An Avirulence Gene Cluster in the Wheat Stripe Rust Pathogen (Puccinia striiformis f. sp. tritici) Identified through Genetic Mapping and Whole-Genome Sequencing of a Sexual Population

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ABSTRACT Puccinia striiformis f. sp. tritici, the causal agent of wheat stripe (yellow) rust, is an obligate, biotrophic fungus. It was difficult to study the genetics of the pathogen due to the lack of sexual reproduction. The recent discovery of alternate hosts for P. striiformis f. sp. tritici makes it possible to study inheritance and map genes involved in its interaction with plant hosts. To identify avirulence (Avr) genes in P. striiformis f. sp. tritici, we developed a segregating population by selfing isolate 12-368 on barberry (Berberis vulgaris) plants under controlled conditions. The dikaryotic sexual population segregated for avirulent/virulent phenotypes on nine Yr single-gene lines. The parental and progeny isolates were whole-genome sequenced at ∼30× coverage using Illumina HiSeq PE150 technology. A total of 2,637 high-quality markers were discovered by mapping the whole-genome sequencing (WGS) reads to the reference genome of strain 93-210 and used to construct a genetic map, consisting of 41 linkage groups, spanning 7,715.0 centimorgans (cM) and covering 68 Mb of the reference genome. The recombination rate was estimated to be 1.81 ± 2.32 cM/10 kb. Quantitative trait locus analysis mapped six Avr gene loci to the genetic map, including an Avr cluster harboring four Avr genes, AvYr7, AvYr43, AvYr44, and AvYrExp2. Aligning the genetic map to the reference genome identified Avr candidates and narrowed them to a small genomic region (~200 kb). The discovery of the Avr gene cluster is useful for understanding pathogen evolution, and the identification of candidate genes is an important step toward cloning Avr genes for studying molecular mechanisms of pathogen-host interactions.

IMPORTANCE Stripe rust is a destructive disease of wheat worldwide. Growing resistant cultivars is the most effective, easy-to-use, economical, and environmentally friendly strategy for the control of the disease. However, P. striiformis f. sp. tritici can produce new virulent races that may circumvent race-specific resistance. Therefore, understanding the genetic basis of the interactions between wheat genes for resistance and P. striiformis f. sp. tritici genes for avirulence is useful for improving cultivar resistance for more effective control of the disease. This study developed a high-quality map that facilitates genomic and genetic studies of important traits related to pathogen pathogenicity and adaptation to different environments and crop cultivars carrying different resistance genes. The information on avirulence/virulence genes identified in this study can be used for guiding breeding programs to select

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combinations of genes for developing new cultivars with effective resistance to mitigate this devastating disease.

**KEYWORDS** avirulence, genetics, host-pathogen interaction, *Puccinia striiformis*, QTL mapping, wheat stripe rust, whole-genome sequencing

Rust diseases, caused by fungi in the order *Pucciniales*, are a large threat to food security and impact ecosystems (1). Economically important rusts include leaf rust in coffee (2), soybean rust (3), crown rust in oat (4), flax rust (5), poplar leaf rust (6), and wheat rusts (7). Numerous epidemics of these rusts have been recorded in diverse agriculture systems and in many countries. Extensive efforts have been made to incorporate resistance genes into cultivars to protect plants from attacks of rust pathogens. However, rapidly evolving rust fungi are highly capable of evading plant immunity systems, resulting in ineffective host resistance. To avoid resistance failure and elongate the effectiveness of resistance genes, understanding the molecular mechanisms underlying host-pathogen interactions is essential.

Plant-pathogen interactions were initially studied by Harold Flor and explained by his gene-for-gene concept (8). In this concept, host defense responses are activated by the recognition of a pathogen avirulence (*Avr*) gene by a cognate resistance (*R*) gene in the host. This concept has been supported by the fact that many *Avr* genes from bacteria, oomycetes, and fungi and *R* genes from different plant hosts have been cloned, and direct or indirect interactions between the products of some of the cloned *R* and *Avr* genes have been demonstrated (9). Such pioneering works have considerably increased our understanding of host-pathogen interactions. While *Avr* gene recognition is often referred to as effector-triggered immunity (ETI), pathogen-associated molecular pattern (PAMP)-triggered immunity (PTI) is generally thought of as a different type of resistance (10). PTI is activated by the recognition of conserved pathogen PAMPs such as chitin in the fungal cell wall. Pathogen effectors encoded by pathogenicity genes then suppress host PTI, enabling the pathogen to infect and cause disease. During coevolution, host plants gain *R* genes to detect evading pathogens. The recognition of *Avr* proteins by *R* proteins leads to ETI. As a coevolutionary arms race, a pathogen avoids the perception of a host *R* protein by mutating its *Avr* genes or developing new *Avr* genes to overcome or suppress host ETI.

Classical approaches for cloning *Avr* genes and effectors include reverse-genetics and mapping-based positional methods. The reverse-genetics approach has been used to successfully clone many *Avr* genes in different pathosystems, including *Cladosporium fulvum*-tomato, from which the first fungal *Avr* gene, *avr9*, was identified and cloned (11, 12). Even though many effectors from oomycetes and fungi have been cloned using this approach, the reverse-genetics approach for *Avr* identification is not suitable for wheat-*Puccinia* pathosystems because the techniques usually used in reverse genetics, such as transformation, effector delivery systems, and RNA interference, are still not mature for rust fungi (13).

An alternate approach for cloning *Avr* genes in plant pathogens is based on genetic mapping. Briefly, in the genetic mapping-based approach, an *Avr* locus is genetically mapped along with molecular markers; next, the genomic interval between two flanking markers is completely sequenced; and finally, the *Avr* gene is identified, followed by functional validation. The efficiency of this approach has been well demonstrated in cloning avirulence genes in the ascomycete fungus *Leptosphaeria maculans*, the causal agent of stem canker/black leg of oilseed rape and canola (14–18). Recently, this approach has been complemented by comparative genomics approaches, which has significantly accelerated the identification of *Avr* genes in *L. maculans* (19, 20). The mapping-based cloning approach has also been successful in basidiomycetes. In fact, this approach was successfully used to clone *UhAvr1* from the barley smut pathogen, *Ustilago hordei*, the first avirulence gene cloned from basidiomycete fungi (21, 22). Particularly in *Melampsora lini*, the flax rust fungus, which is also in the order *Pucciniales* containing cereal rust pathogens, several *Avr* genes that follow
the gene-for-gene relationship with flax resistance genes have been genetically mapped since the work of Flor and cloned using genome sequencing technology (23, 24). More recently, two Avr genes (AvrSr35 and AvrSr50) in *Puccinia graminis* f. sp. *tritici*, the causal agent of stem rust of wheat and barley, were cloned. AvrSr35 was identified through whole-genome sequencing (WGS) and comparison of chemically mutagenized mutants with a natural isolate (25), while AvrSr50, located in a loss-of-heterozygosity region, was identified by analyzing the genome variation and gene expression of spontaneous mutants (26).

Among the rust fungi, *Puccinia striiformis* Westend. f. sp. *tritici* Erikk. causes wheat stripe (yellow) rust and is recognized as one of the most serious plant pathogens threatening global food security (27–30). *P. striiformis* f. sp. *tritici* is a macrocyclic, heteroecious fungus having five spore stages in its complete life cycle. Its urediniospores (n + n) are produced on and can reinfect primary hosts (wheat and grasses), on which teliospores (2n) are usually produced in the late crop season. Teliospores germinate to produce basidiospores (n) after meiosis, and basidiospores infect alternate hosts (*Berberis* spp. and *Mahonia* spp.), on which pycniospores (n) are produced, and fertilize receptive hyphae (n) to produce aeciospores (n + n). Aeciospores infect the primary host and produce urediniospores (31–33). Although economically important, avirulence genes have not been molecularly identified and characterized in *P. striiformis* f. sp. *tritici*. Cloning of Avr genes has not been conducted in *P. striiformis* f. sp. *tritici*, and the potential of a genetic mapping-based approach has been impeded, mainly due to the unknown alternate hosts of *P. striiformis* f. sp. *tritici* until the recent discovery of its alternate hosts in the genera *Berberis* and *Mahonia* (31–33). Since then, sexual populations of *P. striiformis* f. sp. *tritici* have been developed, and genetic studies of the inheritance of virulence phenotypes have been conducted (34–36). However, no Avr genes could be precisely defined due to the limited number of codominant molecular markers and the fragmented reference genomes. To conquer these limitations, we developed a segregating population through self-fertilizing a *P. striiformis* f. sp. *tritici* isolate on barberry and developed a high-density genetic map consisting of a large number of genome-wide molecular markers by whole-genome sequencing of the progeny population using next-generation sequencing technology. We mapped six Avr genes, including four in a gene cluster. Comparison of the high-density map regions with a reference genome of the pathogen enabled us to identify Avr candidates in narrow genome regions. The results set the basis for cloning the Avr genes for understanding the molecular mechanisms underlying rapid virulence changes in the wheat stripe rust fungus.

**RESULTS**

**Virulence phenotyping.** The *P. striiformis* f. sp. *tritici* isolate 12-368 was selected to generate a self-fertilized sexual population based on its capability of producing abundant teliospores and high heterozygosity revealed by molecular markers, representing a different race group from those of our previously established sexual populations. The parental isolate and progeny isolates were kept as urediniospores that have two nuclei and can be asexually reproduced on susceptible wheat plants for a large quantity. The dikaryotic uredinial stage was genetically treated as diploid in the present study and used for virulence phenotyping and genomic DNA sequencing. Based on avirulence/virulence characterization of the set of 18 wheat *Yr* (yellow rust) single-gene differentials, isolate 12-368 was identified as belonging to race PStV-4, with avirulence to the resistance genes *Yr5*, *Yr7*, *Yr8*, *Yr10*, *Yr15*, *Yr24*, *Yr32*, *Yr43*, *Yr44*, *YrTr1*, and *YrExp2* and virulence to the genes *Yr1*, *Yr6*, *Yr9*, *Yr17*, *Yr27*, *YrSP*, and *Yr76* (37).

