

Pm55, a developmental-stage and tissue-specific powdery mildew resistance gene introgressed from *Dasypyrum villosum* into common wheat

Ruiqi Zhang¹ · Bingxiao Sun¹ · Juan Chen¹ · Aizhong Cao¹ · Liping Xing¹ · Yigao Feng¹ · Caixia Lan² · Peidu Chen¹

Received: 20 April 2016 / Accepted: 9 July 2016 / Published online: 15 July 2016
© Springer-Verlag Berlin Heidelberg 2016

Abstract

Key message Powdery mildew resistance gene *Pm55* was physically mapped to chromosome arm 5VS FL 0.60–0.80 of *Dasypyrum villosum*. *Pm55* is present in T5VS·5AL and T5VS·5DL translocations, which should be valuable resources for wheat improvement.

Abstract Powdery mildew caused by *Blumeria graminis* f. sp. *tritici* is a major wheat disease worldwide. Exploiting novel genes effective against powdery mildew from wild relatives of wheat is a promising strategy for controlling this disease. To identify novel resistance genes for powdery mildew from *Dasypyrum villosum*, a wild wheat relative, we evaluated a set of Chinese Spring-*D. villosum* disomic addition and whole-arm translocation lines for reactions to powdery mildew. Based on the evaluation data, we concluded that the *D. villosum* chromosome 5V controls post-seedling resistance to powdery mildew. Subsequently, three introgression lines were developed and confirmed by molecular and cytogenetic analysis following ionizing radiation of the pollen of a Chinese Spring-*D. villosum* 5V disomic addition line. A homozygous T5VS·5AL translocation line (NAU421) with good plant vigor and full fertility was further characterized using sequential genomic in situ hybridization, C-banding, and EST-STS marker analysis.

A dominant gene permanently named *Pm55* was located in chromosome bin 5VS 0.60–0.80 based on the responses to powdery mildew of all wheat-*D. villosum* 5V introgression lines evaluated at both seeding and adult stages. This study demonstrated that *Pm55* conferred growth-stage and tissue-specific dependent resistance; therefore, it provides a novel resistance type for powdery mildew. The T5VS·5AL translocation line with additional softness loci *Dina/Dinb* of *D. villosum* provides a possibility of extending the range of grain textures to a super-soft category. Accordingly, this stock is a new source of resistance to powdery mildew and may be useful in both resistance mechanism studies and soft wheat improvement.

Introduction

Powdery mildew, caused by *Blumeria graminis* (DC.) E.O. Speer f. sp. *tritici* Em. Marchal is one of the most economically important diseases in many wheat (*Triticum aestivum* L.) growing regions with cool or maritime climates. The denser canopies of modern semi-dwarf cultivars produced under high fertility conditions favor powdery mildew development with consequent losses in yield and grain quality. Resistant cultivars are considered to be a cost-effective and environment friendly way of controlling this disease. Although more than 70 genes/alleles (*Pm* genes) conferring powdery mildew resistance have been identified in wheat (McIntosh et al. 2013), most of them are ineffective due to the presence of virulence in the pathogen. Hence, identification of new resistance sources in adapted germplasm is an important and long-term objective in achieving durable and broad-spectrum resistance.

Powdery mildew resistance in wheat has heavily relied on the species of the tertiary gene pool to discover new

Communicated by S. S. Xu.

✉ Ruiqi Zhang
zrq@njau.edu.cn

¹ College of Agronomy, Nanjing Agricultural University/
National Key Laboratory of Crop Genetics and Germplasm
Enhancement/JCIC-MCP, Nanjing 210095, China

² International Maize and Wheat Improvement Center
(CIMMYT), 06600 Mexico, DF, Mexico

resistance sources. Among the powdery mildew resistance genes that are currently available, *Pm7*, *Pm8*, *Pm17*, and *Pm20* were introduced from cereal rye (*Secale cereale* L.) (McIntosh et al. 2013), *Pm40* (Luo et al. 2009), *Pm43* (He et al. 2009), and *PmL962* (Shen et al. 2015) were introgressed from *Thinopyrum intermedium* (Host) Barkworth & D. R. Dewey and *Pm51* came from *Th. ponticum* (Podp.) Barkworth & D.R. Dewey (Zhang et al. 2014a), suggesting alien gene transfer is an important means of increasing the genetic diversity of powdery mildew resistance in common wheat. In the tertiary gene pool, *Dasypyrum villosum* (L.) Candargy is a valuable species for wheat improvement because of its resistance to cereal cyst nematodes (Zhang et al. 2012a), wheat streak mosaic virus (Zhang et al. 2005), stem rust (Qi et al. 2011), cereal eyespot (Yildirim et al. 1998), and powdery mildew (Chen et al. 1995). The all-stage powdery mildew resistance gene *Pm21*, originating from chromosome arm 6VS of *D. villosum*, has remained as an effective source of resistance for more than 20 years and has been used widely in breeding powdery mildew resistant cultivars in China (Chen et al. 1995; Li et al. 2007). Except for chromosome 6V, it is not clear if other chromosomes of *D. villosum* have powdery mildew resistance genes.

