Theoretical and Applied Genetics (2021) 134:887–896
https://doi.org/10.1007/s00122-020-03739-1

ORIGINAL ARTICLE

Characterization of a new gene for resistance to wheat powdery mildew on chromosome 1RL of wild rye *Secale sylvestre*

Huagang He[1,2,3] · Haonan Du[2] · Renkang Liu[2] · Tianlei Liu[1] · Lijun Yang[4] · Shuangjun Gong[4] · Zongxiang Tang[5] · Haimei Du[5] · Cheng Liu[6] · Ran Han[6] · Weihong Sun[2] · Lei Wang[7] · Shanying Zhu[3]

Received: 7 August 2020 / Accepted: 18 November 2020 / Published online: 2 January 2021
© Springer-Verlag GmbH Germany, part of Springer Nature 2021

Abstract

Key message ***PmSESY*, a new wheat powdery mildew resistance gene was characterized and genetically mapped to the terminal region of chromosome 1RL of wild rye *Secale sylvestre*.**

Abstract The genus *Secale* is an important resource for wheat improvement. The *Secale* species are usually considered as non-adapted hosts of *Blumeria graminis* f. sp. *tritici* (*Bgt*) that causes wheat powdery mildew. However, as a wild species of cultivated rye, *S. sylvestre* is rarely studied. Here, we reported that 25 *S. sylvestre* accessions were susceptible to isolate BgtYZ01, whereas the other five confer effective resistance to all the tested isolates of *Bgt*. A population was then constructed by crossing the resistant accession SESY-01 with the susceptible accession SESY-11. Genetic analysis showed that the resistance in SESY-01 was controlled by a single dominant gene, temporarily designated as *PmSESY*. Subsequently, combining bulked segregant RNA-Seq (BSR-Seq) analysis with molecular analysis, *PmSESY* was mapped into a 1.88 cM genetic interval in the terminus of the long arm of 1R, which was closely flanked by markers *Xss06* and *Xss09* with genetic distances of 0.87 cM and 1.01 cM, respectively. Comparative mapping demonstrated that the corresponding physical region of the *PmSESY* locus was about 3.81 Mb in rye cv. Lo7 genome, where 30 disease resistance-related genes were annotated, including five NLR-type disease resistance genes, three kinase family protein genes, three leucine-rich repeat receptor-like protein kinase genes and so on. This study gives a new insight into *S. sylvestre* that shows divergence in response to *Bgt* and reports a new powdery mildew resistance gene that has potential to be used for resistance improvement in wheat.

Communicated by Beat Keller.

✉ Huagang He
hghe@mail.ujs.edu.cn

✉ Shanying Zhu
zhushanying@mail.ujs.edu.cn

1 School of Life Sciences, Jiangsu University, Zhenjiang 212013, China

2 School of Food and Biological Engineering, Jiangsu University, Zhenjiang 212013, China

3 School of Environment, Jiangsu University, Zhenjiang 212013, China

4 Institute of Plant Protection and Soil Science, Hubei Academy of Agricultural Sciences, Wuhan 430064, China

5 College of Agronomy, Sichuan Agricultural University, Chengdu 611130, China

6 Crop Research Institution, Shandong Academy of Agricultural Sciences, Jinan 250100, China

7 INDEL Biological Technology Corporation, Nanjing 210000, China

Introduction

Modern cultivated wheat (*Triticum aestivum* L., $2n=6x=42$, AABBDD) is one of the most cultivated cereal crops that possesses abundant germplasm resources for improvement of its various desirable traits (Li et al. 2019a, b). Usually, wheat germplasm resources are classified into primary, secondary, and tertiary gene pools. The primary gene pool consists of hexaploid species containing AABBDD genome and their tetraploid and diploid progenitors, such as common wheat (AABBDD), *T. spelta* (AABBDD), *T. dicoccoides* (AABB), *T. durum* (AABB), *T. urartu* (AA), and *T. tauschii* (DD). The secondary gene pool includes the relatives sharing at least one homoloeogous genome with cultivated wheat, such as *T. timopheevii* (AAGG) and *Aegilops* species containing S genome related to B genome. Except the species from the primary and secondary gene pools, other distant species in *Triticeae* belong to the tertiary gene pool, such as *Secale cereale* (RR) and *Dasypyrum villosum* (VV) (Feuillet et al. 2007).

Blumeria graminis f. sp. *tritici* (*Bgt*) is a biotrophic fungal pathogen that causes powdery mildew in wheat and subsequent yield losses ranging from 5 to 40%. Exploring effective powdery mildew resistance genes and development of resistant wheat cultivars are important for controlling this disease (He et al. 2018; Li et al. 2020a). Up to now, 86 formally designated wheat powdery mildew resistance genes/alleles have been characterized from the primary (63), secondary (9), and tertiary (14) gene pools of wheat (McIntosh et al. 2017; Li et al. 2020b; He et al. 2020). Among the genes from the tertiary gene pool, *Pm7*, *Pm8*, *Pm17*, *Pm20*, and *Pm56* are derived from *S. cereale* (Friebe et al. 1994; Singh et al. 2018; Hao et al. 2018), *Pm21*, *Pm55*, *Pm62*, and *Pm67* from *D. villosum* (Chen et al. 1995; Zhang et al. 2016; Zhang et al. 2018a, b), *Pm40* and *Pm43* from *Thinopyrum intermedium* (Luo et al. 2009; He et al. 2009), *Pm51* from *Th. ponticum* (Zhan et al. 2014), *Pm2b* from *Agropryron cristatum* (Ma et al. 2015), and *Pm29* from *Ae. ovata* (Zeller et al. 2002).

The genus *Secale* (rye) consists of four species, including *S. cereale*, *S. vavilovii*, *S. strictum* (syn. *S. montanum*), and *S. sylvestre* (syn. *S. fragile*, Tibetan rye). Among them, *S. sylvestre* is an annual and autogamous wild species. It grows in sandy regions of rivers, shores, and steppe ecosystems, distributing from Hungary to Mongolia. Because morphological, cytogenetic, and molecular characters are obviously different from those of other rye species, *S. sylvestre* is considered to be highly divergent from other rye species (Tang et al. 2011). In wheat breeding, different species in the genus *Secale* are important germplasm resources; however, *S. sylvestre* is rarely concerned by breeders and researchers so far.

In the present study, we exploited the possibility of using *S. sylvestre* as a genetic resource for wheat improvement and identified five accessions conferring effective resistance against different isolates of *Bgt*. Using a population derived from the cross between the resistant accession SESY-01 and the susceptible accession SESY-11 of *S. sylvestre*, a new powdery mildew resistance gene, *PmSESY*, was mapped to the terminus of the long arm of chromosome 1R.