The sexual reproduction of the parental isolate 12-368 through self-fertilization on barberry plants produced 117 progeny isolates (Fig. 1). Besides the above-mentioned 18 *Yr* single-gene lines for differentiating *P. striiformis* f. sp. *tritici* races, the parental and progeny isolates were also tested on 16 additional wheat lines, each with a single different resistance gene (see Materials and Methods). Virulence tests showed that the parental and progeny isolates were all avirulent to 12 *Yr* genes, including *Yr5*, *Yr10*, *Yr15*,
Yr24, Yr32, YrTr1, Yr26, YrCV, YrTr1, Yr45, Yr53, and Yr64, indicating that the Avr genes corresponding to these Yr genes were homozygous in the parental isolate. Similarly, the parental and progeny isolates were all virulent to 13 Yr genes, including Yr1, Yr2, Yr6, Yr9, Yr21, Yr25, Yr28, Yr29, Yr31, Yr76, YrA (i.e., Yr73 plus Yr74), Yr74 (in the Avocet Susceptible [AvS] cultivar), and YrSP, suggesting that the virulence loci corresponding to these Yr genes were also homozygous in the parental isolate. Therefore, these avirulence or virulence loci could not be mapped in this study. Detailed infection types (ITs) of parental and progeny isolates are provided in Data Set S1 in the supplemental material.

In contrast, the avirulence/virulence phenotypes of the parental isolate to nine Yr genes (Yr7, Yr8, Yr17, Yr27, Yr35, Yr41, Yr43, Yr44, and YrExp2) were segregating in the progeny population (Table 1). Thus, the Avr genes corresponding to these resistance genes could be mapped. Since the dikaryotic (two unfused nuclei in a cell) urediniospores are heterozygous at these loci, the parental isolate was considered F1, and the progeny isolates produced through self-fertilization of the parental isolate were considered F2. Therefore, the avirulence/virulence phenotypes of progeny isolates should

### TABLE 1 Segregation of avirulence/virulence in the progeny isolates derived from selfing parental isolate 12-368 of *Puccinia striiformis* f. sp. *tritici* on wheat Yr single-gene lines

| Wheat Yr gene line | IT* of 12-368 | No. of progeny isolates | Expected ratio (A/V) | \( p^b \) | Avirulence gene(s) |
|--------------------|---------------|------------------------|----------------------|-------|-------------------|
| Yr7                | 3 (A)         | 86 31                  | 3:1                  | 0.71  | AvYr7            |
| Yr43               | 4 (A)         | 91 36                  | 3:1                  | 0.20  | AvYr43           |
| Yr44               | 3 (A)         | 91 36                  | 3:1                  | 0.20  | AvYr44           |
| YrExp2             | 3 (A)         | 82 35                  | 3:1                  | 0.22  | AvYrExp2         |
| Yr8                | 2 (A)         | 105 12 15:1            | 0.07                 | AvYr8-1, AvYr8-2 |
| Yr27               | 7 (V)         | 31 86                  | 1:3                  | 0.71  | avYr27           |
| Yr17               | 8 (V)         | 21 95                  | 1:3                  | 0.09  | avYr17           |
| Yr41               | 8 (V)         | 16 101                 | 3:13                 | 0.16  | AvYr41, AvYr41-Inh |
| Yr35               | 7 (V)         | 48 69                  | 7:9                  | 0.55  | avYr35-1, avYr35-2 |

*aIT, infection type based on a scale from 0 to 9, with 0 to 6 being avirulent (A) and 7 to 9 being virulent (V).  
b\( p \), probability of goodness of fit by a \( \chi^2 \) test.
follow the segregation patterns in an F₂ population. On wheat lines carrying Yr7, Yr8, Yr17, Yr41, Yr43, Yr44, and YrExp2, the avirulence/virulence phenotypes of progeny isolates fit the models that would be expected if the avirulence phenotype were dominant, whereas on the wheat lines with Yr27 or Yr35, to which the parental isolate was virulent, phenotypes of the progeny isolates were segregating, suggesting that the virulence phenotypes were dominant (Table 1).

The segregations of the parental avirulent phenotypes on wheat lines with Yr7, Yr43, Yr44, and YrExp2 fit the 3:1 avirulent/virulent (A/V) ratio, suggesting that each of the avirulent phenotypes of the parental isolate was controlled by a dominant gene. Therefore, the avirulence genes were designated AvYr7, AvYr43, AvYr44, and AvYrExp2, respectively. The segregation of avirulence on the wheat line with Yr8 fit the 15:1 A/V ratio, indicating two dominant avirulence genes, designated AvYr8-1 and AvYr8-2. The virulence phenotypes on wheat lines possessing Yr17 and Yr27 segregated at the 1:3 A/V ratio, indicating a recessive avirulence or a dominant virulence gene corresponding to each of the resistance genes. These P. striiformis f. sp. tritici genes were designated avYr17 and avYr27, respectively. The segregation of virulence to Yr41 best fit the 3:1 A/V ratio, indicating an epistatic interaction of a dominant inhibitor (AvYr41-Inh) over a dominant avirulence gene (AvYr41). On the wheat line with Yr35, the observed 7:9 A/V ratio indicated two independent recessive genes for avirulence, designated avYr35-1 and avYr35-2.

Genotyping by whole-genome sequencing. To identify molecular markers for genetic mapping, Illumina HiSeq 150-bp paired-end (PE) technology was used to sequence the whole genomes of all 117 progeny isolates as well as the parental isolate. Twenty-six million pairs of reads (7.89 Gb in total) and 1.5 billion pairs of reads (471 Gb in total) were generated from the parental and progeny isolates, respectively. The numbers of filtered reads, percentages of mapped reads, and mapping coverages and qualities for the parental and all progeny isolates are summarized in Table S1. On average, 4.02-Gb sequences were generated for each progeny isolate. The high-quality reads of progeny isolates were mapped to the reference genome of isolate P. striiformis f. sp. tritici 93-210 (38). On average, 95.72% of reads were mapped to the reference genome. The mapping coverage of the progeny isolates ranged from 24.16× to 50.99×, with a mean coverage of 35.45×. The deep sequencing and high-quality reads enabled us to identify genome-wide variations for genetic mapping.

In total, 2,487 heterozygous single nucleotide polymorphisms (SNPs) and 150 indels in the parental isolate genome were obtained based on their segregation at the 1:2:1 (AA:AB:BB) ratio (P ≤ 0.05 by a chi-squared test) in the progeny population (Fig. 2A). Contamination analysis suggested that 23 of the progeny isolates had abnormal numbers of crossover events, and these were therefore excluded from subsequent analyses. A total of 2,637 codominant markers were selected from 251 (out of 492) contigs, covering 71.35 Mb (out of 84.62 Mb) of the reference genome (Fig. 2B). The mean distance between two markers in the reference genome was 42.93 kb. Detailed genotypes of parental and progeny isolates are listed in Data Set S2.

Genetic map of P. striiformis f. sp. tritici. After correcting allele switches and filtering potentially contaminated isolates, a genetic map was generated using the minimum spanning tree algorithm at a P value of 1E–10, which contained 2,631 markers in 41 linkage groups (LGs) (LG-1 to LG-41) (Table 2; Data Set S3); the remaining 6 markers could not be linked in the genetic map. The genetic map spanned a total of 7,715.0 centimorgans (cM), with individual LGs of up to 1,011.0 cM (LG-1, with 312 markers). The average genetic distance between markers was 2.94 cM/10 kb (Table 3). This rate was higher than those of the flax
rust fungus *M. lini* (1.18 cM/10 kb) and *Zymoseptoria tritici* (1.25 cM/10 kb) and much higher than that of *Fusarium graminearum* (0.3 to 0.5 cM/10 kb) (Table 3).

To investigate potential genome features that might contribute to the relatively large *P. striiformis* f. sp. *tritici* genetic map and high recombination rate, we estimated the numbers of single- and double-crossover events in each isolate, with averages of 133.15 and 35.94, respectively (Fig. 2C and D). We also calculated the genome coverage by CpG islands in *P. striiformis* f. sp. *tritici* and compared this value with those of a few other plant-pathogenic fungi. The CpG islands in the *P. striiformis* f. sp. *tritici* reference isolate covered 7.26% of the genome (Table 3). The CpG island coverage was higher than those of *M. lini* (5.09%), *Fusarium graminearum* (2.87%), and *Zymoseptoria tritici* (1.64%) but slightly lower than that of the pine fusiform rust fungus *Cronartium quercuum* f. sp. *fusiforme* (7.37%) (Table 3). The average recombination rate of the *P. striiformis* f. sp. *tritici* CpG islands was estimated to be 1.81 cM/10 kb, the same as the
The segregating avirulence/virulence phenotypes of the progeny population were used to calculate the confidence interval of the QTL mapping for avirulence loci. The genetic map and infection type (IT) data of the segregating avirulence/virulence phenotypes of the progeny population were used to calculate the confidence interval of the QTL mapping. Six avirulence genes (AvYr7, AvYr43, AvYr44, AvYrExp2, AvYr8-1, and AvYr7) were mapped to three LGs (Table 4). AvYr8-1 was mapped to LG-19, at the 6.82- to 31.09-cM region flanked by markers C085_283131 and C182_5504. AvYr27 was mapped to LG-4, between the 446.65- and 528.51-cM positions flanked by markers C162_20837 and C086_188415, respectively (Data Set S3). The QTL confidence interval of AvYr8-1 covered three contigs, contig 1.085 (from kb 200 to kb 283), contig 1.137 (from kb 12 to kb 29), and contig 1.182 (from kb 5 to kb 14) in the reference genome of isolate 93-210. The AvYr27 interval covered two contigs, contig 1.086 (from kb 10 to kb 188) and contig 1.162 (from kb 1 to kb 164). Four avirulence genes, AvYr44, AvYr7, AvYr43, and AvYrExp2, were mapped to the same LG region.