Several wheat-*D. villosum* chromosome addition and substitution lines have been developed in the past 30 years (Gradzielewska 2006; De Pace et al. 2011). However, when the targeted alien genes from the *D. villosum* V genome are used in wheat improvement, they need to be in the form of a cytologically stable, compensating wheat-alien whole-arm (Robertsonian) translocation (Qi et al. 2007). Liu et al. (2011) developed a set of compensating *T. aestivum*-*D. villosum* Robertsonian translocation lines, with the exception of the 2VS and 5VL arms. In addition, eight whole-arm translocations, including T1VS·1BL, T1VL·1DL, T2VS·2DL, T2VL·2DS, T4VS·4DL, T5VS·5DL, T6VS·6AL, and T6VL·6AS (Chen et al. 1995; Zhang et al. 2005, 2010, 2013, 2014b), which originated from the *D. villosum* accession GP005 (introduced from the Cambridge Botanical Garden, UK), have been developed in our laboratory. The T5VS·5DL translocation line carrying grain softness genes *Dina/Dinb* from *D. villosum* has been transferred into current varieties by backcrossing (Zhang et al. 2015). Agronomic characteristics showed that T5VS·5DL translocation lines had higher powdery mildew resistance at the early flowering and grain filling stages than its recurrent parents, implying that a resistance gene may locate on the alien chromatin.

To confirm this resistance, *T. aestivum*-*D. villosum* 5V introgression lines and a set of wheat-*D. villosum* addition and whole-arm translocation lines were evaluated for response to powdery mildew in both seedling and adult stages. The resistance characteristics and physical location

of a novel powdery mildew resistance gene on the short arm of chromosome 5V were investigated in this study.

Materials and methods

Plant materials

The lines used in this study included *T. aestivum* cv. Chinese Spring (CS), Yangmai 15, Zhengmai 9405, *D. villosum* accession GP005, *T. durum* cv. 1286 (AABB), *T. durum* cv. 1286-*D. villosum* (GP005) amphiploid (AABBVV), and other *T. aestivum*-*D. villosum* genetic stocks listed in Table 1. *D. villosum* accession GP005 is the donor of the V chromatins in the present study and its chromosomes were numbered #4 by De Pace et al. (2011). All of these materials were maintained at the Cytogenetics Institute at Nanjing Agricultural University (CINAU). Three nulli-tetrasomic lines (N5AT5D, N5BT5D, and N5DT5A), kindly provided by the Wheat Genetics and Genomics Resource Center, Kansas State University, were also used in the study.

Development of *T. aestivum*-*D. villosum* 5V introgression lines

To develop *T. aestivum*-*D. villosum* 5V introgression lines, flowering spikes of CS-*D. villosum* disomic addition 5V line, DA5V, were cut off retaining flag leaves and irradiated with 1200 rad of γ -rays from a ^{60}Co source. The matured pollen freshly harvested from irradiated spikes within 2 days after irradiation was pollinated to emasculated florets of CS. M_1 and M_2 plants were backcrossed to CS. In BC_2F_2 , three CS-5V introgression lines, including a Robertsonian translocation T5VS·5AL (NAU421), were identified using genomic in situ hybridization (GISH), sequential chromosome C-banding, and molecular markers.

Cytogenetic analysis

The protocol of chromosome preparation of root tip cells (RTCs) and C-banding followed Gill et al. (1991) and GISH analysis of RTCs and pollen mother cells (PMCs) were according to Chen et al. (1995). The genomic DNA of *D. villosum* was labeled with fluorescein-12-dUTP, and used as a probe to detect the chromosome fragment of 5V. After hybridization with the probes, chromosomes were counterstained with propidium iodide (PI) and mounted in Vectashield (Roche Co., Burlingame, CA, USA). Signals were examined with an Olympus BX60 epifluorescence microscope (Olympus Co., Tokyo, Japan). GISH images of RTCs and PMCs were captured with a SPOT Cooled Color Digital Camera (Diagnostic Instruments, Sterling Heights, MI, USA).