Materials and methods

Plant materials

S. sylvestre accessions were kindly provided by the National Centre for Plant Genetic Resources, Polish Genebank (NCPGR) (10), Genebank Information System of the IPK Gatersleben (GBIS-IPK) (12), and Germplasm Resources Information Network (GRIN) (8) (Table 1). *S. strictum* (PI 401405), *S. vavilovii* (PI 573649), *S. cereale* cv. Petkus (PI 428373), and cv. Kustro (PI 392065) were obtained from GRIN. The *S. sylvestre* accession SESY-01 (original accession number: 31356 in NCPGR) immune to isolate BgtYZ01 was crossed with the highly susceptible accession SESY-11 (original accession number: R 801 in GBIS-IPK), and the generated 345 F_2 individuals and their corresponding $F_{2:3}$ families were utilized to genetically map the powdery mildew resistance gene in SESY-01. All plants used in this study were grown under a daily cycle of 16 h of light and 8 h of darkness at 22 ± 2 °C in a greenhouse.

Evaluation of powdery mildew response to Bgt isolates

All plants of *S. sylvestre* accessions, F_1 and F_2 individuals, and ~ 50 seedlings of each $F_{2:3}$ line generated from the cross SESY-01/SESY-11 were inoculated with *Bgt* isolate BgtYZ01 at one-leaf stage. The powdery mildew responses were evaluated at eight days after inoculation. The responses of the resistant accessions to another 15 *Bgt* isolates, collected from different regions of China, were also assessed, using the susceptible accession SESY-11 as control. Infection types (IT) were scored according to a 0 to 4 scale (Li et al. 2020b).

Non-denaturing fluorescence in situ hybridization (ND-FISH) analysis

S. sylvestre accessions SESY-01, SESY-11, *S. strictum*, *S. vavilovii*, *S. cereale* cv. Petkus, and cv. Kustro were used for ND-FISH assay. Root-tip metaphase chromosomes were prepared using the method described by Han et al. (2006). ND-FISH combined with oligonucleotide (oligo) probes Oligo-pSc119.2-1(Tang et al. 2014) and $(AAC)_6$ was used to identify individual rye chromosomes. The ND-FISH procedure was carried out as described by Fu et al. (2015).

Molecular detection of rye species

The primers HAdh2e1 and HAdh8e1r were used for amplification of the partial *Adh1* gene from *S. sylvestre* accessions (Petersen and Seberg 1998; Petersen et al. 2004). PCR amplification was carried out using the PrimeSTAR Max Premix (TaKaRa, Shiga, Japan). The obtained PCR products were extracted from agarose gel and then sequenced using the Sanger method. The phylogenetic tree was constructed by the neighbor-joining method in the MEGA7.0 software (Kumar et al. 2016).

Bulked segregant RNA-Seq (BSR-Seq)

The BSR-Seq method was conducted on the F_2 individuals derived from the cross SESY-01/SESY-11. After estimating of the powdery mildew responses of F_2 plants at one-leaf stage, equal size of the second leaves of 50 resistant and 50 susceptible plants was sampled as the R

Table 1 *S. sylvestre* germplasms and their responses to *Bgt* isolate BgtYZ01

Code	Powdery mildew response	Origin	Original accession	Provider
SESY-01	0	Unknown	31356	NCPGR
SESY-02	4	Unknown	31357	NCPGR
SESY-03	4	Unknown	31358	NCPGR
SESY-04	4	Unknown	31359	NCPGR
SESY-05	4	Unknown	31360	NCPGR
SESY-06	4	Unknown	31361	NCPGR
SESY-07	4	Unknown	31362	NCPGR
SESY-08	4	Unknown	31363	NCPGR
SESY-09	4	Unknown	31364	NCPGR
SESY-10	4	Unknown	31365	NCPGR
SESY-11	4	Former Soviet Union	R 801	GBIS-IPK
SESY-12	4	Unknown	R 802	GBIS-IPK
SESY-13	4	Unknown	R 806	GBIS-IPK
SESY-14	4	Poland	R 807	GBIS-IPK
SESY-15	4	Russia	R 812	GBIS-IPK
SESY-16	0	Unknown	R 873	GBIS-IPK
SESY-17	4	Unknown	R 891	GBIS-IPK
SESY-18	4	Unknown	R 901	GBIS-IPK
SESY-19	0	Unknown	R 953	GBIS-IPK
SESY-20	4	Unknown	R 956	GBIS-IPK
SESY-21	4	Unknown	R 957	GBIS-IPK
SESY-22	4	Hungary	R 1033	GBIS-IPK
SESY-23	0	Ukraine	PI 592294	GRIN
SESY-24	4	Ukraine	PI 614647	GRIN
SESY-25	4	Ukraine	PI 614648	GRIN
SESY-26	4	Hungary	PI 615332	GRIN
SESY-27	4	Hungary	PI 615334	GRIN
SESY-28	0	Ukraine	PI 618674	GRIN
SESY-29	4	Bulgaria	PI 618675	GRIN
SESY-30	4	Poland	PI 618676	GRIN

NCPGR National Centre for Plant Genetic Resources, Polish Genebank; *GBIS-IPK* Genebank Information System of the IPK Gatersleben; *GRIN* Germplasm Resources Information Network

and S pools, respectively. Total RNA extraction, RNA-Seq analysis, quality control, and SNP/InDel calling were described in Wu et al. (2018) and He et al. (2020). SNP/InDel was called using the genome of rye cv. Lo7 (Martis et al. 2013; Rabanus-Wallace et al. 2019) as a reference and assessed by smoothed *G* (*G'*) value (Lott et al. 2009).

Development of molecular markers

In the target genomic region harboring powdery mildew resistance gene in SESY-01, rye genes (Martis et al. 2013; Rabanus-Wallace et al. 2019) containing the InDels and SNPs, revealed by BSR-Seq analysis, were used for developing molecular markers. Primers of InDel markers were designed according to the conserved sequences surrounding the polymorphism sites using Primer Premier 5.0. Primers of SNP markers were designed using the CAPS/dCAPS designer (Li et al. 2018). Polymorphic markers between the two parents SESY-01 and SESY-11 are listed in Table 2.

Marker analysis

Genomic DNA solution of each plant was prepared by the TE-boiling method and PCR amplified as described by He et al. (2017). PCR products of the InDel markers were directly

Table 2 Powdery mildew responses of *S. sylvestre* accessions to different isolates of *Bgt*

Bgt isolate	SESY-01	SESY-16	SESY-19	SESY-23	SESY-28	SESY-11
Bgt01(BgtYZ01)	0	0	0	0	0	4
Bgt02	0	0	0	0	0	4
Bgt03	0	1	1	0	0	4
Bgt04	0	0	0	0	0	4
Bgt05	0	0	0	0	0	4
Bgt06	0	0	0	1	0	4
Bgt07	0	0	0	0	0	4
Bgt08	0	0	1	0	0	4
Bgt09	0	0	0	0	0	4
Bgt10	0	0	0	0	0	4
Bgt11	0	0	0	0	1	4
Bgt12	0	1	0	0	0	4
Bgt13	0	0	0	0	0	4
Bgt14	0	0	0	0	0	4
Bgt15	0	0	0	0	1	4
Bgt16	0	0	0	0	0	4

separated in 10% non-denaturing polyacrylamide gel electrophoresis (PAGE), and those of the SNP markers were cut with the corresponding restriction enzymes (Takara, Shiga, Japan) (Table 2) prior to separation in 10% PAGE. DNA bands were visualized by silver staining.