### TABLE 2 General features of the constructed linkage map

| Chr | No. of markers | Length (cM) | Avg genetic distance between markers (cM) ± SD | No. of contigs | Contig length (bp) | Mean recombination rate (cM/10 kb) ± SD |
|-----|----------------|------------|-----------------------------------------------|----------------|-------------------|----------------------------------------|
| LG-1 | 312            | 1,011.0    | 3.25 ± 3.04                                 | 39             | 10,869,479        | 0.66 ± 3.07                            |
| LG-2 | 288            | 762.7      | 2.65 ± 1.99                                 | 27             | 6,567,233         | 1.94 ± 2.21                            |
| LG-3 | 232            | 696.5      | 3.01 ± 2.23                                 | 21             | 4,370,414         | 1.66 ± 2.43                            |
| LG-4 | 216            | 655.5      | 3.04 ± 2.54                                 | 24             | 6,208,200         | 1.72 ± 2.37                            |
| LG-5 | 183            | 436.7      | 2.39 ± 2.36                                 | 18             | 5,300,867         | 1.35 ± 1.61                            |
| LG-6 | 190            | 413.4      | 2.18 ± 1.66                                 | 13             | 1,825,696         | 1.48 ± 2.18                            |
| LG-7 | 124            | 387.0      | 3.14 ± 2.75                                 | 13             | 2,688,337         | 1.80 ± 2.20                            |
| LG-8 | 132            | 343.6      | 2.62 ± 1.63                                 | 9              | 2,509,186         | 1.84 ± 2.71                            |
| LG-9 | 101            | 293.7      | 2.93 ± 2.40                                 | 11             | 2,209,512         | 1.99 ± 2.07                            |
| LG-10| 93             | 292.1      | 3.17 ± 2.29                                 | 5              | 943,798           | 1.95 ± 2.01                            |
| LG-11| 67             | 268.9      | 4.07 ± 3.58                                 | 7              | 1,838,758         | 2.11 ± 2.01                            |
| LG-12| 76             | 248.3      | 3.31 ± 2.55                                 | 10             | 2,280,812         | 1.61 ± 1.78                            |
| LG-13| 72             | 232.5      | 3.27 ± 3.00                                 | 11             | 2,224,764         | 1.94 ± 2.02                            |
| LG-14| 51             | 179.0      | 3.58 ± 3.65                                 | 8              | 1,834,719         | 2.11 ± 2.25                            |
| LG-15| 67             | 167.7      | 2.54 ± 1.55                                 | 5              | 1,431,574         | 1.77 ± 2.15                            |
| LG-16| 48             | 153.8      | 3.27 ± 1.74                                 | 7              | 1,390,043         | 2.17 ± 2.78                            |
| LG-17| 49             | 152.1      | 3.16 ± 2.29                                 | 3              | 549,149           | 2.01 ± 2.11                            |
| LG-18| 58             | 147.4      | 2.58 ± 1.46                                 | 7              | 1,158,936         | 1.42 ± 1.70                            |
| LG-19| 30             | 135.0      | 4.65 ± 3.47                                 | 9              | 833,085           | 3.19 ± 3.37                            |
| LG-20| 28             | 81.9       | 3.03 ± 1.28                                 | 2              | 425,950           | 1.05 ± 1.52                            |
| LG-21| 27             | 81.4       | 3.13 ± 2.35                                 | 4              | 923,042           | 1.92 ± 1.86                            |
| LG-22| 13             | 62.0       | 5.14 ± 3.48                                 | 3              | 768,977           | 1.46 ± 1.06                            |
| LG-23| 15             | 54.1       | 3.86 ± 3.43                                 | 1              | 602,119           | 1.60 ± 1.14                            |
| LG-24| 19             | 50.9       | 2.82 ± 1.51                                 | 1              | 512,194           | 1.84 ± 2.48                            |
| LG-25| 19             | 48.4       | 2.68 ± 1.52                                 | 5              | 866,427           | 1.99 ± 1.49                            |
| LG-26| 26             | 43.9       | 1.75 ± 1.79                                 | 3              | 602,608           | 0.76 ± 1.45                            |
| LG-27| 12             | 43.3       | 3.94 ± 2.64                                 | 3              | 287,392           | 1.94 ± 0.93                            |
| LG-28| 9              | 38.3       | 4.78 ± 3.31                                 | 2              | 838,633           | NC                                      |
| LG-29| 14             | 32.2       | 1.16 ± 1.46                                 | 2              | 476,402           | 3.55 ± 2.49                            |
| LG-30| 10             | 31.0       | 3.44 ± 3.26                                 | 2              | 947,870           | 2.58 ± 2.44                            |
| LG-31| 7              | 26.9       | 4.48 ± 1.88                                 | 1              | 295,440           | NC                                      |
| LG-32| 5              | 25.4       | 6.36 ± 2.67                                 | 1              | 336,304           | NC                                      |
| LG-33| 3              | 20.9       | 10.46 ± 3.39                                | 1              | 536,190           | NC                                      |
| LG-34| 7              | 19.4       | 3.23 ± 0.68                                 | 1              | 295,889           | NC                                      |
| LG-35| 6              | 18.7       | 3.73 ± 1.19                                 | 1              | 261,047           | NC                                      |
| LG-36| 6              | 17.3       | 3.45 ± 1.55                                 | 2              | 397,004           | NC                                      |
| LG-37| 5              | 14.4       | 3.59 ± 0.74                                 | 1              | 815,364           | NC                                      |
| LG-38| 3              | 13.5       | 6.74 ± 0.32                                 | 2              | 630,032           | NC                                      |
| LG-39| 4              | 9.5        | 3.16 ± 1.46                                 | 2              | 554,726           | NC                                      |
| LG-40| 2              | 4.1        |                                             | 1              | 181,331           | NC                                      |
| LG-41| 2              | 0.5        |                                             | 1              | 230,054           | NC                                      |

Overall 2,631 7,715.0 2.94 ± 2.39 251 68,819,557 1.81 ± 2.32

Chr, chromosome; NC, not calculated because the linkage has <10 markers.
between the 29.06- and 57.49-cM positions flanked by markers C022_56722 and C022_180222 in LG-22, respectively (Fig. 3A; Data Set S3).

**Genomic location of the AvYr44-AvYr7-AvYr43-AvYrExp2 cluster.** All markers from similar QTL regions for AvYr44, AvYrYr7, AvYr43, and AvYrExp2 were located in a single contig, namely, contig 1,002, in the reference genome of isolate 93-210. The leftmost marker was at bp 6812, and the rightmost marker was at bp 180222, indicating that the AvYr44-AvYr7-AvYr43-AvYrExp2 cluster was most likely within the region of the

### TABLE 3 Genome-wide CpG islands in selected plant pathogens

| Parameter                | C. quercuum f. sp. fusiforme | F. graminearum | M. linic | P. striiformis f. sp. tritici | Z. tritici |
|--------------------------|-----------------------------|----------------|----------|-------------------------------|-----------|
| Genome length (bp)       | 76,567,842                  | 37,946,458     | 189,516,653 | 84,531,325                   | 39,686,251 |
| Mean recombination rate (cM/10 kb) ± SD (reference) | Unknown (51) | 0.3–0.5 (52) | 1.18 (24) | 1.81 ± 2.32 | 1.25 (53) |
| Total no. of CpGs       | 1,310,360                   | 1,846,827      | 4,449,449 | 2,003,593                    | 2,796,481 |
| No. of CpG dinucleotides in CpG islands (%) | 7.37 | 2.87 | 5.09 | 7.26 | 1.64 |
| Island length (bp)      | 189.68 ± 130.65             | 174.07 ± 108.73 | 236.65 ± 164.82 | 207.02 ± 135.58 | 120.05 ± 77.74 |
| Avg island GC% ± SD     | 54.62 ± 7.25                | 60.58 ± 6.92   | 54.85 ± 8.98 | 57.51 ± 2.08              | 66.64 ± 8.07 |
| Avg CpG O/E ratio ± SD  | 1.37 ± 0.35                 | 1.53 ± 0.31    | 1.31 ± 0.36 | 1.37 ± 0.08              | 1.73 ± 0.30 |

*The reference genome was from G11 (https://genome.jgi.doe.gov/portal/Croqu1/download/Croqu1_AssemblyScaffolds.fasta.gz).*

*The reference genome was from isolate RRES (GenBank accession no. HG970335).*

*The reference genome was from isolate CH5 (https://genome.jgi.doe.gov/portal/Melli1/download/Melli1_AssemblyScaffolds.fasta.gz).*

*The reference genome was from isolate PST93-210.*

*The reference genome was from isolate IPO323 (https://ftp.ncbi.nlm.nih.gov/genomes/all/GCF/000/219/625/GCF_000219625.1_MYCGR_v2.0/GCF_000219625.1_MYCGR_v2.0_genomic.fna.gz).*

*Percentage of the genome covered by CpG islands.*

*Reference from which the previously estimated recombination rates were retrieved.*

### TABLE 4 Quantitative trait loci for Avr genes identified in the selfing population of isolate 12-368 of *Puccinia striiformis* f. sp. *tritici*

| QTL         | Linkage group | Genetic position | Interval (cM) | LOD | P value | PVEa |
|-------------|---------------|------------------|---------------|-----|---------|------|
| AvYr8-1     | LG-19         | C085_283131, C182_5504 | 19.00 | 6.82–31.09 | 6.73 | <0.0001 | 30.85 |
| AvYr27      | LG-4          | C162_20837, C086_188415 | 484 | 446.65–528.51 | 6.16 | <0.0001 | 28.65 |
| AvYr44      | LG-22         | C022_56722, C022_180222 | 47.00 | 29.06–57.49 | 12.07 | <0.0001 | 48.40 |
| AvYr7       | LG-22         | C022_56722, C022_180222 | 46.00 | 29.06–57.49 | 10.3 | <0.0001 | 43.14 |
| AvYr43      | LG-22         | C022_56722, C022_180222 | 43.00 | 29.06–57.49 | 10.91 | <0.0001 | 44.98 |
| AvYrExp2    | LG-22         | C022_56722, C022_180222 | 47.00 | 29.06–57.49 | 9.36 | <0.0001 | 40.13 |

*PVE, percentage of variance explained by the QTL, calculated as $1 - 10^{-\frac{1}{2}LOD}$, where n is the total number of individuals.*

*Based on the reference genome of isolate 93-210 (38).*
first 200 kb of contig 1.022 in the reference genome. Our previous annotation of this region of the reference genome (39) identified 47 protein-coding genes, 4 of which have signal peptides in the N terminus (Table 5).

