Theoretical and Applied Genetics (2021) 134:53–62
https://doi.org/10.1007/s00122-020-03681-2

ORIGINAL ARTICLE

Characterization of *Pm68*, a new powdery mildew resistance gene on chromosome 2BS of Greek durum wheat TRI 1796

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Received: 21 June 2020 / Accepted: 4 September 2020 / Published online: 11 September 2020

Abstract

Key message **New powdery mildew resistance gene *Pm68* was found in the terminal region of chromosome 2BS of Greek durum wheat TRI 1796. The co-segregated molecular markers could be used for MAS.**

Abstract Durum wheat (*Triticum turgidum* L. var. *durum* Desf.) is not only an important cereal crop for pasta making, but also a genetic resource for common wheat improvement. In the present study, a Greek durum wheat TRI 1796 was found to confer high resistance to all 22 tested isolates of *Blumeria graminis* f. sp. *tritici* (*Bgt*). Inheritance study on the F_1 plants and the F_2 population derived from the cross TRI 1796/PI 584832 revealed that the resistance in TRI 1796 was controlled by a single dominant gene, herein designated *Pm68*. Using the bulked segregant RNA-Seq (BSR-Seq) analysis combined with molecular analysis, *Pm68* was mapped to the terminal part of the short arm of chromosome 2B and flanked by markers *Xdw04* and *Xdw12*/*Xdw13* with genetic distances of 0.22 cM each. According to the reference genome of durum wheat cv. Svevo, the corresponding physical region spanned the *Pm68* locus was about 1.78-Mb, in which a number of disease resistance-related genes were annotated. This study reports the new powdery mildew resistance gene *Pm68* that would be a valuable resource for improvement of both common wheat and durum wheat. The co-segregated markers (*Xdw05–Xdw11*) developed here would be useful tools for marker-assisted selection (MAS) in breeding.

Introduction

Wheat (*Triticum aestivum* L., $2n=6x=42$, AABBDD) is one of the most widely cultivated cereal crops in the world, which provides about 18% of daily dietary calories consumed by humans (Tan et al. 2018; Li et al. 2019). Wheat production is greatly threatened by powdery mildew, caused by the fungal pathogen *Blumeria graminis* f. sp. *tritici* (*Bgt*) that has complex and variable virulence structures in natural populations (Wicker et al. 2013). Exploiting and utilizing broad-spectrum powdery mildew resistance (*Pm*) genes are important and

Communicated by Evans Lagudah.

Huagang He, Renkang Liu and Pengtao Ma have contributed equally to this work.

Electronic supplementary material The online version of this article (https://doi.org/10.1007/s00122-020-03681-2) contains supplementary material, which is available to authorized users.

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urgent to effectively control the mildew disease. Although more than a hundred *Pm* genes/alleles have been documented, a few can provide resistance to most or all *Bgt* isolates (McIntosh et al. 2017; Chen et al. 2019). Commonly, *Pm* genes originated from distant relatives of wheat have resistance to more isolates, such as *Pm12* from *Aegilops speltoides* Tausch (Song et al. 2009; Zhang et al. 2019) and *Pm21* from *Dasypyrum villosum* L. Candagy (Chen et al. 1995; He et al. 2018). Some rare natural variations occurred in common wheat genes can also confer broad-spectrum resistance to powdery mildew, such as *Pm24* (Lu et al. 2020), *Pm38/Yr18/Lr34/Sr57* (Krattinger et al. 2009) and *Pm46/Yr46/Lr67/Sr55* (Moore et al. 2015), among which the latter two genes also provide durable resistance to other diseases.

Durum wheat (*Triticum turgidum* L. var. *durum* Desf., simply *T. durum*, $2n=4x=28$, AABB) is a tetraploid wheat species that is a relatively small cereal crop mainly used for pasta making. Durum wheat possesses good resistance to leaf rust, stem rust and stripe rust and has been used for wheat improvement (Miedaner et al. 2019). However, most durum wheat accessions are susceptible to powdery mildew. From this crop, only three powdery mildew resistance genes have been identified in the past decades, including *Mld*, *Pm3h* and *PmDR147*. *Mld* is a recessive gene reported on 4B that has been combined with other powdery mildew resistance gene, such as *Pm2*, and used for wheat breeding (Bennett 1984). *Pm3h* is a dominant resistance gene located on chromosome 1AS, probably originated from an Ethiopian durum wheat accession (Srichumpa et al. 2005). *Pm3h* has been cloned and confirmed to be identical to *Pm3d* at the nucleotide sequence level (Yahiaoui et al. 2006). *PmDR147* is another dominant gene identified on 2AL of durum wheat accession DR147 (Zhu et al. 2004).

BgtYZ01, a super virulent isolate, was originally collected from Yangzhou, Jiangsu Province, China, where *Bgt* pathogen is prevailing (He et al. 2016, 2017). To effectively control this isolate, we attempted to screen new resistance resources from different relatives of wheat. From 100 durum wheat accessions, one resistant landrace TRI 1796 collected from Greece was obtained. TRI 1796 also conferred resistance to other 21 tested isolates of *Bgt* at the seedling stage and showed high resistance at the adult plant stage in fields of different regions. In the present study, genetic and comparative mapping of *Pm68* in durum wheat TRI 1796 was carried out, which will facilitate its application in both common wheat breeding and durum wheat breeding.