Genetic analysis and comparative mapping

Genetic analysis of powdery mildew resistance gene in accession SESY-01 was conduct on an F_2 population derived from the cross SESY-01/SESY-11. Chi-squared (χ^2) test was used to determine the goodness-of-fit of the observed segregation ratio to the theoretical Mendelian ratio. Rye contigs corresponding to polymorphic markers were used to perform BLAST against the genomes of rye cv. Lo7 (Martis et al. 2013; Rabanus-Wallace et al. 2019) and wheat cv. Chinese Spring (IWGSC et al. 2018) to generate comparative genomics maps. Gene annotations of rye and wheat genomes were adopted to analyze the gene composition in the corresponding target region of *S. sylvestre* that was closely flanked by markers *Xss06* and *Xss09*.

Results

Powdery mildew responses of different *S. sylvestre* accessions to Bgt isolates

Thirty *S. sylvestre* accessions obtained from different international germplasm resource institutions were tested against BgtYZ01, a virulent *Bgt* isolate prevailing in Yangzhou, Jiangsu province (China). The results demonstrated that five accessions (SESY-01, SESY-16, SESY-19, SESY-23, and SESY-28) were immune (IT 0), whereas the others were all complete susceptible (IT 4) (Fig. 1; Table 1). The resistance spectra of the above five resistant accessions were then assessed with another 15 *Bgt* isolates, collected from different regions of China. The five BgtYZ01-resistant accessions were still highly resistant, among which, accession SESY-01 conferred immunity to all isolates tested (Table 2).

Morphological, cytological and molecular characterization of *S. sylvestre* accessions

All plants of *S. sylvestre* accessions used in this study had slender culms and set slender seeds. These morphological

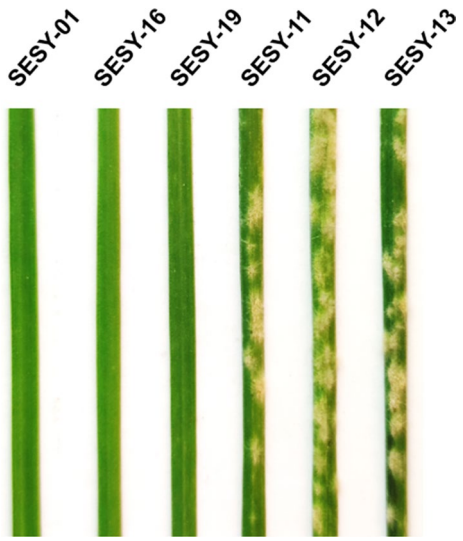

Fig. 1 Powdery mildew responses of different accessions of *S. sylvestre* to *Bgt* isolate BgtYZ01

characteristics were obviously distinguished from those of the other rye species (Tang et al. 2011). ND-FISH assay showed that the probe $(AAC)_6$ produced signals on the satellites of 1RS arms of *S. sylvestre* accessions SESY-01 and SESY-11; however, these signals disappeared from the other four *Secale* accessions (Fig. 2a). This result was consistent with the findings of Cuadrado and Jouve (2002). In the phylogenetic tree based on the partial sequence of *Adh1* gene, SESY-01, SESY-11, and *S. sylvestre* accession H4416 (AY294170) were clustered in the same clade (Fig. 2b). Taken together, accessions SESY-01 and SESY-11 belong to the species *S. sylvestre*.

Genetic characteristics of the powdery mildew resistance in accession SESY-01

S. sylvestre accession SESY-01 immune to all tested *Bgt* isolates was crossed to the highly susceptible accession SESY-11 generating F_1, F_2, and F_3 populations. After inoculation with isolate BgtYZ01, all the F_1 plants displayed immunity. In the F_2 population consisting of 345 individuals, 257 and 88 were resistant and susceptible, respectively, which fits to the ratio 3:1 ($\chi^2 = 0.047$, $P = 0.828$). In the F_3 families, 90 were homozygous resistant, 167 were segregating, and 88 were homozygous susceptible, fitting to the ratio 1:2:1 ($\chi^2 = 0.374$, $P = 0.829$). Hence, it was concluded that the powdery mildew resistance in accession SESY-01 is governed by a single dominant gene, temporarily designated *PmSESY*.

BSR-Seq analysis of PmSESY

Using the RNA-Seq method, a total of 38,604,908 and 30,192,360 raw reads were obtained from the resistant and susceptible bulks, respectively. After quality control, 21,236,142 of 38,576,518 high-quality reads from the resistant pool and 17,521,940 of 30,156,107 high-quality reads from the susceptible pool were uniquely mapped to the genome of rye cv. Lo7, respectively. A total of 574,339 SNPs and InDels between the resistant and susceptible bulks were identified by variant calling, and 37,455 of them had a depth > 6. The results demonstrated that 157 SNPs and 15 InDels distributed in different chromosomes, of which, 137 SNPs and 13 InDels distributed on chromosome 1R. Further analysis revealed that most SNPs (84) and InDels (5) lied in 695–722 Mb on the long arm of chromosome 1R (1RL) (Fig. 3a, b), suggesting that this region provides powdery mildew resistance in SESY-01.

Genetic mapping of *PmSESY*

Based on the results obtained by the BSR-Seq analysis, rye genes located in 695–722 Mb of chromosome 1RL, which contained InDels and SNPs, were used for development of molecular markers. As a result, a total of 15 polymorphic markers between the two parents SESY-01 and SESY-11 were obtained, among which, 3 (*Xss01*, *Xss02*, and *Xss*15) were InDel markers and 12 (*Xss03–Xss14*) were SNP markers (Fig. 4; Table 3). These markers were then used to