To validate the QTL mapping results and to identify putative casual variations, we performed a genome-wide association study (GWAS) for each of the AvrYr44, AvrYr7, AvrYr43, and AvrYrExp2 genes. Instead of using only markers in nontransposable elements (non-TEs) and without significant distortion for the 1:2:1 ratio in the QTL analysis, all markers and all 117 progeny isolates were used for the GWAS. In this way, 609 markers from contig 1.022 were obtained and used in the GWAS. Seventeen out of the 18 significantly associated SNPs were within the first 200 kb on contig 1.022. Surprisingly, a major GWAS signal peak was within the first 5-kb region (Fig. 3B). This peak matched the gene PSTG_03388, and several of the SNPs within the gene were nonsynonymous (Table 6). Thus, both QTL analyses and GWASs located the AvrYr7-AvrYr43-AvrYrExp2 locus to the first 200-kb region of contig 1.022.

We calculated the SNP density and GC content in contig 1.022. The region harboring the AvrYr44-AvrYr7-AvrYr43-AvrYrExp2 cluster had 49 SNPs within the first 5 kb and only 4 between kb 5 and kb 20. The GC content was 47% within the first 20 kb, relatively in balance with the AT content along the contig (Fig. 3C). We further investigated whether AT-rich genomic regions exist in the P. striiformis f. sp. tritici genome and whether contig 1.022 is located in an AT-rich region. The unimodal distributions of GC content indicated that there were no distinct AT-rich segments in either the whole genome or...
Therefore, we conclude that the AvYr44-AvYr7-AvYr43-AvYrExp2 cluster is not located in an AT-rich region. Next, we attempted to investigate the genomic environment of the AvYr44-AvYr7-AvYr43-AvYrExp2 cluster. Considering that this cluster resides in one of the contig terminal regions, we tried to extend contig 1.022 by aligning it to three other well-assembled genomes, those of isolates 104E 137A/H11002, 11-281, and 93TX-2, in addition to the reference genome (93-210) (Fig. S2). Surprisingly, the homologous contigs of these genomes terminated around the AvYr44-AvYr7-AvYr43-AvYrExp2 cluster, except for the genomes of the parental and reference isolates (Fig. 3D). In fact, the first 10-kb region of contig 1.022 in the reference genome of isolate 93-210 was mostly or partially absent in the 104E 137A/H11002, 93TX-2, and 11-281 genomes (Fig. S2). Besides the AvYr44-AvYr7-AvYr43-AvYrExp2 cluster, the remaining regions were highly conserved among these three genomes. Most SNPs associated with the Avr cluster were found within the first 2,600 bp of the contig in the parental isolate.

### TABLE 5: Candidate genes of the AvYr44-AvYr7-AvYr43-AvYrExp2 cluster in the confidence interval defined by QTL analysis

| Gene name | Functional annotation | Secreted | Mature length (amino acids) | % cysteine |
|-----------|------------------------|----------|-----------------------------|------------|
| PSTG_03388 | Hypothetical protein | No | 291 | 1.03 |
| PSTG_03389 | Enoyl-(acyl carrier protein) reductase | No | 351 | 1.14 |
| PSTG_03390 | ABC transporter | No | 1,053 | 0.66 |
| PSTG_03391 | Hypothetical protein | No | 102 | 1.96 |
| PSTG_03392 | Hypothetical protein | No | 814 | 1.11 |
| PSTG_03393 | Hypothetical protein | No | 199 | 0.50 |
| PSTG_03394 | Hypothetical protein | Yes | 228 | 0.88 |
| PSTG_03395 | Noncatalytic module family expansin | No | 351 | 0.57 |
| PSTG_03396 | Hypothetical protein | No | 545 | 0.37 |
| PSTG_03397 | Hypothetical protein | No | 458 | 1.09 |
| PSTG_03398 | Hypothetical protein | No | 489 | 0.82 |
| PSTG_03399 | Hypothetical protein | Yes | 143 | 1.40 |
| PSTG_03400 | Threonine dehydratase I | No | 486 | 1.03 |
| PSTG_03401 | Hypothetical protein | No | 394 | 0.25 |
| PSTG_03402 | Hypothetical protein | No | 400 | 0.75 |
| PSTG_03403 | Hypothetical protein | No | 414 | 0.97 |
| PSTG_03404 | Hypothetical protein | No | 154 | 0.65 |
| PSTG_03405 | Ca2+/calmodulin-dependent protein kinase | No | 452 | 0.66 |
| PSTG_03406 | zap1 metalloregulator involved in zinc-responsive transcriptional regulation | No | 628 | 1.27 |
| PSTG_03407 | Ubiquitin-specific protease 7 | No | 1,116 | 0.45 |
| PSTG_03408 | Hypothetical protein | Yes | 130 | 2.31 |
| PSTG_03409 | Hypothetical protein | No | 556 | 0.72 |
| PSTG_03410 | RING-H2 finger ATL54-like | No | 243 | 0.82 |
| PSTG_03411 | P-loop-containing nucleoside triphosphate hydrolase | No | 189 | 1.59 |
| PSTG_03412 | Per1-like; involved in manganese homeostasis | No | 458 | 0.87 |
| PSTG_03413 | Inositol monophosphatase | No | 315 | 0.95 |
| PSTG_03414 | Subunit of cytochrome bd ubiquinol oxidase | No | 128 | 0.00 |
| PSTG_03415 | Hypothetical protein | Yes | 338 | 0.30 |
| PSTG_03416 | Hypothetical protein | No | 148 | 1.35 |
| PSTG_03417 | Hypothetical protein | No | 173 | 0.58 |
| PSTG_03418 | Hypothetical protein | No | 135 | 0.00 |
| PSTG_03419 | Hypothetical protein | No | 189 | 0.53 |
| PSTG_03420 | 3-Deoxy-D-arabinoheptulosonate 7-phosphate (DAHP) synthetase I family | No | 435 | 1.38 |
| PSTG_03421 | Hypothetical protein | No | 678 | 1.18 |
| PSTG_03422 | tRNA | No | 696 | 1.01 |
| PSTG_03423 | Hypothetical protein | No | 420 | 1.43 |
| PSTG_03424 | Hypothetical protein | No | 380 | 1.05 |
| PSTG_03425 | Hypothetical protein | No | 142 | 1.41 |
| PSTG_03426 | Hypothetical protein | No | 193 | 0.52 |
| PSTG_03427 | Hypothetical protein | No | 301 | 0.66 |
| PSTG_03428 | Hypothetical protein | No | 409 | 1.22 |
| PSTG_03429 | Hypothetical protein | No | 23 | 0.00 |
| PSTG_03430 | Hypothetical protein | No | 269 | 0.74 |
| PSTG_03431 | Hypothetical protein | No | 90 | 0.00 |
| PSTG_03432 | Hypothetical protein | No | 241 | 0.41 |
| PSTG_03433 | RNA polymerase I-specific transcription initiation factor rrn11 | No | 270 | 1.48 |
and within the first 2,700 bp of the contig in the reference genome. The first seven SNPs were within the first exon identified in the contig. In summary, the results suggested that the \textit{AvYr44-AvYr7-AvYr43-AvYrExp2} cluster resides in a genetically complex region attached to a highly conserved genomic region.

**DISCUSSION**

In the present study, we used Illumina sequencing technology to construct a high-density genetic map for mapping \textit{Avr} genes in the wheat stripe rust fungus. We generated a \textit{P. striiformis} f. sp. \textit{tritici} sexual population for genetic mapping by self-fertilizing \textit{P. striiformis} f. sp. \textit{tritici} isolate 12-368 (race PSTv-4). Whole-genome deep sequencing of progeny isolates generated 2,637 high-quality codominant molecular markers, which enabled us to construct a high-density genetic map for \textit{P. striiformis} f. sp. \textit{tritici} comprising 41 LGs. QTL analysis mapped six \textit{Avr} genes in three LG regions. Moreover, an avirulence gene cluster carrying four \textit{Avr} genes was identified and located at a single contig in the \textit{P. striiformis} f. sp. \textit{tritici} reference genome. Aligning the genetic map to the reference genome enabled us to further locate the \textit{Avr} candidates at a small genomic region ($\leq$200 kb). This study provides the resources for functional cloning of \textit{Avr} genes and a better understanding of the genomic basis of the rapid evolution of virulence in the wheat-\textit{P. striiformis} f. sp. \textit{tritici} pathosystem.

### Isolate-dependent inheritance of avirulence/virulence in \textit{P. striiformis} f. sp. \textit{tritici}

The segregation patterns of phenotypes in the progeny population suggest a

**TABLE 6** SNPs within the \textit{AvYr44-AvYr7-AvYr43-AvYrExp2} cluster significantly associated with avirulent (\textit{Avr}) and virulent (\textit{avr}) phenotypes