Table 1 Wheat-*D. villosum* genetic stocks used in this study

Accession no.	Chromosome structure	Description/background	Reference
DA1V	DA1V	Chinese Spring- <i>D. villosum</i> disomic addition 1V	Zhang et al. (2013)
DA2V	DA2V	Chinese Spring- <i>D. villosum</i> disomic addition 2V	
DA3V	DA3V	Chinese Spring- <i>D. villosum</i> disomic addition 3V	
DA4V	DA4V	Chinese Spring- <i>D. villosum</i> disomic addition 4V	
DA5V	DA5V	Chinese Spring- <i>D. villosum</i> disomic addition 5V	
DA6V	DA6V	Chinese Spring- <i>D. villosum</i> disomic addition 6V	
DA7V	DA7V	Chinese Spring- <i>D. villosum</i> disomic addition 7V	
NAU1V-4	T1VS·1BL	Chinese Spring	Zhang et al. (2014b)
NAU1V-6	T1VL·1DL	Chinese Spring	
NAU422	T2VS·2DL	Chinese Spring	Zhang et al. (2015)
NAU2V-4	T2VL·2DS	Chinese Spring	
NAU4V-1	T4VS·4DL	Chinese Spring	Zhang et al. (2005)
NAU415	T5VS·5DL	Chinese Spring	Zhang et al. (2010)
92R137	T6VS·6AL	Yangmai5	Chen et al. (1995)
NAU423	T6VL·6AS	Chinese Spring	Zhang et al. (2013)
NAU222	T5VS·5DL	(NAU415 × Yangmai 15)/Yangmai 15 BC ₄ F ₄	Zhang et al. (2015)
NAU5VS-4	T5VS·W·W	FL:0.6–1.0, Zhengmai 9405 genetic background	Zhang et al. (2012b)
NAU5VS-5	T5VS·6AS·6AL	FL:0.8–1.0, Zhengmai 9405 genetic background	
NAU421	T5VS·5AL	Chinese Spring	This study
NAU111	DT5VS	Chinese Spring telosomic addition 5VS	
NAU122	T5VL·W	Chinese Spring	

Molecular marker analysis

Six previously reported 5V-specific EST-PCR markers (*CINAU41*, *CINAU42*, *CINAU43*, *5EST-237*, *5EST-238*, and *Xwmc233*) were used to screen the alien chromatin in wheat genetic background (Cao et al. 2009; Zhang et al. 2012b). Among them, *CINAU42* is specific for chromosome arm 5VL and the others are specific for 5VS. Genomic DNA was isolated from young leaves according to instructions accompanying the DNAsure Plant Kit (Tiangen Biotech Co., Ltd., Beijing, China). PCR amplifications were conducted in a 25 µL reaction mixture containing 1 × Taq DNA polymerase buffer, 0.8 mmol/L MgCl₂, 0.8 mmol/L dNTPs, 200 µmol/L primers, 2 units DNA polymerase, and 50 ng genomic DNA as template. The samples were denatured at 95 °C for 4 min and subjected to 35 cycles of 30 s of denaturation at 94 °C, 55–60 °C (depending on the specific primers) for 45 s, and 1.2 min elongation at 72 °C, with a final extension at 72 °C for 10 min. The PCR products were analyzed on 10 % nondenaturing polyacrylamide gels with a 39:1 ratio of acrylamide: bisacrylamide. Gels were silver stained and photographed.

Field trials

The wheat-*D. villosum* genetic stocks and their genetic background parents (Table 1) were planted in the field

nurseries in Nanjing, China, consecutively for 3 years. Each line was planted in a four-row (1 m long) plot, with 25 cm spacing between rows. The planting rate was 10 plants per row. To investigate the inheritance of powdery mildew resistance introgressed from *D. villosum* chromosome arm 5VS, T5VS·5AL (NAU421) was backcrossed to the powdery mildew susceptible cultivar (cv.) Yangmai 15. The BC₁F₁ (NAU421/2*Yangmai 15) and F₂ (NAU421/Yangmai 15) populations were planted in the field nurseries in Nanjing in 2014 growing season and F_{2:3} populations were evaluated in 2015 growing season. Common wheat ‘Sumai 3’ was used to increase Bgt inoculum and as the susceptible control. All plants were covered with plastic sheeting for protection during the winter seasons. A local field collection of Bgt (mixed races) was used to infect all materials at the jointing stage. In addition, adult-plant reactions to powdery mildew on NAU415 (T5VS·5DL) and NAU421 (T5VS·5AL) were simultaneously assessed at the Institute of Plant Protection, Chinese Academy of Agricultural Sciences in Beijing, China. Also, NAU222 (T5VS·5DL) and NAU421 (T5VS·5AL), along with their respective recurrent parents Yangmai 15 and CS, were tested at Zhengzhou in Henan province, China, using local Bgt field inoculum during 2015. Reactions evaluated at the heading and grain filling stages were scored on a 0–4 infection type (IT) scale, and were then separated into two classes, resistant (R, IT 0–2) and susceptible (S, IT 3–4).