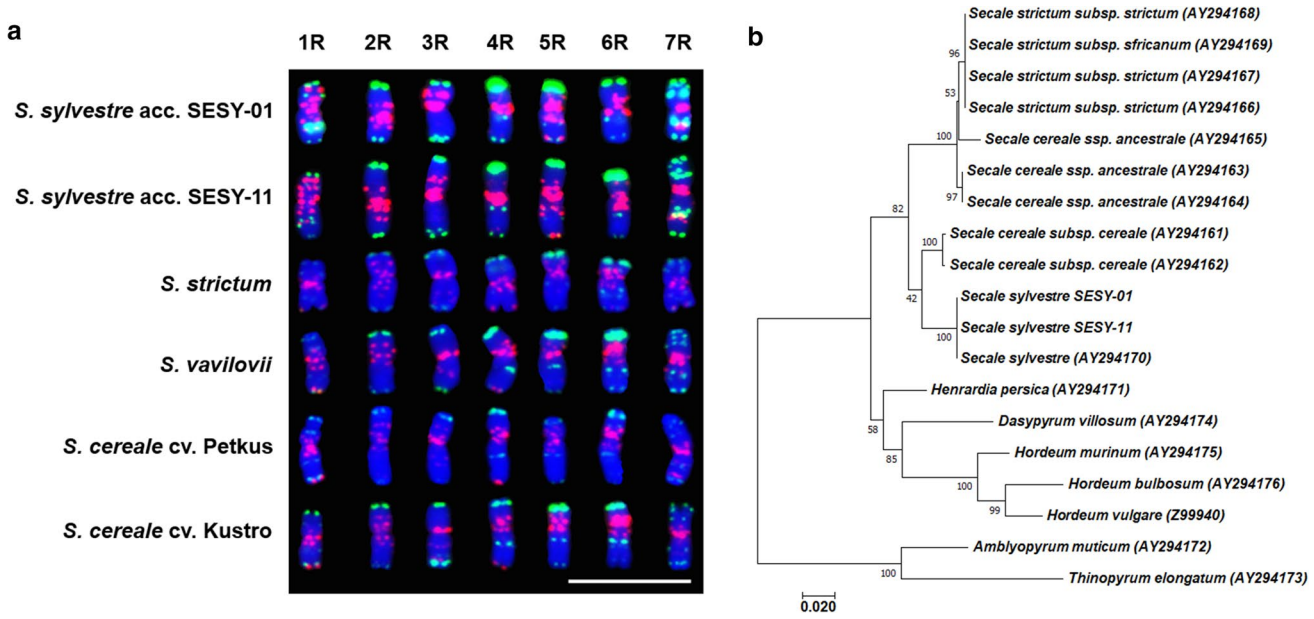

Fig. 2 Cytological and molecular detection of *S. sylvestre* accessions. **a** ND-FISH analysis of mitotic metaphase chromosomes of six *Secale* accessions using oligo probes Oligo-pSc119.2–1 (green) and $(AAC)_6$ (*red*). Chromosomes were counterstained with DAPI (*blue*). Scale bar: 10 μm. **b** Phylogenetic tree based on the partial sequences of the *Adh1* gene. GenBank accession numbers are shown in brackets

genotype 345 F_2 individuals derived from the cross SESY-01/SESY-11. *PmSESY* was closely flanked by markers *Xss06* and *Xss09* with the corresponding genetic distances of 0.87 cM and 1.01 cM, respectively. In addition, two markers *Xss07* and *Xss08* were confirmed to co-segregate with *PmSESY* (Fig. 5a).

Comparative mapping of *PmSESY* among the genomes of *S. sylvestre*, rye and wheat

Fifteen gene-derived markers, *Xss01–Xss15*, were used to carry out comparative genomics analysis among the genomes of *S. sylvestre*, rye cv. Lo7 and wheat cv. Chinese Spring. All the 15 markers were mapped to chromosome 1RL of the rye genome assembly. Furthermore, except the corresponding gene of marker *Xss13*, which could not be found on wheat 1AL, the corresponding genes of all the other markers could be well assigned to wheat chromosomes 1AL, 1BL, and 1DL (Fig. 5b–d). These results indicated that there is a good collinearity relationship among the tested genomic regions of *S. sylvestre* 1RL, rye 1RL, wheat 1AL, 1BL, and 1DL.

In the rye cv. Lo7 genome, the corresponding genes of flanking markers *Xss06* and *Xss09* were SECCE1Rv1G0061930 (Chr1R: 717,893,790–717,896,874) and SECCE1Rv1G0062920 (Chr1R: 721,703,553–721,707,206), respectively. Hence, *PmSESY* could be narrowed to a 3.81-Mb genomic region in the terminus of rye chromosome 1RL, where 98 genes (excluding SECCE1Rv1G0061930 and SECCE1Rv1G0062920) exist. Among them, 30 genes were involved in plant disease defense according to the annotation of rye genome, including five nucleotide-binding leucine-rich-repeat receptor (NLR)-type disease resistance genes, three kinase family protein genes, three leucine-rich repeat receptor-like protein kinase genes, three E3 ubiquitin-protein ligase genes, nine F-box protein genes, two zinc finger BED domain-containing protein genes, as well as one pathogen-related protein gene, lectin receptor kinase gene, calmodulin gene, calcium-binding protein gene, and flavin-containing monooxygenase gene each (Table 4). It was suggested that the *PmSESY* locus lies in a genomic region enriched with disease resistance-related genes.

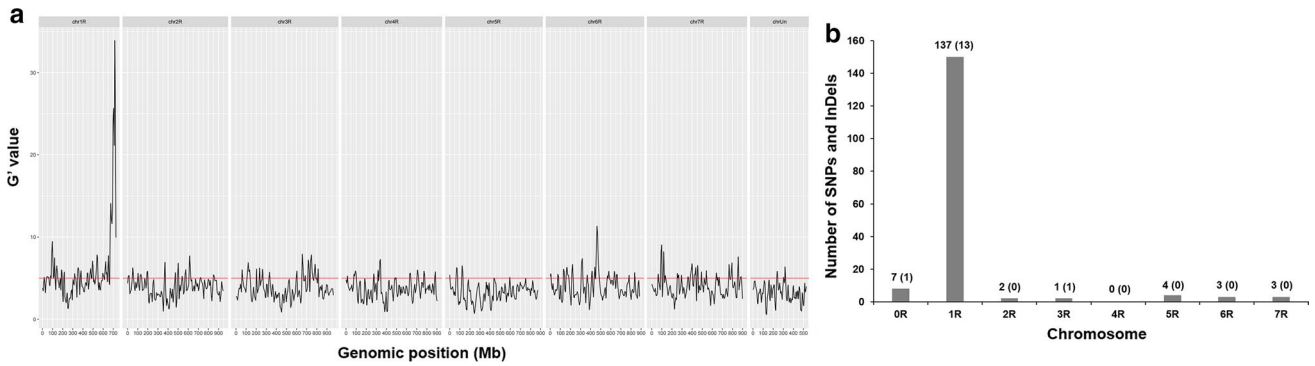

Fig. 3 BSR-Seq analysis of *PmSESY*. **a** Distribution of SNPs and InDels on different rye chromosomes. **b** Number of polymorphic SNPs on different rye chromosomes. The number of InDels in each chromosome is shown in bracket

