sears, E. R. (1953). Addition of the genome of *Haynaldia villoosa* to *Triticum aestivum*. *Am. J. Bot.* 40, 168–174. doi: 10.2307/2436774

Steuernagel, B., Peniyannan, S. K., Hernández-Pinzón, I., Witek, K., Rouse, M. N., Yu, G., et al. (2016). Rapid cloning of disease-resistance genes in plants using mutagenesis and sequence capture. *Nat. Biotechnol.* 34, 652–655. doi: 10.1038/nbt.3543

Wiersma, A. T., Pulman, J. A., Brown, L. K., Cowger, C., and Olson, E. L. (2017). Identification of Pm58 from *Aegilops tauschii*. *Theor. Appl. Genet.* 130, 1123–1133. doi: 10.1007/s00122-017-2874-8

Zeng, X. Y., Zhang, Z. Y., Du, L. P., Xin, Z. Y., and Chen, X. (2005). Development of wheat germplasms with multi-resistance to powdery mildew, stripe rust and yellow dwarf virus by molecular marker-assisted selection (in Chinese). *Sci. Agric.* 38, 2380–2386.

Zhang, R., Sun, B., Chen, J., Cao, A., Xing, L., Feng, Y., et al. (2016). Pm55, a developmental-stage and tissue-specific powdery mildew resistance gene introgressed from *Dasypyrum villosum* into common wheat. *Theor. Appl. Genet.* 129, 1975–1984. doi: 10.1007/s00122-016-2753-8

**Conflict of Interest Statement:** The authors declare that the research was conducted in the absence of any commercial or financial relationships that could be construed as a potential conflict of interest.

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