Greenhouse test

The seedlings of NAU421 (T5VS-5AL) and a set of lines carrying known Pm genes or gene combinations were tested for their reactions to 24 single-pustule-derived Bgt virulent isolates (E01, E02, E05, E06, E07, E09, E11, E13, E15, E16, E17, E18, E20, E21, E23-1, E23-2, E26, E30-1, E30-2, E32, E49, E50, E60, and E69) using separate artificial inoculation in a temperature-controlled greenhouse at the Institute of Plant Protection, the Chinese Academy of Agricultural Sciences, Beijing, China (Zhou et al. 2002). In addition, the powdery mildew responses of lines NAU415 (T5VS-5DL), NAU421 (T5VS-5AL), CS, Yangmai 15, and NAU222 (T5VS-5DL) were simultaneously evaluated at the one- to seven-leaf stages in Nanjing. Six seeds per line were planted in a single pot, with one Sumai 3 plant being used as the susceptible control in every pot. A Nanjing field collection of Bgt conidiospores and single spore-derived isolates, E20, E26, and E31 were used for separate tests under controlled greenhouse conditions. Inoculations were carried out by shaking conidiospores from susceptible seedlings of Sumai 3 held above the test plants. The response of each plant was recorded on a 0–4 IT scale system at 15 days after inoculation.

Results

Origin of the powdery mildew resistance

Seedling reactions of a set of *T. aestivum*-*D. villosum* chromosomes 1V to 7V disomic addition lines and eight whole-arm translocation lines (Table 1) to the local Bgt isolates in Nanjing were evaluated in the greenhouse. Results showed that the disomic addition line DA6V and T6VS-6AL translocation line 92R137 had a high level of resistance (IT 0), which was similar to the donor *D. villosum* GP005 while other lines were susceptible (IT 3–4) at the seedling stage. When the lines were tested in the field at adult stages, in addition to DA6V and 92R137, disomic addition line DA5V and T5VS-5DL translocation line NAU415 also exhibited a high level of resistance (IT 0; –1). These results suggested that in addition to Pm21 on chromosome arm 6VS (Chen et al. 1995), chromosome 5V may carry adult-plant resistance to powdery mildew.

Development of *T. aestivum*-*D. villosum* 5V introgression lines

To confirm if *D. villosum* chromosome 5V carries a resistance gene to powdery mildew, pollen of DA5V was irradiated with γ -rays. Three CS-*D. villosum* 5V introgression lines were developed in the progenies following irradiated

pollen treatment. Two of the lines, NAU122 and NAU421, were Robertsonian translocation lines with $2n = 42$, pairing as 21 bivalents at meiotic metaphase I. The 5VL-specific marker CINAU42 was present in NAU122, but all five 5VS-specific markers were absent, indicating that NAU122 was a T5VL-W translocation line (Fig. 1a, b). In contrast, all five 5VS-specific markers were present and CINAU42 was absent in NAU421, indicating that it was a T5VS-W translocation line (Fig. 1c, d). Absence of 5AS-specific bands of CINAU-41, 5EST-237, and 5EST-238 in NAU421 (Fig. 2) indicated that chromosome arm 5AS had been replaced by 5VS. Sequential chromosome C-banding (Fig. 1f) and GISH (Fig. 1g) showed that the opposite arm of the translocated chromosome in NAU421 was chromosome 5AL (Gill et al. 1991). Thus, the translocated chromosome in NAU421 was T5VS-5AL. NAU111 was a telosomic addition line ($2n = 42 + 2t$) (Fig. 1e). Molecular marker analysis showed that all five 5VS-specific markers were present whereas the 5VL-specific marker CINAU42 was absent, indicating that NAU111 was a disomic chromosome 5VS telosomic addition line. The three *T. aestivum*-*D. villosum* 5V introgression lines developed were used to evaluate the powdery mildew response in detail.