Materials and methods

Plant materials

A collection of 100 accessions of *T. durum* were kindly provided by Genebank Information System of the IPK Gatersleben (GBIS-IPK) (82) and Germplasm Resources Information Network (GRIN) (18). The Greek *T. durum* accession TRI 1796 highly resistant to isolate BgtYZ01 was crossed with susceptible Canadian *T. durum* accession PI 584832, and the generated 224 F_2 individuals and their corresponding $F_{2:3}$ families were used to map the powdery mildew resistance gene in TRI 1796. The resistant wheat cv. Yangmai 18 carrying *Pm21* and susceptible wheat cv. Yangmai 23 were kindly provided by Dr. Tongde Bie (Yangzhou Academy of Agricultural Sciences, China). The wheat lines carrying *Pm26* and *Pm42* were kindly provided by Prof. Zhiyong Liu (Institute of Genetics and Developmental Biology, Chinese Academy of Sciences, China).

Evaluation of powdery mildew response to isolate BgtYZ01

All *T. durum* accessions, F_1 and F_2 individuals as well as ~ 40 seedlings of each $F_{2:3}$ line derived from the cross TRI 1796/PI 584832 at one-leaf stage were inoculated with isolate BgtYZ01 that is a highly virulent isolate collected from Yangzhou (He et al. 2016). The inoculated plants were grown under a daily cycle of 16 h of light and 8 h of darkness at 22 ± 2 °C in a greenhouse. The powdery mildew responses were assessed at 8 days after inoculation. The responses of parents TRI 1796 and PI 584832 to a set of 22 *Bgt* isolates collected from different regions of China were also detected. The resistance spectrum of TRI 1796 was compared with those of the wheat lines carrying *Pm21*, *Pm26* and *Pm42*. Infection types (IT) were scored according to a 0–4 scale. ITs 0, 0, 1 and 2 were considered resistant, while those with an IT score of 3 and 4 were considered as susceptible (Li et al. 2020). Powdery mildew response of TRI 1796 at the adult plant stage in field was also estimated in Zhenjiang (Jiangsu Province), Yantai (Shandong Province) and Jinan (Shandong Province) in the 2018–2019 and 2019–2020 sowing seasons. Twenty seeds of each material were sown in a 1-m row. The flanking rows of TRI 1796 and PI 584832 were planted susceptible wheat cv. Yangmai 23 as the spreader. A mixture of *Bgt* isolates collected from the local site was inoculated on TRI 1796, PI 584832 and the spreader wheat at the jointing stage. At the milk stage, powdery mildew responses of the flag leaves were assessed on a 0–9 scale. Plants with

scale 0, 1–2, 3–4, 5–6 and 7–9 were considered immune, highly resistant, moderately resistant, moderately susceptible and highly susceptible, respectively (Li et al. 2011).

Bulked segregant RNA-Seq (BSR-Seq)

The BSR-Seq method was conducted on the F_2 individuals derived from the cross TRI 1796/PI 584832. Powdery mildew responses of the F_2 individuals were assessed at one-leaf stage. Then, equal size of the second leaves of 60 resistant and 60 susceptible individuals were pooled separately. Total RNA of the two bulks of leaf samples was separately extracted using Illumina TruSeq RNA Sample Prep Kit (Illumina, Inc., San Diego, CA, United States) to be used for RNA-Seq analysis using the platform of Illumina HiSeq 4000 (Beijing Southern Genome Research Technology Co., Ltd., Beijing, China). The raw sequencing reads generated were quality controlled using software Trimmomatic v0.36 (Bolger et al. 2014) with default parameters. Using software STAR v2.5.1b (Dobin et al. 2013), the clean reads were aligned to the genome assembly sequences of durum wheat cv. Svevo (https://www.interomics.eu/durum-wheat-genome; Maccaferri et al. 2019) with the mismatch rate of less than 5%. The uniquely mapped read pairs were used in further analysis. The read alignments were masked for PCR duplications and split for reads spanning introns before they were used to call SNPs and InDels using module "HaplotypeCaller" of software GATK v3.6 (McKenna et al. 2010). The resulting SNPs and InDels with sequencing depth less than 6 were discarded, and the remaining ones were applied to BSA. Only variants with allele frequency difference (AFD) > 0.6 and *P* value of Fisher's exact test on read count data $< 1e^{-8}$ were classified as resistance-associated variants and used as templates for marker development. The genome of durum wheat cv. Svevo was further used as a reference to call SNPs and InDels.

Development of molecular markers

A total of 56 SSR markers reported to be located on chromosome 2BS (Somers et al. 2004; Hua et al. 2009; Liu et al. 2012) were used to screen polymorphisms between two parents TRI 1796 and PI 584832. The genes showed polymorphic SNP and their corresponding 3000-bp upstream and 3000-bp downstream sequences in the target region revealed by BSR-Seq were retrieved from the reference genome of durum wheat cv. Svevo (https://www.interomics.eu/durum-wheat-genome; Maccaferri et al. 2019) and used to perform BLAST against the genome of durum wheat cv. Kronos (https://opendata.earlham.ac.uk/opendata/data/Triticum_turgidum/EI/v1.1). The conserved sequences flanking the InDel regions between durum wheat cv. Svevo and cv. Kronos were then used to design primer pairs. A total of 171 gene-derived primer pairs were used to detect the polymorphisms between TRI 1796 and PI 584832.

Marker analysis

The crude genomic DNA solution was prepared using the TE-boiling method (He et al. 2017). PCR amplification was performed with polymorphic markers between the two parents TRI 1796 and PI 584832. Each reaction mixture (25 µl) contained 1 × PCR buffer (Mg^{2+} free), 2.7 mM of $MgCl_2$, 0.2 mM of dNTP, 2 µM of each primer, 1 U of *Taq* DNA polymerase (Takara, Shiga, Japan) and 1 µl of DNA solution. DNA amplification was programmed at 94 °C for 3 min; 35 cycles of 94 °C for 20 s, 60 °C for 30 s, 72 °C for 1 min; at 72 °C for 5 min. PCR products of InDel markers were separated in 8% non-denaturing polyacrylamide gels, followed by silver staining. DNA fragment of *TRITD2Bv1G010030* harboring a SNP site was obtained by PCR with marker *Xdw04* and the polymorphism was then detected by Sanger sequencing.