| \textit{Avr} gene | SNP          | \textit{P} \_\textit{FDR adj} | Genotype(s) | Annotation                  |
|------------------|--------------|-------------------------------|-------------|-----------------------------|
| \textit{AvYr44}  | C022_2562    | 3.30E–05                      | GA AA       | Intergenic                  |
|                  | C022_170166  | 0.00144                       | TC TT       | Intergenic                  |
| \textit{AvYr7}   | C022_1634    | 0.00018                       | GA GG       | PSTG\_03388, nonsynonymous, Pro/Leu |
|                  | C022_1643    | 0.00018                       | TC CC       | PSTG\_03388, nonsynonymous, Glu/Gly |
|                  | C022_2343    | 0.00018                       | TG TT       | Intergenic                  |
|                  | C022_2562    | 0.00018                       | GA AA       | Intergenic                  |
|                  | C022_2568    | 0.00018                       | GA AA       | Intergenic                  |
|                  | C022_2576    | 0.00018                       | AC AA       | Intergenic                  |
|                  | C022_2584    | 0.00018                       | GA AA       | Intergenic                  |
|                  | C022_8283    | 0.00064                       | GC, CC GG   | PSTG\_03390, nonsynonymous, Asp/His |
|                  | C022_2099    | 0.00109                       | GT TT       | PSTG\_03388, nonsynonymous, Pro/Gln |
|                  | C022_1555    | 0.00172                       | TA AA       | PSTG\_03388, synonymous     |
|                  | C022_1561    | 0.00172                       | GA AA       | PSTG\_03388, synonymous     |
| \textit{AvYr43}  | C022_1555    | 1.12E–10                      | TA AA       | PSTG\_03388, synonymous     |
|                  | C022_188343  | 0.00045                       | AT AA       | Intergenic                  |
|                  | C079_150209  | 0.00226                       | GA AA       | PSTG\_03421, intron         |
| \textit{AvYrExp2}| C022_1634    | 5.47E–05                      | GA GG       | PSTG\_03388, nonsynonymous, Pro/Leu |
|                  | C022_1643    | 5.47E–05                      | TC CC       | PSTG\_03388, nonsynonymous, Glu/Gly |
|                  | C022_2343    | 5.47E–05                      | TC CC       | Intergenic                  |
|                  | C022_2370    | 5.54E–05                      | CT TT       | Intergenic                  |
|                  | C022_2562    | 8.70E–05                      | GA AA       | Intergenic                  |
|                  | C022_2568    | 8.70E–05                      | GT TT       | Intergenic                  |
|                  | C022_2576    | 8.70E–05                      | AC CC       | Intergenic                  |
|                  | C022_2584    | 8.70E–05                      | GA AA       | Intergenic                  |
|                  | C022_2282    | 0.00034                       | AG GG       | Intergenic                  |
|                  | C022_2294    | 0.00034                       | TC CC       | Intergenic                  |
|                  | C022_82080   | 0.00034                       | AG, GG AA   | PSTG\_03406, synonymous     |
|                  | C022_1531    | 0.00038                       | GT TT       | PSTG\_03388, synonymous     |
|                  | C022_1890    | 0.00038                       | CG GG       | PSTG\_03388, nonsynonymous, Glu/Gln |
|                  | C022_2099    | 0.00051                       | GT TT       | PSTG\_03388, nonsynonymous, Pro/Gln |
|                  | C022_2676    | 0.00051                       | AC, CC AA   | Intergenic                  |
|                  | C022_320118  | 0.00059                       | TT, T–      | Intergenic                  |
|                  | C022_8283    | 0.00096                       | GC, CC GG   | PSTG\_03390, nonsynonymous, Asp/His |
|                  | C022_46983   | 0.00096                       | TA, AA TT   | PSTG\_03396, synonymous     |
complex inheritance of avirulence/virulence in *P. striiformis* f. sp. *tritici*, which is consistent with previous observations (34–36). First, different phenotypes of avirulence/virulence to individual *Yr* genes could be controlled by one or two genes in a single isolate. In isolate 12-368 used in the present study, the avirulence phenotypes to *Yr*7, *Yr*43, *Yr*44, and *Yr*Exp2 were each controlled by a single dominant gene, whereas the avirulence phenotype to *Yr*8 was controlled by two dominant genes. Different numbers of genes controlling avirulence/virulence phenotypes in a single isolate have also been observed in other *P. striiformis* f. sp. *tritici* selfing populations (34–36) as well as in other fungi (42). One possible explanation is that there may be an unidentified resistance gene(s) in the wheat *Yr* gene lines used in the genetic studies of *P. striiformis* f. sp. *tritici*, even though many of these wheat lines are nearly isogenic in the Avocet Susceptible (AvS) background. AvS was reported to have *Yr*74, which is complementary to *Yr*73, to provide resistance to some *P. striiformis* f. sp. *tritici* races in Australia (43). It is likely that some of the *Yr* near-isogenic lines also have *Yr*74. Yuan et al. (36) reported that the virulence phenotype in the parental isolate (08-220, race PSTv-11) was heterozygous and segregated in its selfed progeny population (36). In contrast, the parental isolate and the progeny isolates in the present study were all virulent on AvS (*Yr*74). Therefore, the presence of *Yr*74 in the AvS background of many *Yr* single-gene lines did not influence the segregation ratios of the wheat lines with an AvS background. Thus, it is possible for two avirulence genes to interact with a single *Yr* gene. Second, the particular avirulence/virulence phenotype to a single *Yr* gene could be controlled by one gene in one isolate but by two or more genes in another isolate. Third, the complex inheritance in *P. striiformis* f. sp. *tritici* is also suggested by the fact that the avirulence/virulence phenotype to a single *Yr* gene could be dominant in one isolate but recessive in another. For example, the avirulence phenotype on *Yr*7 was determined by a single dominant avirulence gene in isolate 12-368 in the present study and in isolate GS-2013 (34) but was determined by a single dominant virulence gene in isolates CY32 (35) and PSTv-11 (36). A possible explanation for this is the underestimation of the interaction between a dominant avirulence gene and a dominant inhibitor gene by misclassification of the segregation of the 3:13 ratio as 1:3, at least in some cases. Taken together, the inheritance of avirulence/virulence and the interactions between avirulence/virulence-controlling genes in *P. striiformis* f. sp. *tritici* are isolate dependent.

The complex interactions between fungal *Avr* genes and their corresponding *R* genes have been described in many plant pathosystems (9). The isolate-dependent nature of avirulence/virulence inheritance observed in *P. striiformis* f. sp. *tritici* has also been reported in other rust fungi (44–46). Until now, no universal genetic models have been available to explain such complex inheritance. Therefore, further identification and comparison of genes and genomic regions between different isolates showing different avirulence/virulence inheritance are needed to test the association of complex inheritance and the plasticity of the genomic environment.

**Avirulence gene cluster in *P. striiformis* f. sp. *tritici**. The QTL analysis and the GWAS mapped four *Avr* genes in a small genomic region (Table 4 and Fig. 3A and B), indicating a cluster of *Avr* genes in *P. striiformis* f. sp. *tritici* isolate 12-368. The existence of *Avr/Vir* clusters has also been revealed from different *P. striiformis* f. sp. *tritici* mapping populations in previous studies (34–36). However, due to the limited number of molecular markers (34, 35) or the lack of codominant markers (36), either previous studies were not able to identify cosegregating markers or the flanking markers were too far away from the cluster to precisely define the genomic intervals of the cluster. In contrast, QTL mapping with the highly improved reference genome enabled us to map an *Avr* cluster, *AvrYr44-AvYr7-AvYr43-AvYrExp2*, in LG-22 to a single contig of the reference genome. *Avr* and effector genes usually reside in plastic genomic regions, and gene clusters are often located in such regions (47). Such *Avr* gene clusters are not uncommon in cereal rust fungi. For example, the *Avr* genes in the flax rust fungus *M. lini* were genetically mapped to four small regions, and the genes within each region were tightly linked and inherited as a unit (5). Several *Avr* genes have been cloned from...
these clusters. Interestingly, single Avr genes (e.g., AvrL567 and AvrM14) in M. lini controlling avirulence to several R genes have been reported (23, 24). Such a single Avr gene (allele) recognized by multiple R genes has also been reported in the pathosystem of Leptosphaeria maculans-oilseed rape (48). Syntenic analysis revealed that the AvYr44-AvYr7-AvYr43-AvYrExp2 cluster resides in one of the contig termini, which was difficult to assemble in different isolates (Fig. 3D; Fig. S1). It will be interesting to determine whether this Avr cluster is located in a subtelomere-like region, similar to AvrPita from Pyricularia oryzae (49) and AvrStb6 from Z. tritici (50), or a region adjacent to a repetitive region of the genome. Further work using linked- or long-read sequencing, e.g., bacterial artificial chromosome sequencing, PacBio technology, or nanopore sequencing, is needed to dissect the cluster of AvYr44-AvYr7-AvYr43-AvYrExp2 loci for cloning the Avr genes and for providing a genomic basis for the rapid avirulence changes in P. striiformis f. sp. tritici. The genes identified in the first 20 kb of the contig can be studied for expression to determine if they are functionally associated with avirulence.

**Large genetic map with a high recombination rate of P. striiformis f. sp. tritici.** Generally, our genetic map was consistent with the physical map (Data Set S3). For example, the markers of each large contig (>200 kb in length) were always located nearby in the genetic map. However, we noticed two types of inconsistencies. First, some of the markers from different contigs were interwoven in the genetic map, especially the markers from short contigs. We speculate that these might be due to either the highly repetitive nature of the P. striiformis f. sp. tritici genome or the high heterozygosity between two marker loci. Second, even though markers from the same contig were located nearby in the genetic map, their locations were not linearly correlated. This inconsistency might be caused by structural variations (e.g., genome rearrangement) between the parental and reference isolates or by possible genotyping errors from intrinsic sequencing bias and errors. An improved and haplotype-solved reference genome, especially from the parental isolate, as well as long-read sequencing with high accuracy (e.g., PacBio HiFi), is needed to solve such inconsistencies.

We took the advantage of our previously assembled high-continuity reference genome for comparison between the genetic map and the physical map (38, 39). This enabled us to discover new genetic features from a sexual P. striiformis f. sp. tritici population. Our study showed that P. striiformis f. sp. tritici has a large genetic map with a total genetic distance of 7,715.0 cM, which is comparable to other rust fungi. In the pine fusiform rust fungus (C. quercuum f. sp. fusiforme), a genetic map of 3,006 cM was constructed using 421 (including 208 randomly amplified polymorphic DNA [RAPD], 34 simple sequence repeat [SSR], and 184 amplified fragment length polymorphism [AFLP]) markers (51). Similarly, Anderson et al. (24) generated a genetic map of 5,860 cM using 13,412 restriction site-associated DNA sequence (RADseq) markers in the flax rust fungus (M. lini). One of the factors contributing to the slightly larger map in the present study might be the genotyping platform used since markers from whole-genome sequencing could potentially cover the whole genome. In the present study, 68.81 Mb, out of the 84.53-Mb (81.4%) genome, were covered by the markers from whole-genome sequencing. In the M. lini study, a slightly lower coverage (68.9%) of the genome was anchored to the genetic map using RADseq markers (24). We also noticed that P. striiformis f. sp. tritici has a slightly higher recombination rate (1.81 cM/10 kb) (Table 2) than other fungal pathogens, e.g., 0.3 to 0.5 cM/10 kb in F. graminearum (52), 1.25 cM/10 kb in Z. tritici (53), and 1.18 cM/10 kb in M. lini (24). We speculate that the higher recombination rate in P. striiformis f. sp. tritici is associated with the high number of detected single- and double-crossover events. Compared with the average of 114.6 crossovers per F2 individual in M. lini, P. striiformis f. sp. tritici has a relatively high number of crossovers per individual, at 133.15 (Fig. 2C). In addition to the high number of crossover events, we also noticed that rust fungi with high recombination rates also have high percentages of CpG islands in their genomes. It has been proposed that the depletion of nucleosome occupancy in particular functional features such as CpG islands increases the accessibility of the recombination machinery (54). We found that the percentages of genome coverage by CpG islands in the basidiomycete rust fungi...
are higher than those in the ascomycete fungi in our comparison. We noticed that Z. tritici was an exception in its slightly higher recombination rate than that of M. lini but much lower CpG island coverage (Table 3). Because only a few plant-pathogenic fungi have genome-wide recombination rates available, it is not possible to explicitly determine the role of CpG islands in the variation of recombination rates across different plant pathogens. Further studies are needed to test this hypothesis when more plant pathogens have their genome-wide recombination rates available. It is also useful to further investigate the genomic features of recombination hot spot regions besides the CpG islands to explain the recombination rate variation across the genome and among different plant pathogens.