Powdery mildew responses in different stages and conditions

Powdery mildew tests at early flowering and grain filling stages in Beijing field conditions showed that the leaves, stems, and spikes of NAU415 (T5VS-5DL; Zhang et al. 2010) and NAU421 (T5VS-5AL) were all highly resistant (IT, 0–0). When tested under field conditions in Zhengzhou, NAU421 (T5VS-5AL) and NAU222 (T5VS-5DL; Zhang et al. 2015) also exhibited a highly resistant response (IT, 0–1) on all tissues while their respective recurrent parents CS and Yangmai 15 were susceptible (IT, 4) (Fig. 3). Under field conditions in Nanjing, DA5V, NAU415 (T5VS-5DL), NAU421 (T5VS-5AL), NAU222 (T5VS-5DL), and NAU111 (Dt5VS) showed a highly resistant response (IT, 0–1) on leaves and stems but had a susceptible phenotype (IT, 2–3) on spikes when tested in 2013, 2014, and 2015. The non-5VS control lines NAU122 (T5VL-W), CS, and Yangmai 15 had highly susceptible IT on all tissues and this resistance, therefore, was confirmed to be derived from the chromosome arm 5VS.

The seedlings of NAU415 and a set of wheat genotypes carrying known Pm genes or gene combinations were tested for reaction to 24 virulent Bgt isolates at the one- to two-leaf stages. The results of the mildew response from NAU415 showed susceptibility to all 24 virulent isolates with an IT score of 3–4. In contrast, the T6VS-6AL line 92R137 carrying Pm21 was highly resistant to these isolates with IT scores of 0–0. To investigate the growth