Data analysis

Genetic analysis was performed on an F_2 population derived from the cross TRI 1796/PI 584832. Chi-squared (χ^2) test was used to determine the goodness-of-fit of the observed segregation ratio to theoretical Mendelian ratio.

Comparative genomics analysis

The corresponding genes of markers *Xdw03* and *Xdw16* linked to *Pm68* were used to BLAST against the genomes of durum wheat cv. Svevo and common wheat cv. Chinese Spring (http://www.wheat-urgi.versailles.inra.fr; IWGSC et al. 2018). Gene annotations in the target intervals of the two genomes were provided by the online databases. For comparative mapping, the genomic information of *T. dicoccoides* (https://wheat.pw.usda.gov/GG3/wildemmer; Avni et al. 2017), *T. urartu* (http://202.194.139.32; Lin et al. 2018) and *Aegilops tauschii* (http://aegilops.wheat.ucdavis.edu/ATGSP/index.php; Luo et al. 2017) were also considered.

Results

Powdery mildew responses of different durum wheat accessions against isolate BgtYZ01

A collection of 100 accessions of durum wheat was used to assess powdery mildew responses to *Bgt* isolate BgtYZ01 at the seedling stage. The results showed that only one Greek landrace TRI 1796 was highly resistant (IT 0), whereas all the others were completely susceptible (IT

4). The resistance spectrum of TRI 1796 was further analyzed. The results demonstrated that TRI 1796 conferred effective resistance to 20 isolates (including BgtYZ01) at the level of IT 0; and 2 isolates at the level of IT 1. It was also found that isolates BgtYZ01, Bgt10 and Bgt16 completely infected the wheat line carrying *Pm26* and isolates BgtYZ01, Bgt10, Bgt13 and Bgt19 completely infected the wheat line carrying *Pm42* (Fig. 1a; Table 1). These results revealed that the resistance in TRI 1796 differs from those of *Pm26* and *Pm42*. TRI 1796 and PI 584832 were also planted in fields of different regions during 2018–2020. Powdery mildew responses of their flag leaves were investigated at the milk stage. In each environment, TRI 1796 was immune (scale 0) to the mixture of *Bgt* isolates, whereas PI 584832 was highly susceptible (scale 7–9) (Fig. 1b). Hence, it was indicated that TRI 1796 could confer effective resistance against powdery mildew at the seedling and adult plant stages.

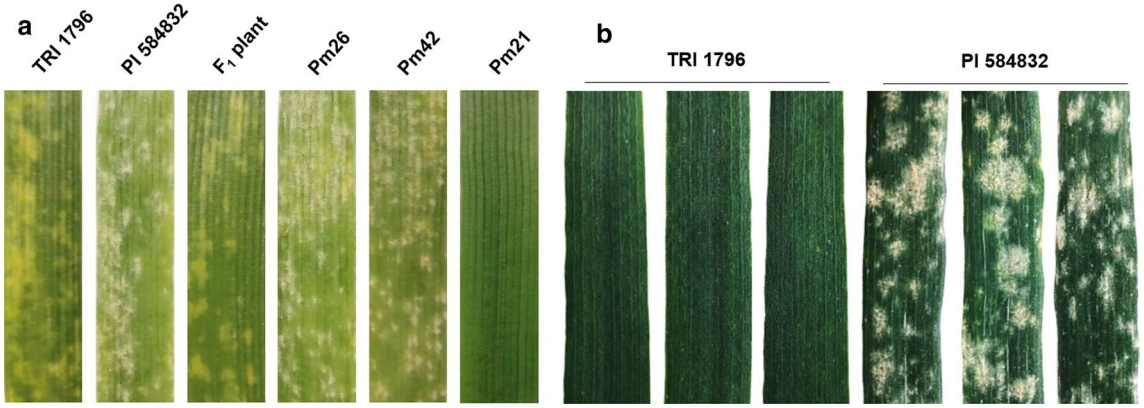

Fig. 1 Powdery mildew responses of durum wheat accessions TRI 1796 and PI 584832. **a** Powdery mildew responses of TRI 1796 and PI 584832 to *Bgt* isolate BgtYZ01 at one-leaf stage, compared with wheat lines carrying *Pm26*, *Pm42* and *Pm21*. **b** Powdery mildew responses of TRI 1796 and PI 584832 to *Bgt* natural population in field at the adult plant stage. The flag leaves shown here were collected from Yantai (Shandong Province) in the 2019–2020 sowing season

Table 1 Powdery mildew responses of durum wheat TRI 1796 and PI 584832 to different *Bgt* isolates from different regions of China, compared with the wheat lines carrying *Pm26*, *Pm42* or *Pm21*

Bgt isolate	Origin	TRI 1796	PI 584832	*Pm26*	*Pm42*	*Pm21*
Bgt01(BgtYZ01)	Yangzhou, Jiangsu Province	0	4	4	4	0
Bgt02	Yangzhou, Jiangsu Province	0	4	0	2	0
Bgt03	Zhenjiang, Jiangsu Province	0	4	0	0	0
Bgt04	Nanjing, Jiangsu Province	0	4	1	0	0
Bgt05	Yantai, Shandong Province	0	4	0	1	0
Bgt06	Yantai, Shandong Province	0	4	0	0	0
Bgt07	Jinan, Shandong Province	1	4	0	0	0
Bgt08	Kaifeng, Henan Province	0	4	1	0	0
Bgt09	Zhengzhou, Henan Province	0	4	0	0	0
Bgt10	Handan, Hebei Province	0	4	4	4	0
Bgt11	Shijiazhuang, Hebei Province	0	4	0	0	0
Bgt12	Shijiazhuang, Hebei Province	0	4	0	0	0
Bgt13	Baoding, Hebei Province	0	4	0	4	0
Bgt14	Xingtai, Hebei Province	0	4	0	0	0
Bgt15	Tianshui, Gansu Province	0	4	0	0	0
Bgt16	Lanzhou, Gansu Province	0	4	4	0	0
Bgt17	Taiyuan, Shanxi Province	0	4	0	0	0
Bgt18	Chengdu, Sichuan Province	1	4	0	0	0
Bgt19	Chengdu, Sichuan Province	0	4	2	0	0
Bgt20	Mianyang, Sichuan Province	0	4	0	2	0
Bgt21	Beijing	0	4	0	0	0
Bgt22	Wuhan, Hubei Province	0	4	0	0	0