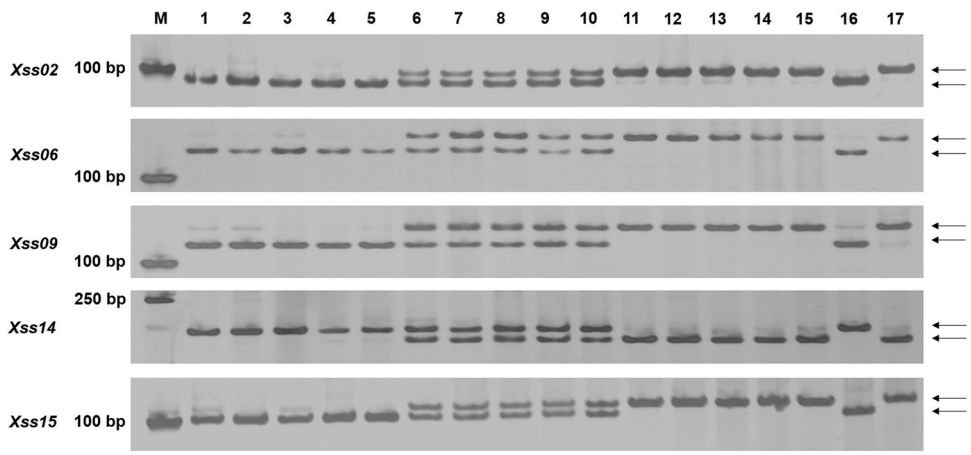

Fig. 4 Polymorphic patterns of five representative markers (*Xss02*, *Xss06*, *Xss09*, *Xss14*, and *Xss15*). M, DL2000 DNA marker. 1–5, homozygous resistant F_2 plants. 6–10, heterozygous resistant F_2 plants. 11–15, homozygous susceptible F_2 plants. 16, resistant accession SESY-01. 17, susceptible accession SESY-11. The polymorphic DNA bands are pointed by arrows

Table 3 Molecular markers used for genetic mapping of *PmSESY*

Marker	Type	Enzyme	Forward primer	Reverse primer	Rye gene for primer designing
Xss01	InDel	–	AGGATGAAAGAGAGAAGCCACGT	CGCTGGGCCTTAGTCGGT	SECCE1Rv1G0058490
Xss02	InDel	–	GAGGAGGTCGAAGCGCTTG	TCTCGCTGTTCGCGCAGCA	SECCE1Rv1G0060750
Xss03	SNP	Sma I	CGGCTCTGCGCAGTGTGAACCCG	CTACAGCGACAACGGAACAA	SECCE1Rv1G0060900
Xss04	SNP	BamHI	TTCTTCACCTTCTACCTGGGATC	CCATGATCTCGTAGGCCACT	SECCE1Rv1G0061550
Xss05	SNP	Mlu I	TCTTGCAGAAGATCATCGACGCG	TGGTGTGGACTATGGTGGTG	SECCE1Rv1G0061800
Xss06	SNP	Sma I	GGCGCAGATGGTGTGATCCCCGG	CCCCCGCAAAAGATAAAAAT	SECCE1Rv1G0061930
Xss07	SNP	Kpn I	TCAGTCTCTTGAGCGTGGCGGTA	TTGTGTGACAACGGCGTATT	SECCE1Rv1G0062310
Xss08	SNP	Mlu I	GAACTCCGGGGTTTATGTAAACG	AGTCACCAGCAACTCCGACT	SECCE1Rv1G0062320
Xss09	SNP	Sma I	TGCAACCATGGTTTCTGCGTCCC	GCCATCACCTGATCCAAGAT	SECCE1Rv1G0062920
Xss10	SNP	Pst I	CTCGCAGAATTCCTGAGAGGCTC	AAAAGGTGGTACCTTCGGCT	SECCE1Rv1G0062970
Xss11	SNP	Xho I	CTCGCAGAATTCCTGAGAGGCTC	AAAAGGTGGTACCTTCGGCT	SECCE1Rv1G0062980
Xss12	SNP	Nhe I	ATACATTTCCTGTACGTCGCTAG	TTGTGCAGAGGAATGACAGC	SECCE1Rv1G0063000
Xss13	SNP	Pvu II	GCTAGGTGCTAGGCAGGGCAGCT	CCCCTTCCCTTGTTACGATT	SECCE1Rv1G0063040
Xss14	SNP	EcoR I	CCCATTTCCGCACCGCTTGAATT	CATCGTACAGCGACAACACC	SECCE1Rv1G0063260
Xss15	InDel	–	AAGTTCTCCAGCTCCAACGTGA	CACCTGGAACACATGGCGAGT	SECCE1Rv1G0063490

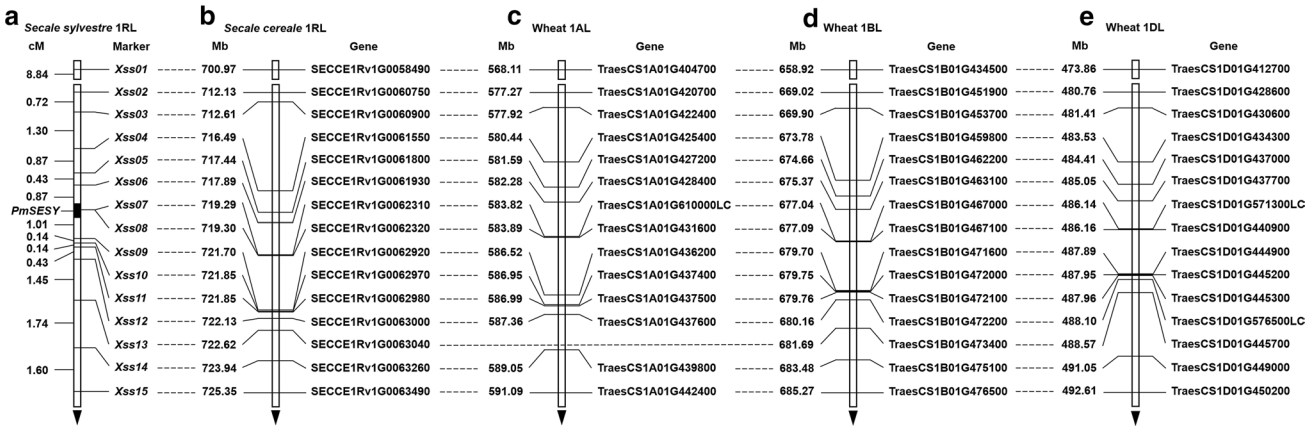

Fig. 5 Genetic and comparative mapping of *PmSESY*. **a** Genetic map of *PmSESY* using the F_2 population derived from the cross between the resistant accession SESY-01 and the susceptible accession SESY-11. **b–d** Comparative maps of the *PmSESY* locus corresponding to the orthologous regions on 1RL of rye cv. Lo7, and 1AL, 1BL and 1DL of wheat cv. Chinese Spring, respectively