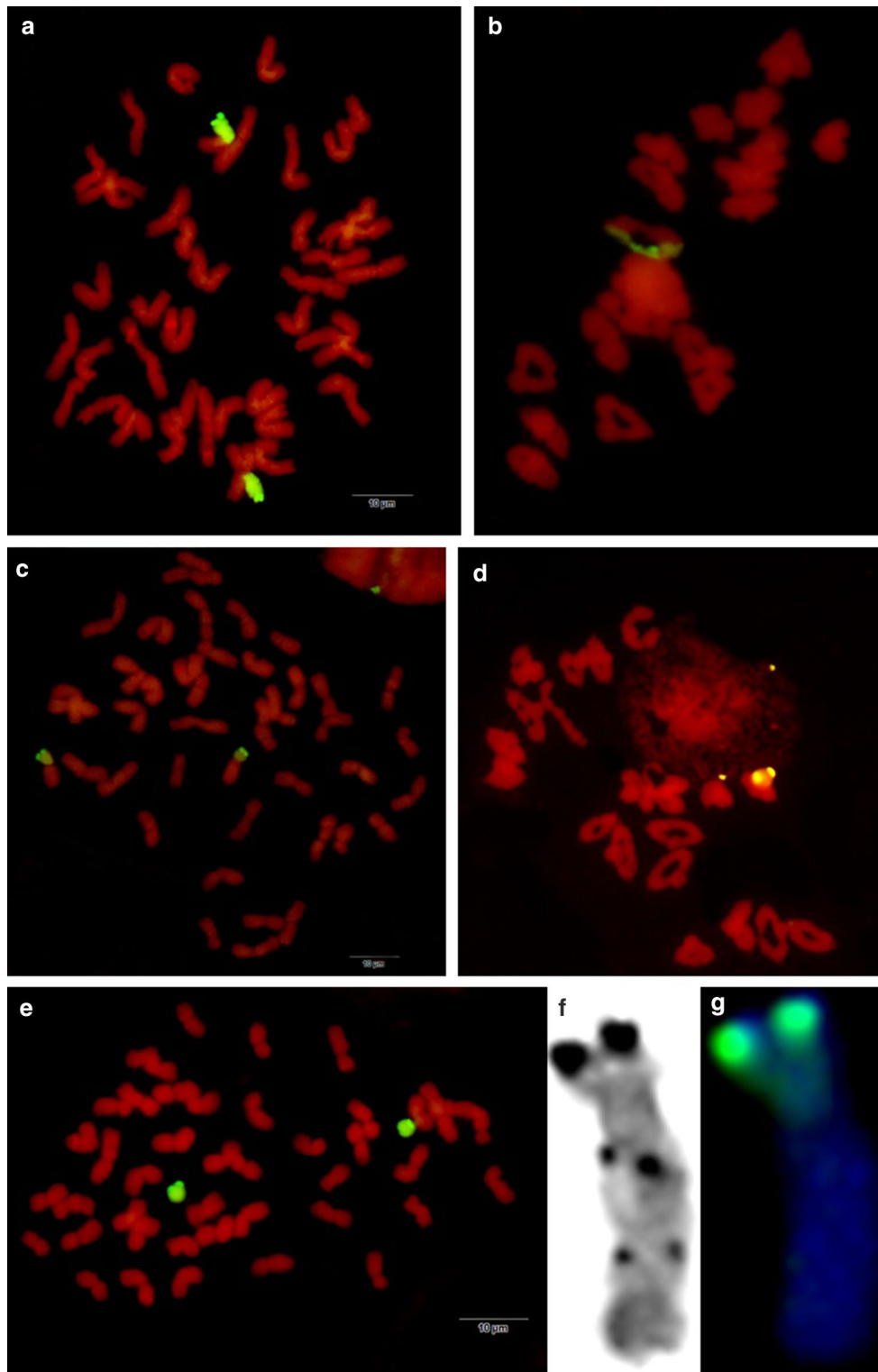


Fig. 1 Mitotic and meiotic GISH analyses of wheat-*D. villosum* chromosome 5V introgression lines. Total genomic DNA of *D. villosum* labeled with digoxigenin-11-dUTP was used as probe. *D. villosum* chromatin fluoresced with a yellowish-green color and wheat chromatin fluoresced red. a, b Mitotic and meiotic GISH patterns of NAU122 containing a pair of wheat-*D. villosum* chromosome arm

5VL Robertsonian translocation chromosomes; c, d GISH patterns of NAU421 with a pair of wheat-*D. villosum* 5VS Robertsonian translocation chromosomes; e mitotic GISH patterns of NAU111, a ditelosomic 5VS addition line; f, g sequential C-banding and GISH patterns of the translocated chromosome in NAU421

Fig. 2 Electrophoresis patterns of EST-PCR marker 5EST-237. Chromosome associations of individual bands are shown on the *right*. The 5VS-specific fragment is present in NAU415, NAU222, NAU421, and NAU111. The 5DS-specific fragment is absent in NAU415 and NAU222; the 5AS-specific fragment is absent in NAU421

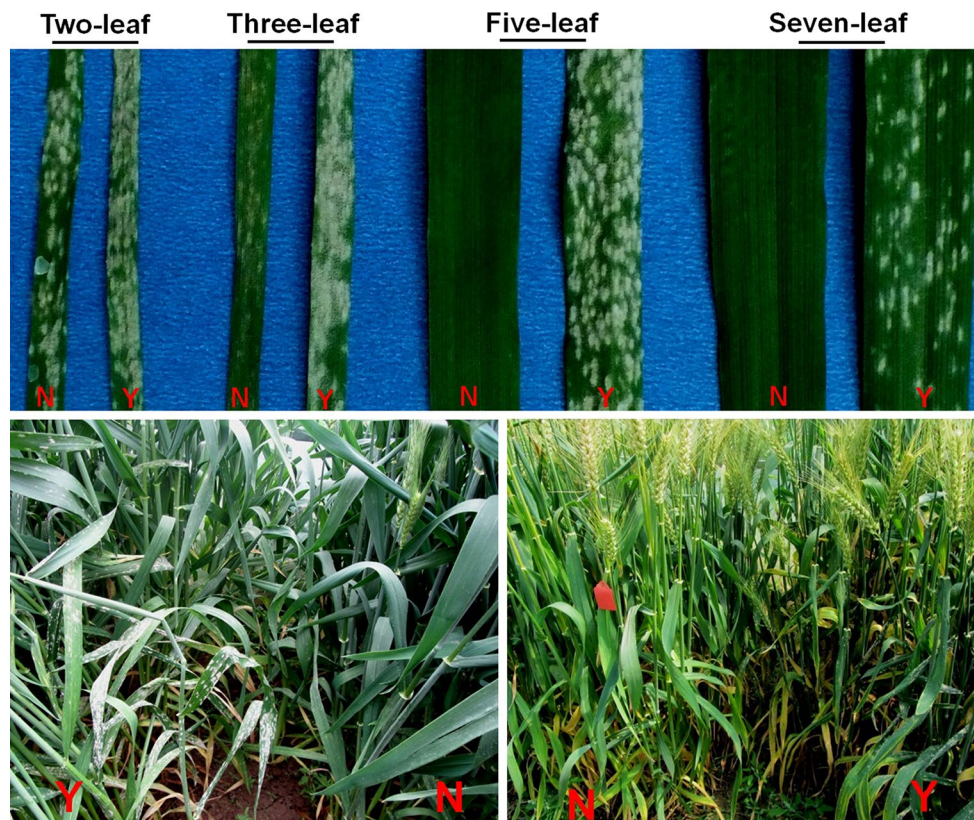
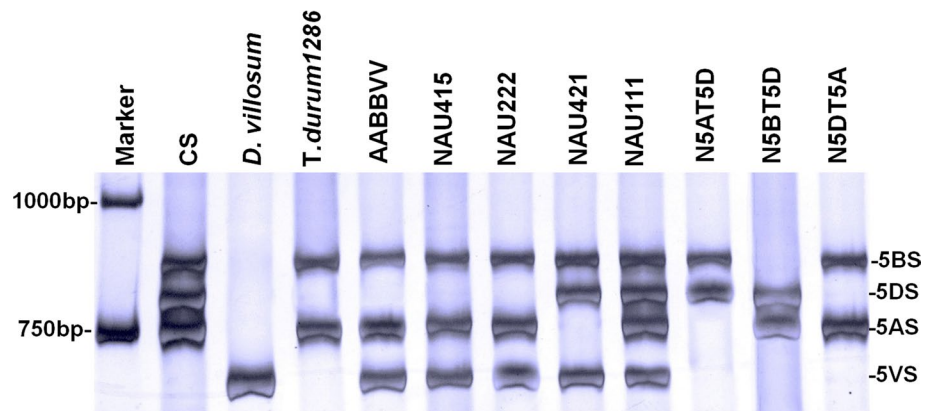