Genetic characteristics of the powdery mildew resistance in TRI 1796

The resistant Greek durum wheat TRI 1796 was then crossed with the susceptible Canada durum wheat PI 584832 (IT 4). When inoculated with isolate BgtYZ01, all the F_1 plants showed resistance at the level of IT 0; (Fig. 1a) and the individuals of the F_2 population showed resistance at the level of IT 0; or susceptibility at the level of IT 4. Among the 224 F_2 plants, the segregation ratio of the resistant (166) and susceptible (58) individuals fits for 3:1, the theoretical Mendelian segregation ratio ($\chi^2 = 0.10$, $P = 0.76$). The F_3 families segregated 56 homozygous resistant: 110 segregating: 58 homozygous susceptible, fitting for the ratio 1:2:1 ($\chi^2 = 0.19$, $P = 0.91$). Therefore, it was concluded that the powdery mildew resistance in TRI 1796 was controlled by a single dominant gene, which has been designated *Pm68* based on the following analyses.

BSR-Seq analysis of *Pm68* in durum wheat

Using RNA-Seq, a total of 28,722,281 and 27,111,764 raw reads were obtained from the resistant bulk and the susceptible bulk, respectively. After quality control, 23,969,694 of 28,703,103 high-quality reads from the resistant bulk and 21,673,784 of 27,095,223 high-quality reads from the susceptible bulk were uniquely mapped to the genome of durum wheat cv. Svevo, respectively. A total of 141,335 SNPs and InDels between the resistant and susceptible bulks were identified by variant calling, and 40,584 of them had a depth > 6. The result showed that 116 SNPs distributed in different chromosomes may be associated with the resistance provided by *Pm68*. Among them, 42 SNPs were enriched in an about 8.6-Mb region (16.2–24.8 Mb) on the short arm of chromosome 2B (2BS) (Fig. 2).

Genetic mapping of *Pm68*

To screen polymorphic markers between two parents TRI 1796 and PI 584832, 56 SSR markers previously reported on 2BS were first used but none of them showed polymorphism. Then, 171 primer pairs were designed based on the InDels of the genes in the target regions of durum wheat cv. Svevo and cv. Kronos and 18 of them showed co-dominant polymorphisms between TRI 1796 and PI 584832. In addition, one SNP marker, *Xdw04*, corresponding to *TRITD2Bv1G010030*, was also developed (Fig. 3a, b; Table 2). Subsequently, these markers were used to genotype 224 F_2 individuals derived from the cross between resistant TRI 1796 and susceptible PI 584832. *Pm68* was closely flanked by markers *Xdw04* and *Xdw12*/*Xdw13*, and both the genetic distances between *Xdw04* and *Pm68* and *Xdw12*/*Xdw13* and *Pm68* were 0.22 cM (Fig. 4a). Seven markers (*Xdw05–Xdw11*) were confirmed to co-segregate with *Pm68*. Among them, *Xdw06*, *Xdw07* and *Xdw08* were resistance gene analog (RGA) markers that were developed based on NBS-LRR-like genes *TRITD2Bv1G010130*, *TRITD2Bv1G010230* and *TRITD2Bv1G010240*, respectively.

Comparative mapping of *Pm68* among related genomes

Fourteen gene-derived markers (*Xdw04–Xdw17*) were further used to perform comparative genomics analysis among the genomes of durum wheat cv. Svevo, common wheat cv. Chinese Spring, *T. dicoccoides*, *T. urartu*, and *Ae. tauschii* (Fig. 4b–f). The data demonstrated relatively good collinearity relationship between B genomes of durum wheat cv. Svevo and common wheat cv. Chinese Spring. In durum wheat, the corresponding genes of flanking markers *Xdw04* and *Xdw12* were *TRITD2Bv1G010030* (Chr2B:

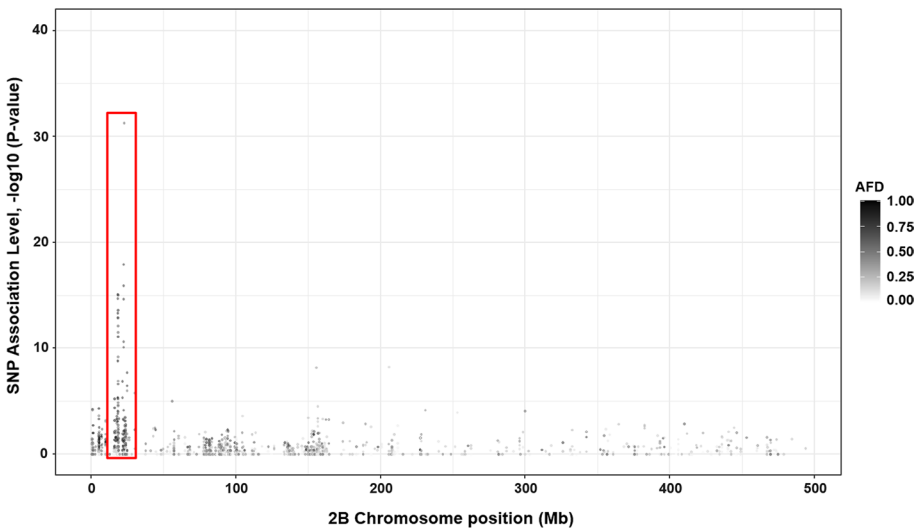

Fig. 2 Distribution of SNPs on chromosome 2B revealed by BSR-Seq. The red box shows the SNP-rich region