Discussion

Blumeria graminis is a fungal pathogen that attacks species of the grass family, Poaceae. This pathogen is thought to be a single species but it can be classified into different *forma speciales* (*f.sp.*) according to host specialization. In general, one *forma specialis* infects only one specific host species (Troch et al. 2014; Menardo et al. 2017). For instance, *B. graminis* f. sp. *tritici* (*B.g. tritici*, *Bgt*) can infect wheat but not rye, whereas *B. graminis* f. sp. *secalis* (*B.g. secalis*, *Bgs*) can infect rye but not wheat. Recently, Menardo et al. (2016) reported that a *forma specialis B.g. triticale* can colonize on wheat, rye, and triticale, which originated from the hybridization between *Bgt* and *Bgs* in Europe where rye and triticale are widely planted. In China, both rye and triticale are small crops only planted in certain northern regions. As a result, *B.g. triticale* would not be prevailing in China. In this study, we found that *S. sylvestre* accession SESY-11 is highly susceptible to all the tested *Bgt* isolates collected from different wheat-producing regions of China. It was suggested that the susceptibility of SESY-11 is caused by losing resistance gene rather than by emergence of the new *forma specialis B.g. triticale*. We also found that most accessions of *S. sylvestre* examined are completely susceptible to *Bgt* isolate BgtYZ01. This is the first report that a species of the genus

Table 4 Potential disease resistance-related genes in the *PmSESY* locus on chromosome 1RL of rye cv. Lo7

Gene	Physical location (bp)	Predicted protein
SECCE1Rv1G0061940	717,952,187–717,953,380	F-box protein
SECCE1Rv1G0061970	718,098,036–718,098,374	BED zinc finger family protein
SECCE1Rv1G0061980	718,098,548–718,098,990	Zinc finger BED domain-containing protein
SECCE1Rv1G0061990	718,099,823–718,101,393	F-box domain containing protein
SECCE1Rv1G0062010	718,226,761–718,227,500	F-box family protein
SECCE1Rv1G0062180	718,540,909–718,545,469	E3 ubiquitin-protein ligase MARCH6
SECCE1Rv1G0062210	718,631,797–718,632,006	Flavin-containing monooxygenase
SECCE1Rv1G0062230	718,641,406–718,647,922	E3 ubiquitin-protein ligase MARCH6
SECCE1Rv1G0062240	718,649,195–718,650,681	F-box family protein
SECCE1Rv1G0062250	719,003,349–719,008,332	E3 ubiquitin-protein ligase MARCH6
SECCE1Rv1G0062270	719,129,615–719,130,612	Pathogen-related protein
SECCE1Rv1G0062300	719,215,450–719,219,656	NLR-type disease resistance protein
SECCE1Rv1G0062310	719,291,889–719,293,532	Leucine-rich repeat receptor-like protein kinase
SECCE1Rv1G0062340	719,399,788–719,404,203	Lectin receptor kinase
SECCE1Rv1G0062490	719,681,439–719,685,431	Kinase family protein
SECCE1Rv1G0062550	720,280,940–720,285,001	Protein kinase family protein
SECCE1Rv1G0062560	720,415,309–720,415,620	NLR-type disease resistance protein
SECCE1Rv1G0062570	720,423,721–720,426,778	Leucine-rich repeat receptor-like protein kinase
SECCE1Rv1G0062580	720,436,434–720,444,242	NLR-type disease resistance protein
SECCE1Rv1G0062600	720,449,544–720,453,693	Leucine-rich repeat receptor-like protein kinase
SECCE1Rv1G0062700	720,935,452–720,936,576	F-box protein
SECCE1Rv1G0062720	720,993,859–720,994,419	Calmodulin
SECCE1Rv1G0062740	721,010,502–721,011,062	Calcium-binding protein
SECCE1Rv1G0062750	721,021,612–721,023,278	Kinase family protein
SECCE1Rv1G0062840	721,488,561–721,489,987	F-box family protein
SECCE1Rv1G0062850	721,500,325–721,501,038	F-box family protein
SECCE1Rv1G0062860	721,505,687–721,507,609	NLR-type disease resistance protein
SECCE1Rv1G0062890	721,626,733–721,630,125	NLR-type disease resistance protein
SECCE1Rv1G0062900	721,699,654–721,700,163	FBD-associated F-box protein
SECCE1Rv1G0062910	721,700,483–721,701,673	F-box/RNI/FBD domains-containing protein

Secale can be colonized by wheat *Bgt*. Genetic analysis revealed that the resistance in *S. sylvestre* accession SESY-01 is controlled by a single dominant gene, *PmSESY*. Combining BSR-Seq analysis with genetic mapping, *PmSESY* was narrowed to a 1.88-cM genetic interval in the terminal region of the long arm of chromosome 1R (1RL), where no other powdery mildew resistance gene has been found before. Therefore, it was concluded that *PmSESY* is a novel gene conferring resistance against wheat powdery mildew.

The *PmSESY* locus corresponded to a 3.81-Mb region in rye cv. Lo7 genome, in which, 98 genes have been annotated (Rabanus-Wallace et al. 2019). Among them, 30 genes, such as NLR-type disease resistance genes and leucine-rich repeat receptor-like protein kinase genes, may serve as candidates involving in *PmSESY* resistance. Comparative analyses of the differences in sequences and transcriptional level of these genes between resistant SESY-01 and susceptible SESY-11 and then performing virus-induced gene silencing of differential genes in SESY-01 would allow to narrow down the *PmSESY* candidate(s). Further development of more high-density molecular markers according to precise reference genome of rye and larger population would contribute to fine genetic mapping of *PmSESY*. Moreover, using more markers in the target region carrying *PmSESY* to carry out association analysis on all resistant and susceptible accessions of *S. sylvestre* would also benefit to finding the *PmSESY* candidate(s).

The reference genome of rye cv. Lo7 may contribute to isolating *PmSESY* from *S. sylvestre*. However, it is not excluded that in the orthologous regions of the *PmSESY* locus, there may be different genomic structures and gene organizations between *S. sylvestre* and cultivated rye because the two species have diverged greatly during evolution (Tang et al. 2011). Recently, multiple cloning strategies have been used successfully to clone genes from wheat and barley. For example, through combination of mutagenesis with sequence capture, MutRenSeq has been adopted to identify wheat stem rust resistance genes *Sr22* and *Sr45* (Steuernagel

et al. 2016). Based on mutagenesis, chromosome sorting, and next-generation sequencing, MutChromSeq has been used to clone the wheat powdery mildew resistance gene *Pm2* (Sánchez-Martín et al. 2016) and barley leaf rust resistance gene *Rph1* (Dracatos et al. 2019). These methods are independent of genetic analysis and positional cloning, even independent of reference genome. Therefore, creating susceptible mutants of *S. sylvestre* accession SESY-01 and using the above new methods may provide alternative ways to clone *PmSESY*.