In summary, our high-density genetic map reveals a generally high recombination rate of P. striiformis f. sp. tritici. More studies are needed to investigate the contribution of the high number of sexual recombination events to P. striiformis f. sp. tritici genome architecture and the rapid evolution of virulence. Moreover, our high-quality genetic map with dense markers will provide a valuable resource for anchoring genomic contigs to chromosomes.

In conclusion, using molecular markers generated through whole-genome sequencing of a self-fertilized population, we generated a high-density genetic map for P. striiformis f. sp. tritici comprising 41 lineage groups. Moreover, the SNP and indel markers are attached to the sequences of fragments that have been mapped to the reference genome, which allows direct comparison with data from future similar studies. The high-density genetic map will be valuable to further anchor fragmented contigs to chromosomes. Furthermore, the avirulence gene cluster of AvYr44-AvYr7-AvYr43-AvYrExp2 was identified from QTL mapping and located at a short genome region through genome comparison. Further studies on the detailed genomic environment of this Avr cluster and cloning of these genes will shed light on the genomic basis of the rapid virulence changes of this destructive pathogen.

MATERIALS AND METHODS

Isolate selection, urediniospore multiplication, and teliospore production. P. striiformis f. sp. tritici isolate 12-368 (race PSTv-4), collected from Washington State in the United States in 2012, was selected to generate a segregating population based on its avirulence/virulence profile and its abilities to produce telia and infect barberry plants. The purification, urediniospore multiplication, and teliospore production of the isolate were conducted according to a previously described procedure (36). Briefly, the pure isolate was obtained from a single uredinium. Urediniospores from a single isolate were multiplied on seedlings of the winter wheat cultivar Nugaines (37). The spring wheat cultivar Avocet Sensitive (AvS) was inoculated at the flag-leaf stage and grown under controlled conditions to produce telia.

Developing a sexual population. A sexual population was developed by self-fertilization of isolate 12-368 according to procedures described previously (36, 55) (Fig. 1). Briefly, wheat leaves bearing telia were removed by removing the epidermal layers and then placed on moist filter paper in petri dishes. Teliospore germination and basidiospore production were checked under a microscope periodically. Suspensions of germinated teliospores and produced basidiospores were used to inoculate 10-day-old barberry leaves. After incubation in a dew chamber at 10°C in the dark with 100% relative humidity for 36 to 48 h, the inoculated barberry plants were transferred to a growth chamber for pycnial production. For self-fertilization, the nectar containing pycniospores from one pycnium was transferred to another pycnium. To avoid duplicated fertilization of a pycnium, the transfer of nectar was conducted in one direction such that the nectar from the first pycnium was transferred to the second pycnium but the nectar from the second pycnium was never retransferred back to the first pycnium or another pycnium. As expected, in this way, an ascus was produced only in the opposite leaf surface of the second pycnium but not in the first pycnium. Usually, a cluster of ascial cups was formed after one pycnium was fertilized. When an ascus was mature, only a single ascial cup was excised with a sterile razor blade, the asciospores from the ascial cup were used to inoculate seedlings of wheat cultivar Nugaines, and the leftover cups within one ascus were stored in liquid nitrogen for backup. About 13 to 15 days after asciospore inoculation, a single uredinium from an inoculated Nugaines leaf was isolated, and the urediniospores from the uredinium were used to inoculate Nugaines seedlings again to multiply enough urediniospores, which were considered one progeny isolate produced from infection by a single asciospore. The increased urediniospores of both parental isolate 12-368 and progeny isolates were used for virulence testing and DNA extraction.

Virulence phenotyping and genetic analysis. A total of 34 wheat genotypes, each carrying a single Yr resistance gene, were used to obtain avirulence/virulence phenotypes of isolate 12-368 and the progeny isolates. These 34 Yr genes were Yr1, Yr5, Yr6, Yr7, Yr8, Yr9, Yr10, Yr15, Yr17, Yr24, Yr27, Yr32, Yr43, Yr44, YrSP, YrT1, YrExp2, Yr76, Yr2, Yr21, Yr25, Yr26, Yr28, Yr29, Yr31, Yr35, YrCV, YrTr1, YrCN19, YrA, YrAvS, Yr43, Yr53, and Yn64. Five to seven seeds of each wheat line were planted for each isolate test. About
18 days after inoculation, the IT data of an isolate on each plant were recorded based on a scale of 0 to 9 (56). As genetically pure seeds of each Yr single-gene line were specifically produced and each *P. striiformis* f. sp. *tritici* isolate was obtained initially from a single uredinium and carefully multiplied, the up to seven plants of each isolate in a single isolate test mostly had identical ITs. In cases where different ITs were observed, the identical ITs of most plants or at least three plants were used. An isolate was considered avirulent to a specific Yr gene when the IT was 0 (inoculated leaves showing no visible symptoms), 1 (showing necrotic or chlorotic flecks), 2 (showing necrotic or chlorotic blotches without sporulation), or 3 to 6 (showing necrotic or chlorotic blotches with trace to moderate sporulation) or virulent when the IT was 7 to 9 (showing abundant sporulation with or without necrosis or chlorosis) (37). Since urediniospores of isolate 12-368 are dikaryotic, typical for *P. striiformis* f. sp. *tritici* and other rust fungi, we assumed that the parental isolate is heterokaryotic or “heterozygous” for many loci and therefore treated it as an F1 generation in the present study. The progeny isolates generated by selfing isolate 12-368 were considered the F2 generation. Therefore, the segregation of virulence phenotypes and molecular markers was expected to follow the segregation ratios of an F2 population. The chi-square test was used to determine whether the observed segregation of A/V phenotypes on a particular wheat line fit a theoretical genetic ratio.

**DNA extraction and whole-genome sequencing.** Genomic DNA was extracted from urediniospores using the cetyltrimethylammonium bromide (CTAB) method as previously described (57). The quality and quantity of the extracted DNA were checked using a NanoDrop 1000 spectrophotometer (Thermo Fisher Scientific) and 0.8% agarose (Thermo Fisher Scientific) gel electrophoresis. DNA libraries were constructed and whole-genome sequencing was performed using Illumina Hiseq PE150 technology (Novogene Co. Ltd., Sacramento, CA).

**Genomic variation calling.** For each isolate, Illumina sequencing reads were checked and trimmed using Trimmomatic (version 0.36) (58). Parameters were set as LEADING:10 TRAILING:10 SLIDINGWINDOW:4:15 MINLEN:50 in the paired-end (PE) model. After trimming, reads with lengths of less than 50 bp or that were not paired were excluded from subsequent analyses. Potential sequencing errors in trimmed reads were corrected using Lighter software (59), with the parameter set as –K 21 90000000. The high-quality and error-corrected Illumina reads were used for genomic variation calling. The previously assembled genome of U.S. isolate *P. striiformis* f. sp. *tritici* 93-210 was used as a reference genome (38) since it has a well-assembled genome (38). Also collected from the U.S. Pacific Northwest, the reference isolate was identified as belonging to race PSTv-20 and was avirulent to all 18 wheat Yr single-gene lines, except Yr17, used to differentiate *P. striiformis* f. sp. *tritici* races in the United States (37).

Genome-wide markers were identified according to a previously proposed framework (60). Briefly, the BBurrowes (BWA) tool version 0.7.15 (61) was used to index the sequence reads to the reference genome, and the mem algorithm with default parameters was used to map filtered paired-end Illumina reads to the indexed reference genome. Next, Genome Analysis Toolkit (GATK) version 3.3 (62) was used to identify genomic variations. RealignerTargetCreator software was used to define interval targets for local realignment, and IndelRealigner was used to perform indel realignment. Two rounds of genomic variation calling were performed using GATK HaplotypeCaller with default parameters, as described previously (38, 63). VCFtools software (version 0.1.13) was used to manipulate SNPs and indels stored in vcf format. SNPs and indels of high confidence were filtered using VCFtools, with parameters set as –min-alleles 2 –max-alleles 2 –minQ 1000 –min-meanDP 30 –max-meanDP 60 –max-missing 1 (meaning that only biallelic SNPs and indels with a minimum quality of 1,000, a minimum mean depth of 30, a maximum mean depth of 60, and no missing data were used). By setting –min-meanDP 30 –max-meanDP 60, the variations in repetitive regions could be partially filtered since our sequencing depth ranged from 30× to 60×. To further remove potentially problematic variations in repetitive regions, we filtered SNPs and indels from transposable element (TE) regions as previously defined (38, 39). By doing this, genome-wide variations from only non-TE regions were kept for subsequent analyses. Moreover, duplicate markers (that is, markers with identical genotypes across all isolates) were identified and excluded from the construction of maps. To further reduce the computational burden, only one marker was selected within a 1,000-bp sequence region. The markers were named as contig no._position. For example, C001_1000 is the marker located in contig 1 at bp 1,000 in the reference genome of *P. striiformis* f. sp. *tritici* 93-210 (38).