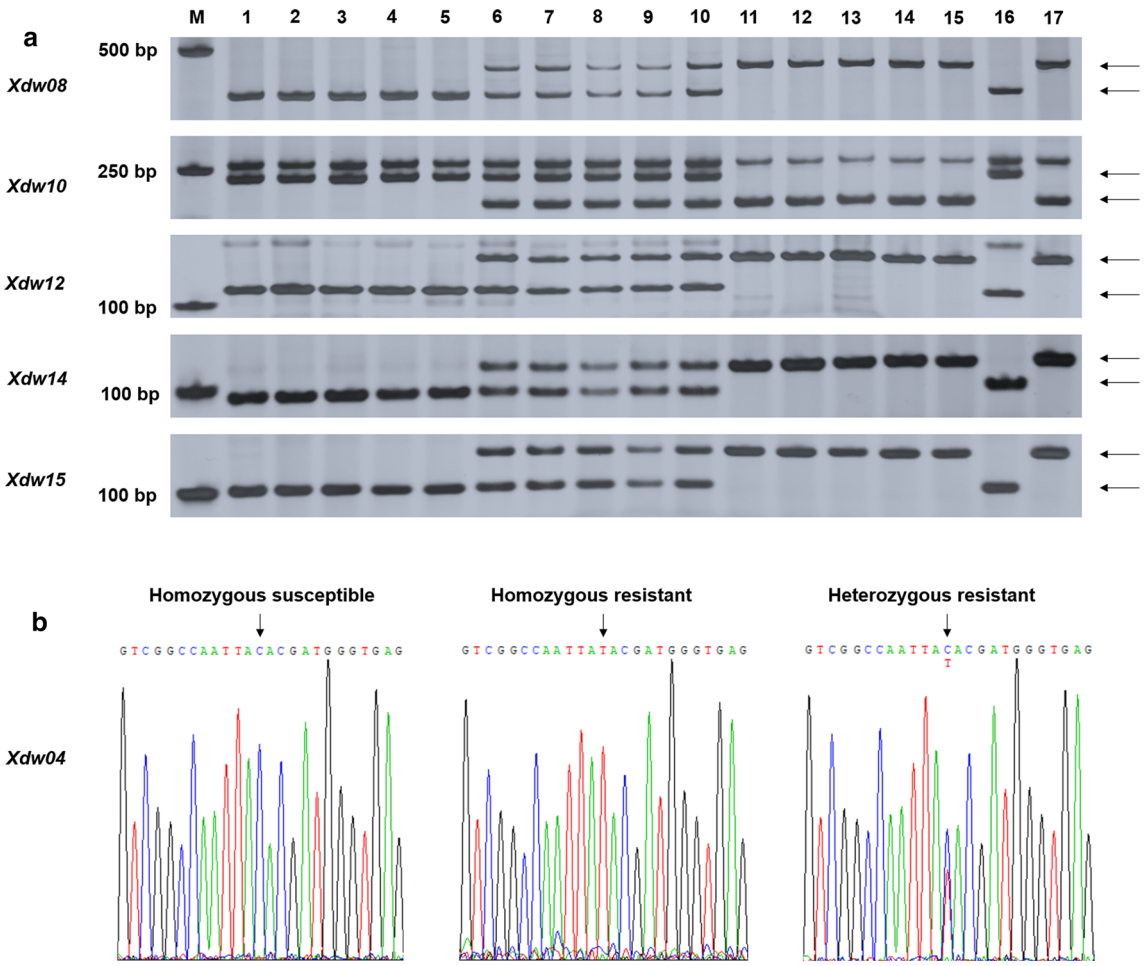

Fig. 3 Polymorphic patterns of six representative markers. **a** PCR amplification patterns of five InDel markers (*Xdw08*, *Xdw10*, *Xdw12*, *Xdw14*, and *Xdw15*). M, DL2000 DNA marker. 1–5, homozygous resistant F_2 plants. 6–10, heterozygous resistant F_2 plants. 11–15, homozygous susceptible F_2 plants. 16, resistant durum wheat TRI 1796. 17, susceptible durum wheat PI 584832. The polymorphic DNA bands are pointed by arrows. **b** DNA sequence chromatograms of PCR products obtained with SNP marker *Xdw04*. The polymorphic nucleotides marked by arrows are C, T and C/T in homozygous susceptible, homozygous resistant and heterozygous resistant individuals, respectively

21587671–21591163) and *TRITD2Bv1G010880* (Chr2B: 23374401–23375310), respectively. Therefore, *Pm68* was narrowed to a 1.78-Mb genomic region on 2BS a durum wheat cv. Svevo. According to the gene annotation published, a total of 84 genes exist in the target physical region. Among them, 23 genes may be associated with plant disease defense, including 8 NBS-LRR-like resistance genes, 6 exocyst complex component genes, 2 LRR receptor-like protein kinase gene, one lectin receptor kinase gene, receptor protein kinase gene, calcineurin B-like gene, F-box/RNI-like superfamily gene, wall-associated kinase gene, chitinase gene and heat shock protein 90 gene each (Table 3).