The five *S. sylvestre* accessions were shown to be effectively resistant to all the tested *Bgt* isolates. It was suggested that *PmSESY* might possess broad-spectrum resistance to wheat powdery mildew and has great value for wheat breeding. Traditional method for utilization of genes originated from wild relatives is transferring them into common wheat through interspecific hybridization (Li et al. 2019b). In the past, *S. sylvestre* was crossed with *Ae. tauschii* and an amphiploid was obtained, suggesting that it is possible to transfer *S. sylvestre* genes to wheat (Yang et al. 2001). Since many wheat-rye addition lines and substitution lines involving chromosome 1R have been developed (Li et al. 2016), crossing *S. sylvestre* accession SESY-01 with such lines would allow the recombination between chromosomes 1R derived from different rye species, which may contribute to developing genetic stocks containing *PmSESY*. Recently, transgenic techniques for wheat have been fast developing and *Agrobacterium tumefaciens*-mediated transformation has been more stable and highly efficient (Zhang et al. 2018a, b), which will contribute to speeding up breeding application of *PmSESY* once it is cloned from *S. sylvestre*.

Acknowledgements This study was supported by grants from Jiangsu Agricultural Science and Technology Innovation Fund [CX(19)2042], State Key Laboratory of Plant Cell and Chromosome Engineering (PCCE-KF-2019-04), Priority Academic Program Development of Jiangsu Higher Education Institutions, Jiangsu Education Department, National Natural Science Foundation of China (31872009), Leading Talents Plan of Hubei Academy of Agricultural Sciences (L2018013), Taishan Scholars Project (tsqn201812123) and State Key Laboratory of Crop Biology in Shandong Agricultural University (2020KF07). The authors are thankful to National Centre for Plant Genetic Resources, Polish Genebank (NCPGR), Genebank Information System of the IPK Gatersleben (GBIS-IPK) and Germplasm Resources Information Network (GRIN) for providing *Secale sylvestre* accessions. The authors are also grateful to Prof. Robert McIntosh (The University of Sydney, Australia) for constructive comments on this manuscript and Prof. Hongjie Li (Chinese Academy of Agricultural Sciences, China) for editing this manuscript.

Author contribution statement HH and SZ conceived and designed the experiments. HH, HD, RL, TL, LY, SG, ZT, HD, CL, RH, WS, and LW performed the experiments. HH and SZ analyzed the data and wrote the paper.

Compliance with ethical standards

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

Ethical Standards The authors state that they follow the Ethical Standards in research. We confirmed that all data in this submission have not been published previously

References

Cuadrado A, Jouve N (2002) Evolutionary trends of different repetitive DNA sequences during speciation in the genus *Secale*. J Hered 93:339–345

Chen PD, Qi LL, Zhou B, Zhang SZ, Liu DJ (1995) Development and molecular cytogenetic analysis of wheat-*Haynaldia villosa* 6VS/6AL translocation lines specifying resistance to powdery mildew. Theor Appl Genet 91:1125–1128

Dracatos PM, Bartoš J, Elmansour H, Singh D, Karafiátová M, Zhang P, Steuernagel B, Svačina R, Cobbin JCA, Clark B et al (2019) The coiled-coil NLR *Rph1*, confers leaf rust resistance in barley cultivar Sudan. Plant Physiol 179:1362–1372

Feuillet C, Langridge P, Waugh R (2007) Cereal breeding takes a walk on the wild side. Trends Genet 24:24–32

Friebe B, Heun M, Tuleen N, Zeller FJ, Gill BS (1994) Cytogenetically monitored transfer of powdery mildew resistance from rye into wheat. Crop Sci 34:621–625

Fu S, Chen L, Wang Y, Li M, Yang Z, Qiu L, Yan B, Ren Z, Tang Z (2015) Oligonucleotide probes for ND-FISH analysis to identify rye and wheat chromosomes. Sci Rep 5:10552

Han F, Lamb JC, Birchler A (2006) High frequency of centromere inactivation resulting in stable dicentric chromosomes of maize. Proc Natl Acad Sci USA 103:3238–3243

Hao M, Liu M, Luo J, Fan C, Yi Y, Zhang L, Yuan Z, Ning S, Zheng Y, Liu D (2018) Introgression of powdery mildew resistance gene *Pm56* on rye chromosome arm 6RS into wheat. Front Plant Sci 9:1040

He H, Ji Y, Zhu S, Li B, Zhao R, Jiang Z, Bie T (2017) Genetic, physical and comparative mapping of the powdery mildew resistance gene *Pm21* originating from *Dasypyrum villosum*. Front Plant Sci 8:1914

He H, Liu R, Ma P, Du H, Zhang H, Wu Q, Yang L, Gong S, Liu T, Huo N et al (2020) Characterization of *Pm68*, a new powdery mildew resistance gene on chromosome 2BS of Greek durum wheat TRI 1796. Theor Appl Genet. https://doi.org/10.1007/s00122-020-03681-2

He H, Zhu S, Zhao R, Jiang Z, Ji Y, Ji J, Qiu D, Li H, Bie T (2018) *Pm21*, encoding a typical CC-NBS-LRR protein, confers broad-spectrum resistance to wheat powdery mildew disease. Mol Plant 11:879–882

He R, Chang Z, Yang Z, Yuan Z, Zhan H, Zhang X, Liu J (2009) Inheritance and mapping of powdery mildew resistance gene *Pm43* introgressed from *Thinopyrum intermedium* into wheat. Theor Appl Genet 118:1173–1180

International Wheat Genome Sequencing Consortium (IWGSC) (2018) Shifting the limits in wheat research and breeding using a fully annotated reference genome. Science 361:eaar7191

Kumar S, Stecher G, Tamura K (2016) MEGA7: molecular evolutionary genetics analysis version 7.0 for bigger datasets. Mol Biol Evol 33:1870–1874

Li H, Dong Z, Xia Q, Tian X, Sehgal S, Koo DH, Friebe B, Ma P, Liu W (2020) A spontaneous wheat-*Aegilops longissima* translocation