Fig. 3 Powdery mildew reactions of T5VS-5DL translocation line NAU222 (N) and its parent Yangmai 15 (Y). *Upper* Phenotypes of NAU222 and Yangmai 15 at about 10 days after inoculation at dif-

ferent leaf development stages; *Lower* powdery mildew reactions of NAU222 (N) and Yangmai 15 (Y) at the early flowering stages (*left*) and grain filling (*right*) at under Zhengzhou field conditions

stage at which the resistance was expressed, lines NAU415 (T5VS-5DL), NAU421 (T5VS-5AL), CS, NAU222 (T5VS-5DL), and Yangmai 15 were tested at the one- to seven-leaf stages with mixed races and three Bgt isolates E20, E26, and E31, respectively. All lines were equally susceptible at the one- and two-leaf stages, but some resistance was expressed at the three-leaf stage, and then increased to a fully effective level at the five-leaf and later stages (Fig. 3). Thus, this powdery mildew resistance gene(s) was

expressed in a specific plant growth stage on targeted tissue in common wheat.

Genetic analysis of powdery mildew resistance

The segregation patterns of the BC₁F₁ population from NAU421/2*Yangmai 15 and F₂ and F_{2,3} populations from NAU421/Yangmai 15 are shown in Table 2. When inoculated with the Nanjing field collection of Bgt conidiospores

Table 2 Segregation ratios for powdery mildew reactions of F₂ and F_{2,3} populations derived from cross NAU421/Yangmai 15 and BC₁F₁ population with a pedigree of NAU421/2*Yangmai 15

Populations	No. of resistant plants/lines	No. of segregating lines	No. of susceptible plants/lines	Expected ratio	χ^2
BC ₁ F ₁	40	–	49	1:1	0.91
F ₂	82	–	38	3:1	3.26
F _{2,3}	16	39	20	1:2:1	0.54

$$\chi_{0.05,1}^2 = 3.84; \chi_{0.05,2}^2 = 5.99$$

at the adult stages, the F₁ plants showed infection types similar to the resistant parent NAU421 (T5VS·5AL), indicating that the resistance was dominant. The BC₁F₁ population segregated in a ratio of 1 resistant:1 susceptible ($\chi^2 = 0.91$, $P > 0.05$) and the F₂'s segregated in 3 resistant:1 susceptible ($\chi^2 = 3.26$, $P > 0.05$) ratio. In addition, the pooled data of 75 F_{2,3} lines from NAU421/Yangmai 15 segregated as expected for single gene ratio:16 homozygous resistant: 39 segregating: 20 homozygous susceptible ($\chi^2 = 0.54$, $P > 0.05$). The combined results from GISH and molecular marker analysis confirmed the presence of a T5VS·5AL translocation chromosome. Similar outcomes were achieved for line NAU415, but in that case a T5VS·5DL translocation chromosome was involved. The results verified that a dominant powdery mildew resistance response gene, permanently designated as Pm55, was located on chromosome arm 5VS.