We further analyzed the evolution of these disease resistance-related genes in the genomes of wheat species. The data showed that all the 23 durum wheat genes had the corresponding homologous genes or perfectly matched genomic sequences in wheat Chinese Spring and 19 of them maintained good collinearity relationship between durum wheat and common wheat. In particular, the 8 NBS-LRR-like resistance genes of durum wheat shared 99–100% identities with those of common wheat. Of the 23 genes of durum wheat, 22 had homologous genes/sequences in the genome of wild emmer and relatively good collinearity was also observed. However, the identities (75–100%) of these genes/sequences between durum wheat and wild emmer were relatively lower than those between durum wheat and common wheat. In *T. urartu* genome, 20 of 23 disease resistance-related genes could be found with 68–97% identities, but they did not keep the same order as shown in durum wheat genome. In addition, most of the 23 durum wheat genes could not match *Ae. tauschii* genes (Supplementary Table S1). In conclusion, most of the predicted candidate

Table 2 Gene-derived molecular markers used for genetic mapping of *Pm68*

Marker	Forward primer	Reverse primer	Gene for primer designing
Xdw01	CTATGGCCGTCCAGCGAGTCCT	GGAGATCCTAATGAAAGCATCTGCCA	*TRITD2Bv1G002880*
Xdw02	GGGATCATCATCGGGTGCT	GAGTTCGATGACGCACCCGAATCCT	*TRITD2Bv1G004850*
Xdw03	GTGCTGCAGGCTGCAGCAGTAC	GCTTGTCAGCTCAGCTTATCAAACTC	*TRITD2Bv1G009130*
Xdw04	CCAAATGCCCACAAGGTGAGTTCGT	GGGCATGCACAATCCAGGGTCTG	*TRITD2Bv1G010030*
Xdw05	CCTTCAAATCCTCCTCTTGTACATG	GTTCGAAAAGAAGTTTGCGGGCTTGA	*TRITD2Bv1G010120*
Xdw06	GGATGCCCACTGGCCAAGA	CACACGGAGAAGACGCTGTTTC	*TRITD2Bv1G010130*
Xdw07	GACAGCAATACCTGAACTTCCAGCA	GGTACAGGCGCGTAAGCTTCTGCA	*TRITD2Bv1G010230*
Xdw08	CCGTACTTTGCTAGTGCCAGT	CCCTACATCTCTCCCCATCCAT	*TRITD2Bv1G010240*
Xdw09	GTCGGACATGTTGGGTTGTGC	CCCTTCTCCTTCAGTCAAATTTGTA	*TRITD2Bv1G010400*
Xdw10	TCAAATCTGAAACCGAAACCTGA	GCAGCTCTTCAACTAGAGAGTGTTG	*TRITD2Bv1G010610*
Xdw11	GGAGTTGAGGACAAGCAGCTAG	GTTGATAGCGGCATTCTACCCA	*TRITD2Bv1G010620*
Xdw12	CAGAAACCTAGTCCCGACTGTTC	GATCGCTTCGATGGCCGAAGT	*TRITD2Bv1G010880*
Xdw13	GAGACAACTACCGCGGTGCCCAGA	GATCGCTTCGATGGCCGAAGTGCT	*TRITD2Bv1G010890*
Xdw14	TAGGGCCGTGAGCCCACGTAT	GACGACGTTGGGTAGGGGTCCA	*TRITD2Bv1G011060*
Xdw15	GCTAATTACTACTCTCTTCGTTCCGA	GAATATGACCCAACAAATATCCGACA	*TRITD2Bv1G011070*
Xdw16	CCACATAGAAGATTTGTTCGAAGTC	ACTTGTGCGCCTGCACTTTCTGCAT	*TRITD2Bv1G011250*
Xdw17	CCACATAGAAGATTTGTTCGAAGTCA	CTTGTGCGCCTGCACTTTCTGCATAC	*TRITD2Bv1G011260*
Xdw18	CTGGATCCTGGATGGTATAGTGCCT	CCCACCATATGCAGGACAGTGTC	*TRITD2Bv1G038240*
Xdw19	CCTGATACCCGAGGGCTGAGGA	CTCCGTCGAGAATTAGCCGTCGGAT	*TRITD2Bv1G042200*

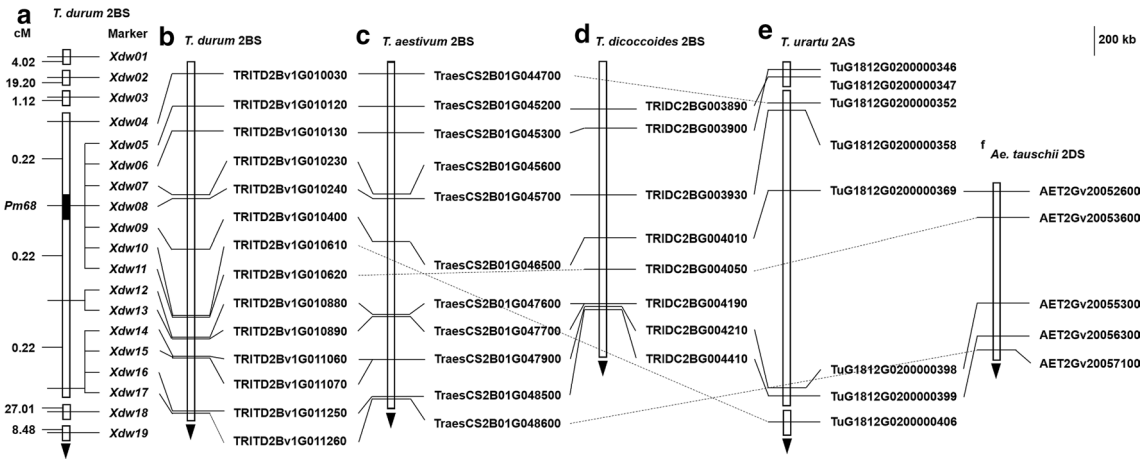

Fig. 4 Genetic map and comparative genomics map of chromosome 2BS carrying *Pm68*. **a** Genetic map of *Pm68* which is closely flanked by markers *Xdw04* and *Xdw12*/*Xdw13*. **b**–**f** Physical maps of homologous/orthologous regions of *Pm68* on 2BS of durum wheat (*T. durum*) cv. Svevo, common wheat cv. Chinese Spring (*T. aestivum*) and wild emmer (*T. dicoccoides*), 2AS of *T. urartu* and 2DS of *Ae. tauschii*, respectively. Bar: 200 kb

genes were highly conserved in the B- and A-subgenomes of wheat species.