**Genetic map construction and QTL mapping.** Before map construction, we first checked the segregation ratios of markers. In general, markers with P values from chi-squared tests of ≥0.05 for the 1 (homozygous AA):2 (heterozygous AB):1 (homozygous BB) ratio expected for a single locus were used for map construction. In total, 2,637 high-quality genome-wide markers and 94 progeny isolates were used for map construction. Genetic map construction was performed using qtl version 1.41-6 (64, 65) and ASMap version 1.0-2 (66) in the R package. The qtl program was used to analyze the genotypes. Even though the progeny population generated from selfing the isolate 12-368 could be considered an F2 population, the linkage phases were unknown due to the lack of paternal and maternal isolates. Therefore, some markers might have switched alleles. This issue was solved by checking logarithm of the odds (LOD) scores against the estimated recombination fractions for all marker pairs using the qtl program. The markers were considered to have alleles switched when they were tightly associated with other markers but had recombination fractions of ≥0.5, which were corrected according to a procedure described by Broman et al. (64). After correcting allele switches, the ASMap package using the minimum spanning tree algorithm was used to infer linkage groups and optimally order markers, with parameters set as dist.fun = “kosambi,” bychr = FALSE, p.value = 1e-10, anchor = TRUE, noMap_.dis = 20, and detectBadData = TRUE. The genotyping errors and rates were calculated using the qtl package, and the number of observed crossovers per individual was estimated using the ASMap package.
package. The genetic map was reconstructed until no genotype errors were detected. In addition, we excluded isolates that were potentially contaminated if their large proportion of alternative alleles was absent from the parental isolate. Analysis with such potentially contaminated isolates showed that these isolates had >3-times-larger numbers of crossover events than other isolates. The number of single- and double-crossover events per individual was calculated using the countXO function in the R/qtl package (64, 65).

After the genetic map was constructed, we explored genome-wide features that might contribute to the high recombination rate of \emph{P. striiformis} f. sp. \emph{tritici}. The AT-rich regions were determined using OcculCut v1.1 with default parameters (67). Since recombination often increases at the CpG islands in mammalian genomes (68), we also analyzed the distribution of CpG islands in \emph{P. striiformis} f. sp. \emph{tritici} and several other plant pathogens with genetic maps available. CpG islands were identified using CpGcluster v2.0 software (69), with a distance threshold of 75 and a P value of 1E−3.

The genetic map generated with ASMap was used for QTL mapping of Avr genes, and QTL mapping was performed using the qtl package. First, the QTL genotype probabilities given the available marker genotypes were calculated using the calc.genoprob function, with parameters set as step=1, error.proba=0.02, mapfunction=“kosambi,” and stepwidth=“fixed.” The recorded ITs were used as pheno-
types for QTL mapping. The QTL mapping approach was used, instead of single-gene locus-like markers, to map Avr genes because it was not always possible to classify isolates into homozygous avirulent, homozygous virulent, and heterozygous as for the genotypic data. Since the ITs did not follow a normal distribution, the nonparametric interval mapping method was selected by setting model=“np” in the scanone function. Instead of using an arbitrary LOD threshold for all phenotypes, we calculated 5% LOD thresholds via permutation tests for each phenotype by setting n.perm=1000. To determine the QTL confidence intervals, the Bayesian credible intervals were calculated using the bayesint function. QTL mapping was performed separately for the segregating phenotype data on each wheat Yr single-gene line.

Association analysis and genomic environment of the \emph{AvYr44-AvYr7-AvYr43-AvYrExp2} cluster. To validate our QTL mapping results and to further identify potential Avr-associated variations, we used all the isolates from the sexual population for association analysis. Here, we focused on \emph{AvYr44}, \emph{AvYr7}, \emph{AvYr43}, and \emph{AvYrExp2} since (i) each of the avirulence phenotypes of these genes was controlled by a single gene and (ii) these four genes were mapped to a cluster in the genetic map and located in a single contig (contig 1.022) (see Results) in the reference genome. Genomic variations in contig 1.022 with <20% missing data were retrieved, and all the sequence variations were used for the GWAS, including those in repetitive regions and those with P values by chi-squared tests of less than 0.05. The FarmCPU method implemented in GAPIT v3 software was used to perform association analysis with three principal components as covariates (70). The associations between genomic variations and virulence phenotypes were considered significant if the P value was <1E−5 and the false discovery rate-adjusted P value (P_FDR_adj) was 0.01. Significantly associated variations were annotated based on our previous 93-210 reference genome (38).

To investigate the genomic environment of the \emph{AvYr44-AvYr7-AvYr43-AvYrExp2} cluster, we com-
pared the homologous regions of this cluster among three well-assembled \emph{P. striiformis} f. sp. \emph{tritici} genomes, namely, isolates 93-210 (38), 11-281 (41), and 104E 137A−3 (40). First, contig 1.022 of 93-210 was subjected to a BLAST search against the 11-281 and 104E 137A−3 genome sequences to detect potential homologous contigs. Next, the three homologous contigs were aligned using progressiveMauve software (71), and the generated alignment in a “.backbone” file was visualized using genoPlotR (72).

Data availability. All data sets generated for this study are included in the figures, tables, and supplemental material of the article. The complete set of sequence data was deposited in the National Center for Biotechnology Information (NCBI) database under accession no. PRJNA599033 and in the SRA under accession no. SRP239501. Further inquiries can be directed to the corresponding authors.

SUPPLEMENTAL MATERIAL

Supplemental material is available online only.

FIG S1, RTF file, 1 MB.
FIG S2, RTF file, 0.4 MB.
TABLE S1, DOCX file, 0.03 MB.
DATA SET S1, XLSX file, 0.03 MB.
DATA SET S2, XLSX file, 1 MB.
DATA SET S3, XLSX file, 0.2 MB.

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REFERENCES

1. Fisher MC, Henk DA, Briggs CJ, Brownstein JS, Madoff LC, McCraw SL, Gurrola CR. 2012. Emerging fungal threats to animals, plant and ecosystem health. Nature 484:186–194. https://doi.org/10.1038/nature10947.

2. Tainihinas P, Batista D, Diniz I, Vieira A, Silva DN, Loureiro A, Tavares S, Pereira AP, Azinheira HG, Guerra-Guimarães L, Várzea V, Silva MDC. 2017. The coffee leaf rust pathogen Hemileia vastatrix: one and a half centuries around the tropics. Mol Plant Pathol 18:1039–1051. https://doi.org/10.1111/plp.12512.

3. Goebeler K, Loebner M, Langenbach C, Conrath U, Koch E, Schaarhuff U. 2010. Phosphopasa pachyrrhizi, the causal agent of Asian soybean rust. Mol Plant Pathol 11:169–177. https://doi.org/10.1111/j.1364-3703.2009.00589.x.

4. Nazareno ES, Li F, Smith M, Park RF, Kianian SF, Figueroa M. 2018. Puccinia coronata f. sp. avenae: a threat to global oat production. Mol Plant Pathol 19:1047–1060. https://doi.org/10.1111/mpp.12608.

5. Lawrence GJ, Dodds PN, Ellis JG. 2007. Rust of flax and linseed caused by Melampsora lini. Mol Plant Pathol 8:349–364. https://doi.org/10.1111/j.1364-3703.2007.00455.x.

6. Duplessis S, Major I, Martin F, Séguin A. 2009. Poplar and pathogen interactions: insights from Populus genome-wide analyses of resistance and defense gene families and gene expression profiling. Crit Rev Plant Sci 28:309–334. https://doi.org/10.1080/07322850903214063.

7. Dean R, van Kan JA, Pretorius ZA, Hammond-Kosack KE, di Pietro A, Spanu PD, Rudd JJ, Dickman M, Kahmann R, Ellis J, Foster GD. 2012. The top 10 fungal diseases in molecular plant pathology. Mol Plant Pathol 13:414–430. https://doi.org/10.1111/j.1364-3703.2011.00783.x.

8. Flor HH. 1971. Current status of the gene-for-gene concept. Ann Rev Phytopathol 9:275–296. https://doi.org/10.1146/annurev.phy.09.091717.001423.

9. Petit-Houdenot Y, Fudal I. 2017. Complex interactions between fungal avirulence genes and their corresponding plant resistance genes and consequences for disease resistance management. Front Plant Sci 8:1072. https://doi.org/10.3389/fpls.2017.01072.

10. Jones JDG, Dangl JL. 2006. The plant immune system. Nature 444:323–329. https://doi.org/10.1038/nature05286.

11. van Kan JAL, van den Ackerveken GFJM, de Wit PJGM. 1991. Cloning and characterization of CDNA of avirulence gene avr9 of the fungal pathogen Cladosporium fulvum, causal agent of tomato leaf mold. Mol Plant Microbe Interact 4:52–59. https://doi.org/10.1094/mpmi-4-052.

12. van den Ackerveken GFJM, Van Kan JAL, de Wit PJGM. 1992. Molecular analysis of the avirulence gene avr9 of the fungal pathogen Cladosporium fulvum fully supports the gene-for-gene hypothesis. Plant J 2:359–366. https://doi.org/10.1046/j.1365-313x.1992.t01-34.00999.x.

13. Yin CT, Hubbert S. 2011. Prospects for functional analysis of effectors from cereal rust fungi. Euphytica 179:57–67. https://doi.org/10.1007/s10681-010-0285-x.

14. Attard A, Goult L, Gourguehs M, Kühn M-L, Schmit J, Laroche S, Ansan-Melayah D, Billault A, Cattolico L, Balesdent MH, Rouxel T. 2002. Analysis of molecular markers genetically linked to the Leptosphaeria maculans avirulence gene AvrLm1 in field populations indicates a highly conserved event leading to virulence on Sr11 genotypes. Mol Plant Microbe Interact 15:672–682. https://doi.org/10.1094/MPMI.2002.15.7.672.

15. Goult L, Fudal I, Kuhn ML, Blaise F, Eckert M, Cattolico L, Balesdent MH, Rouxel T. 2006. Lost in the middle of nowhere: the AvrLm1 avirulence gene of the dothideomycete Leptosphaeria maculans. Mol Microbiol 60:67–80. https://doi.org/10.1111/j.1365-2958.2006.05076.x.