carrying *Pm66* confers resistance to powdery mildew. Theor Appl Genet 133:1149–1159
Li H, Zhou Y, Xin W, Wei Y, Zhang J, Guo L (2019) Wheat breeding in northern China: achievements and technical advances. Crop J 7:718–729
Li J, Chen Q, Zhang P, Lang T, Hoxha S, Li G, Yang Z (2019) Comparative FISH and molecular identification of new stripe rust resistant wheat-*Thinopyrum intermedium* ssp. *trichophorum* introgression lines. Crop J 7:819–829
Li L, Liu J, Xue X, Li C, Yang Z, Li T (2018) CAPS/dCAPS Designer: a web-based high-throughput dCAPS marker design tool. Sci China Life Sci 61:992–995
Li Y, Shi X, Hu J, Wu P, Qiu D, Qu Y, Xie J, Wu Q, Zhang H, Yang L et al (2020) Identification of a recessive gene *PmQ* conferring resistance to powdery mildew in wheat landrace Qingxinmai using BSR-Seq analysis. Plant Dis 104:743–751
Li Z, Ren Z, Tan F, Tang Z, Ren T (2016) Molecular cytogenetic characterization of new wheat-rye 1R(1B) substitution and translocation lines from a Chinese *Secale cereal* L. Aigan with resistance to stripe rust. PLoS One 11:e0163642
Lott GK, Johnson BR, Bonow RH, Land BR, Hoy RR (2009) g-PRIME: a free, Windows based data acquisition and event analysis software package for physiology in classrooms and research labs. J Undergrad Neurosci Educ 8:A50–A54
Luo P, Luo H, Chang Z, Zhang H, Zhang M, Ren Z (2009) Characterization and chromosomal location of *Pm40* in common wheat: a new gene for resistance to powdery mildew derived from *Elytrigia intermedium*. Theor Appl Genet 118:1059–1064
Ma P, Xu H, Xu Y, Li L, Qie Y, Luo Q, Zhang X, Li X, Zhou Y, An D (2015) Molecular mapping of a new powdery mildew resistance gene *Pm2b* in Chinese breeding line KM2939. Theor Appl Genet 128:613–622
Martis MM, Zhou R, Haseneyer G, Schmutzer T, Vrána J, Kubaláková M, König S, Kugler KG, Scholz U, Hackauf B et al (2013) Reticulate evolution of the rye genome. Plant Cell 25:3685–3698
McIntosh RA, Dubcovsky J, Rogers WJ, Morris C, Xia XC (2017) Catalogue of gene symbols for wheat: 2017 supplement (KOMUGI Wheat Genetic Resource Database). https://shigen.nig.ac.jp/wheat/komugi/genes/symbolClassList.jsp
Menardo F, Praz CR, Wyder S, Ben-David R, Bourras S, Matsumae H, McNally KE, Parlange F, Riba A, Roffler S et al (2016) Hybridization of powdery mildew strains gives rise to pathogens on novel agricultural crop species. Nat Genet 48:201–205
Menardo F, Wicker T, Keller B (2017) Reconstructing the evolutionary history of powdery mildew lineages (*Blumeria graminis*) at different evolutionary time scales with NGS data. Genome Biol Evol 9:446–456
Petersen G, Seberg O (1998) Molecular characterization and sequence polymorphism of the alcohol dehydrogenase 1 gene in *Hordeum vulgare* L. Euphytica 102:57–63
Petersen G, Seberg O, Aagesen L, Frederiksen S (2004) An empirical test of the treatment of indels during optimization alignment based on the phylogeny of the genus *Secale* (Poaceae). Mol Phylogenet Evol 30:733–742
Rabanus-Wallace MT, Hackauf B, Mascher M, Lux T, Wicker T, Gundlach H, Báez M, Houben A, Mayer KFX, Guo L et al (2019) Chromosome-scale genome assembly provides insights into rye biology, evolution, and agronomic potential. bioRxiv. https://doi.org/10.1101/2019.12.11.869693
Sánchez-Martín J, Steuernagel B, Ghosh S, Herren G, Hurni S, Adamski N, Vrána J, Kubaláková M, Krattinger SG, Wicker T et al (2016) Rapid gene isolation in barley and wheat by mutant chromosome sequencing. Genome Biol 17:221
Singh SP, Hurni S, Ruinelli M, Brunner S, Sanchez-Martin J, Krukowski P, Peditto D, Buchmann G, Zbinden H, Keller B (2018) Evolutionary divergence of the rye *Pm17* and *Pm8* resistance genes reveals ancient diversity. Plant Mol Biol 98:249–260
Steuernagel B, Periyannan SK, Hernández-Pinzón I, Witek K, Rouse MN, Yu G, Hatta A, Ayliffe M, Bariana H, Jones JDG et al (2016) Rapid cloning of disease-resistance genes in plants using mutagenesis and sequence capture. Nat Biotechnol 34:652–655
Tang Z, Yang Z, Fu S (2014) Oligonucleotides replacing the roles of repetitive sequences pAs1, pSc119.2, pTa-535, pTa71, CCS1, and pAWRC.1 for FISH analysis. J Appl Genet 55:313–318
Tang ZX, Ross K, Ren ZL, Yang ZJ, Zhang HY, Chikmawati T, Miftahudin GJP (2011) *Secale*, Chapter 8. In: Kole C (ed) Wild crop relatives: genomic and breeding resources. Cereals, Springer, Berlin, pp 367–396
Troch V, Audenaert K, Wyand RA, Haesaert G, Höfte M, Brown JKM (2014) *Formae speciales* of cereal powdery mildew: close or distant relatives. Mol Plant Pathol 15:304–314
Wu P, Xie J, Hu J, Qiu D, Liu Z, Li T, Li M, Zhang H, Yang L, Liu H et al (2018) Development of molecular markers linked to powdery mildew resistance gene *Pm4b* by combining SNP discovery from transcriptome sequencing data with bulked segregant analysis (BSR-Seq) in wheat. Front Plant Sci 9:95
Yang Z, Li G, Jiang H, Ren Z (2001) Expression of nucleolus, endosperm storage proteins and disease resistance in an amphiploid between *Aegilops tauschii* and *Secale silvestre*. Euphytica 119:317–321
Zeller FJ, Kong L, Hartl L, Mohler V, Hsam SLK (2002) Chromosomal location of genes for resistance to powdery mildew in common wheat (*Triticum aestivum* L. em Thell.). 7. Gene *Pm29* in line Pova. Euphytica 123:187–194
Zhan H, Li G, Zhang X, Li X, Guo H, Gong W, Jia J, Qiao L, Ren Y, Yang Z, Chang Z (2014) Chromosomal location and comparative genomics analysis of powdery mildew resistance gene *Pm51* in a putative wheat-*Thinopyru ponticum* introgresion line. PLoS ONE 9:e113455
Zhang R, Fan Y, Kong L, Wang Z, Wu J, Xing L, Cao A, Feng Y (2018) *Pm62*, an adult-plant powdery mildew resistance gene introgressed from *Dasypyrum villosum* chromosome arm 2VL into wheat. Theor Appl Genet 131:2613–2620
Zhang R, Sun B, Chen J, Cao A, Xing L, Feng Y, Lan C, Chen P (2016) *Pm55*, a developmental-stage and tissue-specific powdery mildew resistance gene introgressed from *Dasypyrum villosum* into common wheat. Theor Appl Genet 129:1975–1984
Zhang S, Zhang R, Song G, Gao J, Li W, Han X, Chen M, Li Y, Li G (2018) Targeted mutagenesis using the *Agrobacterium tumefaciens*-mediated CRISPR-Cas9 system in common wheat. BMC Plant Biol 18:302

Publisher's Note Springer Nature remains neutral with regard to jurisdictional claims in published maps and institutional affiliations.