Discussion

Three powdery mildew resistance gene, *Mld* (4B), *PmDR147* (2AL) and *Pm3h* (1AS), have been documented in durum wheat (Bennett 1984; Zhu el al. 2004; Srichumpa et al. 2005). In the present study, a dominant powdery mildew resistance gene *Pm68* was identified and mapped on the terminal part of 2BS in a Greek landrace durum wheat TRI 1796. In wheat crops, a total of six powdery mildew resistance genes have been reported on 2BS, including two dominant (*Ml5323* and *PmL962*), three recessive (*Pm26*, *Pm42* and *PmWE99*) and one incomplete dominant (*MlIW170*) genes. Among them, *Pm26*, *Pm42* and *MlIW170* are derived from *T. dicocoides*, *PmWE99* and *PmL962* from *Thinopyrum*

Table 3 Twenty-three predicted disease resistance-related genes in the *Pm68* locus on chromosome 2BS of durum wheat cv. Svevo, not including *TRITD2Bv1G010030* and *TRITD2Bv1G010880*, which corresponded to flanking markers *Xdw04* and *Xdw12*, respectively

Marker	Gene	Physical location (bp)	Predicted function
Xdw04	*TRITD2Bv1G010030*	21587671–21591163	NBS-LRR-like resistance protein
	TRITD2Bv1G010040	21596042–21596362	NBS-LRR-like resistance protein
	TRITD2Bv1G010050	21611497–21617977	Exocyst complex component
	TRITD2Bv1G010060	21628431–21631775	NBS-LRR-like resistance protein
	TRITD2Bv1G010090	21684876–21691842	Exocyst complex component
	TRITD2Bv1G010110	21695620–21696516	NBS-LRR-like resistance protein
	TRITD2Bv1G010120	21815911–21822104	Exocyst complex component
Xdw06	*TRITD2Bv1G010130*	21983992–21987186	NBS-LRR-like resistance protein
	TRITD2Bv1G010140	21994145–21997849	NBS-LRR-like resistance protein
	TRITD2Bv1G010180	22293677–22294875	Exocyst complex component
Xdw07	*TRITD2Bv1G010230*	22398122–22400896	NBS-LRR-like resistance protein
Xdw08	*TRITD2Bv1G010240*	22456742–22460374	NBS-LRR-like resistance protein
	TRITD2Bv1G010250	22463043–22463456	Wall-associated kinase
	TRITD2Bv1G010260	22463905–22464885	Exocyst complex component
	TRITD2Bv1G010290	22622566–22625937	Exocyst complex component
	TRITD2Bv1G010300	22629909–22631735	NBS-LRR-like resistance protein
	TRITD2Bv1G010310	22634154–22634546	Calcineurin B-like protein
	TRITD2Bv1G010330	22656867–22662690	F-box/RNI-like superfamily protein
	TRITD2Bv1G010660	23210823–23214726	Receptor protein kinase
	TRITD2Bv1G010690	23247501–23248373	LRR receptor-like protein kinase
	TRITD2Bv1G010700	23249910–23250431	LRR receptor-like protein kinase
	TRITD2Bv1G010730	23293003–23294124	Lectin receptor kinase
	TRITD2Bv1G010820	23354756–23355374	Chitinase
	TRITD2Bv1G010840	23358720–23361190	Heat shock protein 90
Xdw12	*TRITD2Bv1G010880*	23374401–23375310	Dirigent protein

intermedium, and *Ml5323* from *T. dicoccum* (Rong et al. 2000; Hua et al. 2009; Piarulli et al. 2012; Liu et al. 2012; Liang et al. 2015; Shen et al. 2015; Ma et al. 2016). We comparatively analyzed the chromosomal locations of the six reported genes and found that *Pm42*, *MlIW170*, *Ml5323* and *PmL962* are all in the direction of proximal side of their common markers *Xcau516*/*Xwg516*/*BF202540* (Fig. 5), and *Pm26* is considered to be located in the same genomic region or be allelic to *MlIW170* (Liu et al. 2012; Liang et al. 2015). Marker *Xcau516*/*Xwg516*/*BF202540* responds to the durum wheat gene *TRITD2Bv1G012960* (26.40 Mb). In this study, the closely linked marker *Xgw12* of *Pm68* responds to *TRITD2Bv1G010880* (23.37 Mb). Both *TRITD2Bv1G012960* and *TRITD2Bv1G010880* lie in the proximal direction of *Pm68*. Therefore, the physical location of *Pm68* is obviously different from those of the 5 reported genes *Pm26*, *Pm42*, *MlIW170*, *Ml5323* and *PmL962*.