16. Fudal I, Ross S, Goult L, Blaise F, Kühn ML, Eckert MR, Cattolico L, Bernard-Samain S, Balesdent MH, Rouxel T. 2017. Heterochromatin-like regions as ecological niches for avirulence genes in the Leptosphaeria maculans genome: map-based cloning of AvrLm6. Mol Plant Microbe Interact 20:459–470. https://doi.org/10.1094/MPMI-20-4-0459.

17. Parlange P, Daverdirn G, Fudal I, Kuhn ML, Balesdent MH, Blaise F, Grezes-Besset B, Rouxel T. 2009. Leptosphaeria maculans avirulence gene AvrLm4-7 confers a dual recognition specificity by Rlm4 and Rlm7 resistance genes of oilseed rape, and encumbers Rlm4-mediated recognition through a single amino acid change. Mol Microbiol 71:851–863. https://doi.org/10.1111/j.1365-2988.2008.06547.x.

18. Balesdent M-H, Fudal I, Ollivier B, Bally P, Grandaubert J, Eber F, Chèvre A-M, Leffon M, Rouxel T. 2013. The dispensable chromosome of Leptosphaeria maculans shelters an effector gene conferring avirulence towards Brassica rapa. New Phytopathol 198:887–898. https://doi.org/10.1111/nph.12178.

19. Ghanbarian K, Fudal I, Larkam NJ, Links MG, Balesdent MH, Profotova B, Fernando WGD, Rouxel T, Borhan MH. 2015. Rapid identification of the Leptosphaeria maculans avirulence gene AvrLm2, using an intraspecific comparative genomics approach. Mol Plant Pathol 16:699–709. https://doi.org/10.1111/mpp.12228.

20. Plissonneau C, Daverdirn G, Ollivier B, Blaise F, Degrave A, Fudal I, Rouxel T, Balesdent MH. 2016. A game of hide and seek between avirulence genes AvrLm4-7 and AvrLm3 in Leptosphaeria maculans. New Phytopathol 209:1613–1624. https://doi.org/10.1111/nph.13736.

21. Linning R, Lin D, Lee N, Abdennadher M, Gaudet D, Thomas P, Mills D, Kronstad JW, Bakkeren G. 2004. Marker-based cloning of the region containing the UAhv1 avirulence gene from the basidiomycete barley pathogen Ustilago hordei. Genetics 166:99–111. https://doi.org/10.1534/genetics.166.1.99.

22. Ali S, Laurie JD, Linning R, Cervantes-Chávez JA, Gaudet D, Bakkeren G. 2014. An immunity-triggering effector from the barley smut fungus Ustilago hordei resides in an Ustilaginaceae-specific cluster bearing signs of transposable element-assisted evolution. PLoS Pathog 10:e1004223. https://doi.org/10.1371/journal.ppat.1004223.

23. Dodds PN, Lawrence GJ, Catanzariti A-M, Aylliffe MA, Ellis JG. 2004. The Melampsora lini AvrL567 avirulence genes are expressed in haustoria and their products are recognized inside plant cells. Plant Cell 16:755–768. https://doi.org/10.1105/tpc.020040.

24. Anderson C, Khan MA, Catanzariti AM, Jack CA, Nemri A, Lawrence GJ, Upadhayya NM, Hardham AR, Ellis JG, Dodds PN, Jones DA. 2016. Genome analysis and avirulence gene cloning using a high-density RADseq linkage map of the flax rust fungus, Melampsora lini. BMC Genomics 17:667. https://doi.org/10.1186/s12864-016-3011-9.

25. Saliceo A, Rutter W, Wang S, Akhunova A, Bolus S, Chao S, Anderson N, De Soto MF, Rouse M, Szabo L, Bowden RL, Dubcovsky J, Akhunov E. 2017. Variation in the AvrSr35 gene determines Sr35 resistance against wheat stem rust race U99. Science 358:1604–1606. https://doi.org/10.1126/science.aao7294.

26. Chen J, Upadhayya NM, Ortiz D, Sperschneider J, Li F, Bouton C, Breen S, Dong C, Xu B, Zhang X, Mago R, Newell K, Xia X, Bernoux M, Taylor JM, Steffenson B, Jin Y, Zhang P, Kanyuka K, Figueroa M, Ellis JG, Park RF, Dodds PN. 2017. Loss of AvrSr50 by somatic exchange in stem rust leads to virulence for Sr50 resistance in wheat. Science 358:1607–1610. https://doi.org/10.1126/science.aao4810.
Mahonia

Wang MN, Chen XM. 2013. First report of Oregon grape (Mahonia aquifolium) as an alternate host for the wheat stripe rust pathogen (Puccinia striiformis f. sp. tritici) under artificial inoculation. Plant Dis 97:839. https://doi.org/10.1094/PDIS-09-12-0864-PDN.

Chen W, Wellings C, Chen X, Kang Z, Liu T. 2014. Wheat stripe rust (yellow) rust caused by Puccinia striiformis f. sp. tritici. Mol Plant Pathol 15:433–446. https://doi.org/10.1111/mpp.12116.

Savary S, Willocquet L, Pethybridge SJ, Esker P, McRoberts N, Nelson A. 2019. The global burden of pathogens and pests on major food crops. Nat Ecol Evol 3:430–439. https://doi.org/10.1038/s41559-018-0793-y.

Jin Y, Szabo L, Carson M. 2010. Century-old mystery of wheat stripe rust. Fungal Biol 120:729–744. https://doi.org/10.1016/j.fbi.2009.08.007.

Zhang Z, Marcel TC, Hartmann FE, Ma X, Plissonneau C, Zala M, Ducasse A, Conifais J, Compain J, Lapalu N, Amselem J, McDonald BA, Croll D, Palma-Guerrero J. 2017. A small secreted protein in Zymoseptoria tritici is responsible for avirulence on wheat cultivars carrying the Stb6 resistance gene. New Plant 214:619–631. https://doi.org/10.1111/nph.14434.

Kubiškova TL, Anderson CL, Amerson HV, Smith JA, Davis JM, Nelson CD. 2011. A genomic map enriched for markers linked to Avr1 in Cronartium quercum f. sp. fusiforme. Fungal Genet Biol 48:266–274. https://doi.org/10.1016/j.fgb.2010.09.008.

Laurent B, Palaikostas C, Sputaro C, Moinard M, Zehraoui E, Houston RD, Foulongne-Oriol M. 2018. High-resolution mapping of the recombinant landscape of the phytopathogen Fusarium graminearum suggested two-speed genome evolution. Mol Plant Pathol 19:341–354. https://doi.org/10.1111/mpp.12524.

Lendenmann MH, Croll D, Stewart EL, McDonald BA. 2014. Quantitative trait locus mapping of melanzation in the plant pathogenic fungus Zymoseptoria tritici. G3 (Bethesda) 4:2519–2533. https://doi.org/10.1534/g3.114.015289.

de Castro E, Soriano I, Marin L, Serrano R, Quintales L, Antequera F. 2012. Supplementary resistance genes in wheat selection 'Avocet R' confer resistance to high confidence variant calls: the genome analysis toolkit best practices pipeline. Curr Protoc Bioinformatics 43:10.11–10.33. https://doi.org/10.1002/9780471250953.bi1110s43.

Boiffin A, Leverntz F, Pi-ta AF, Kernytsky AM, Sivachenko AY, Cibulskis K, Gabriel SB, Altshuler D, Daly MJ. 2011. A framework for variation discovery and genotyping using next-generation DNA sequencing data. Nat Genet 43:491–498. https://doi.org/10.1038/ng.806.

Li H, Durbin R. 2010. Fast and accurate long-read alignment with Burrows-Wheeler transform. Bioinformatics 26:589–595. https://doi.org/10.1093/bioinformatics/btp698.

Van der Auwera GA, Carneiro MO, Närbrock B, Cook JM, Krueger R, McKee S, Goodson T, Huber T, Peeperkorn B, Markovic ND, Chen W, Wang M, Liu T, Wang X, Yang F, Stewart RS, Gerstein M, relaxation spots evolution. Next-generation DNA sequencing error correction without counting. Genome Biol 15:509. https://doi.org/10.1186/s12864-014-0040-z.
65. Broman KW. 2010. Genetic map construction with R/qtl. Technical report #214. University of Wisconsin—Madison Department of Biostatistics and Medical Informatics, Madison, WI.

66. Wu Y, Bhat P, Close TJ, Lonardi S. 2008. Efficient and accurate construction of genetic linkage maps from minimum spanning tree of a graph. PLoS Genet 4:e1000212. https://doi.org/10.1371/journal.pgen.1000212.

67. Testa AC, Oliver RP, Hane JK. 2016. OcculterCut: a comprehensive survey of AT-rich regions in fungal genomes. Genome Biol Evol 8:2044–2064. https://doi.org/10.1093/gbe/evw121.

68. Smeds L, Mugal CF, Qvarnström A, Ellegren H. 2016. High resolution mapping of crossover and non-crossover recombination events by whole-genome re-sequencing of an avian pedigree. PLoS Genet 12:e1006044. https://doi.org/10.1371/journal.pgen.1006044.

69. Hackenberg M, Previti C, Luque-Escamilla PL, Carpena P, Martinez-Aroza J, Oliver JL. 2006. CpGcluster: a distance-based algorithm for CpG-island detection. BMC Bioinformatics 7:446. https://doi.org/10.1186/1471-2105-7-446.

70. Liu X, Huang M, Fan B, Buckler ES, Zhang Z. 2016. Iterative usage of fixed and random effect models for powerful and efficient genome-wide association studies. PLoS Genet 12:e1005767. https://doi.org/10.1371/journal.pgen.1005767.

71. Darling AE, Mau B, Perna NT. 2010. progressiveMauve: multiple genome alignment with gene gain, loss and rearrangement. PLoS One 5:e11147. https://doi.org/10.1371/journal.pone.0011147.

72. Guy L, Kultima JR, Andersson SGE. 2010. genoPlotR: comparative gene and genome visualization in R. Bioinformatics 26:2334–2335. https://doi.org/10.1093/bioinformatics/btp413.