The sixth powdery mildew resistance gene is *PmWE99* on 2BS. Although the relationship of *PmWE99* and markers *Xcau516*/*Xwg516*/*BF202540* are not clear, the genetic

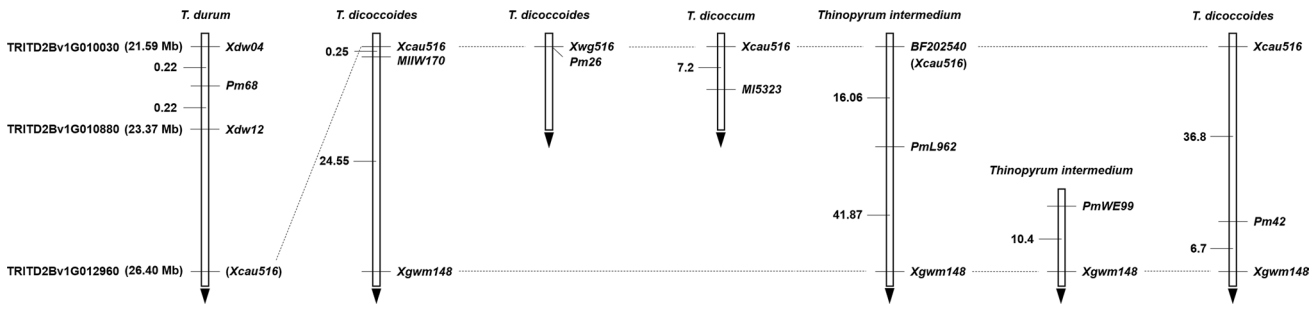

Fig. 5 Linkage map of *Pm68* compared with those of the powdery mildew resistance genes *MlIW170*, *Pm26*, *Ml5323*, *PmL962*, *PmWE99*, and *Pm42* using the linked markers *Xcau516*/*Xwg516*/*BF202540* and *Xgwm148* as references. The arrows point to the centromeres

distance between *PmWE99* and another marker *Xgwm148* is tested to be 10.4 cM (Fig. 5; Ma et al. 2016). *Xgwm148* can match at the position 101.24 Mb in the reference genome of durum wheat which lies between markers *Xgw18* (94.79 Mb) and *Xgw19* (105.45 Mb). In this study, the genetic distance between *Pm68* and *Xgw18* was 27.45 cM which is rather larger than 10.4 cM between *PmWE99* and *Xgwm148*. Furthermore, *Pm68* from durum wheat inherits as a dominant gene, whereas *PmWE99* from *Th. intermedium* is a recessive gene. Hence, it was indicated that *Pm68* also differs from *PmWE99*. Taken together, *Pm68* reported here is a new powdery mildew resistance gene found in wheat crops.

Pm68 was mapped to a 0.44-cM genetic interval, flanked by markers *Xdw04* and *Xdw12*, corresponding to the genes *TRITD2Bv1G010030* and *TRITD2Bv1G010880*, respectively, suggesting the *Pm68* locus responding to the physical region 21.59–23.37 Mb in the reference genome of durum wheat cv. Svevo. In the target region, a total of 84 genes have been annotated. Among them, 8 encode NBS-LRR-like resistance protein, 6 encode exocyst complex components and 9 encode other proteins, such as receptor-like kinase, wall-associated kinase, chitinase and heat shock protein 90. Comparative genomics analysis indicated that most of them were conserved in the B- and A-subgenomes of wheat species. Further comparative analyses of the sequences and the transcription patterns of these genes derived from resistant accession TRI 1796 and susceptible accession PI 584832 would allow to narrow the range of candidates of *Pm68*. However, there are many retrotransposons and transposons in the reference genome of durum wheat, accounting for 33.3%, which have potential impacts on the genome structure. Therefore, it is not excluded that the *Pm68* locus in TRI 1796 has different gene organization from the reference. In addition, more saturated molecular markers and a larger population are required for fine mapping and cloning of *Pm68*.

Powdery mildew is a serious disease of durum wheat. Commonly, excellent resistance resource is lacking for controlling this disease in durum wheat (Bennett 1984). We investigated 100 durum wheat accessions hold by GBIS-IPK (82) and GRIN (18) and found only one accession resistant to virulent isolate BgtYZ01, suggesting that the resistant durum wheat is relatively rare resource. Resistance spectrum analysis demonstrated that Greek accession TRI 1796 carrying *Pm68* possesses effective resistance to all 22 tested isolates of *Bgt* pathogen at the seedling stage, accompanied by hypersensitive response (HR). Interestingly, *Pm68* conferred complete immunity to powdery mildew at the adult plant stage in fields of three regions of China in two sowing seasons (2018–2019 and 2019–2020). It is suggested that *Pm68* resistance might be improved at the adult plant stage or there might be other gene(s) providing adult plant resistance in TRI 1796. Therefore, accession TRI 1796 would be a very useful resource for durum wheat breeding. When we mapped *Pm68*, seven co-segregated markers (*Xdw05–Xdw11*) were developed that would be used as powerful tools in marker-assisted selection (MAS). Furthermore, as one of the direct progenitors of common wheat, durum wheat is easy to cross with common wheat and generate offspring. Hence, *Pm68* in durum wheat TRI 1796 also has potential to be used for common wheat resistance improvement.

Acknowledgements This study was supported by grants from Jiangsu Agricultural Science and Technology Innovation Fund [CX(19)2042], Priority Academic Program Development of Jiangsu Higher Education Institutions, Jiangsu Education Department, Key Research and Development Program of Yantai City (2019YT06000470), National Natural Science Foundation of China (31872009), Leading Talents Plan of Hubei Academy of Agricultural Sciences (L2018013) and State Key Laboratory of Crop Biology in Shandong Agricultural University (2020KF07). The authors are thankful to Genebank Information System of the IPK Gatersleben (GBIS-IPK) and Germplasm Resources Information Network (GRIN) for providing durum wheat accessions, and Prof. Zhiyong Liu (Institute of Genetics and Developmental Biology, Chinese Academy of Sciences, China) for providing the wheat lines carrying *Pm26* and *Pm42*. The authors are also grateful to Prof. Robert McIntosh (The University of Sydney, Australia) for constructive comments on this manuscript.

Author contribution statement HH, YQG and SZ conceived and designed the experiments. HH, RL, PM, HD, HZ, QW, LY, SG and TL performed the experiments. HH, RL, PM and NH analyzed the data. HH, RL, YQG and SZ wrote the paper.

Compliance with ethical standards

Conflict of interest The authors declare that they have no conflict of interest.

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