Physical location of Pm55

To confirm the physical location of Pm55, the lines containing different chromosome 5V segments were evaluated for reactions to powdery mildew in five- to seven-leaf stage plants (Fig. 4). The 5V disomic addition line DA5V, T5VS·5DL translocation line NAU415, T5VS·5AL translocation line NAU421, and 5VS ditelosomic addition line NAU111 all showed high level of resistance (IT, 0–0), whereas the T5VL·W translocation line NAU122 was highly susceptible (IT, 4). The two Zhengmai 9405 intercalary translocation lines NAU5VS-4 and NAU5VS-5 (Zhang et al. 2012b) showed differential reactions. Line NAU5VS-4, which carried a FL 0.60–1.00 5VS segment, was resistant, whereas line NAU5VS-5 with a FL 0.80–1.00 5VS segment was susceptible along with the recurrent parent Zhengmai 9405. The different responses to powdery mildew indicated that the resistance gene is located in the 5VS FL 0.60–0.80 region.

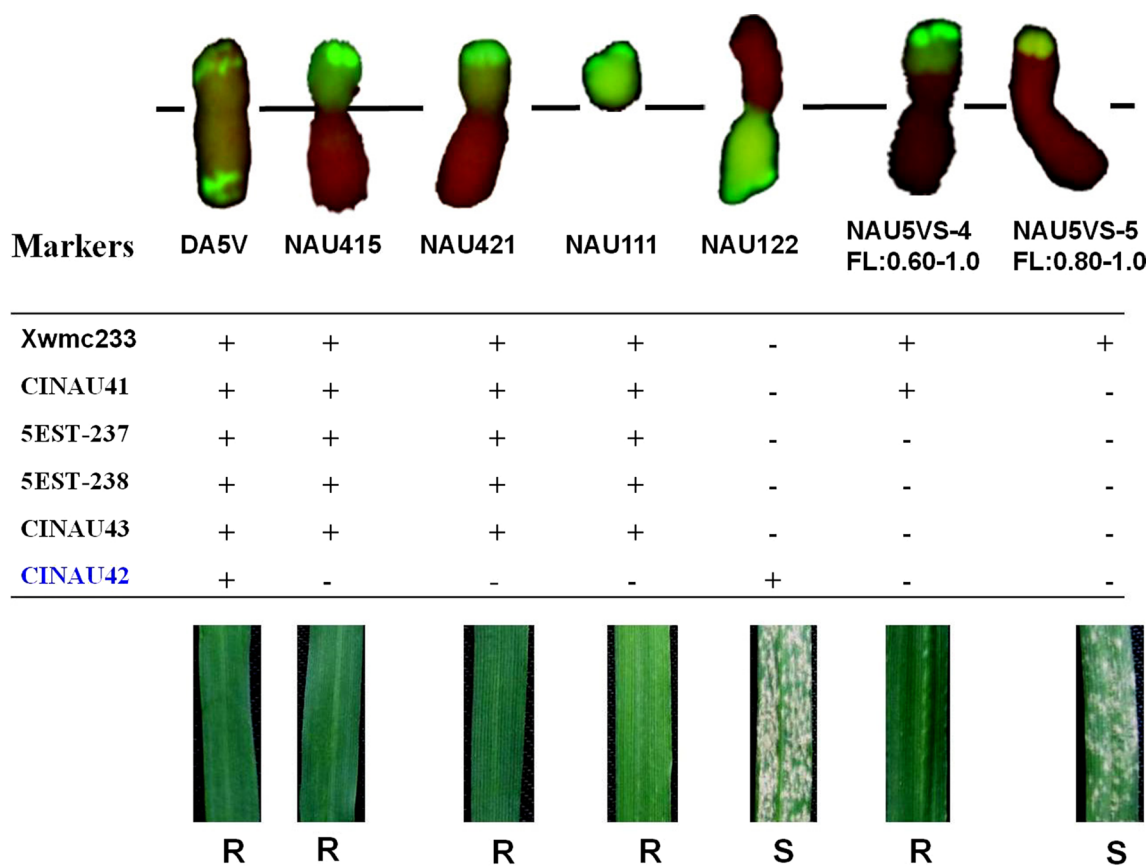
Discussion

Alien gene transfer has played an important role in increasing the genetic diversity for wheat improvement. Several

disease resistance genes have been introduced to hexaploid wheat from *D. villosum* (Qi et al. 2011; Zhang et al. 2005; Chen et al. 1995) by Robertsonian translocations, including the all-stage powdery mildew resistance gene Pm21 located on chromosome 6V. Using a CS-*D. villosum* chromosome 5V disomic addition line irradiated with γ -ray, we successfully developed CS-*D. villosum* chromosome 5V introgression lines NAU415 (T5VS·5DL), NAU421 (T5VS·5AL), NAU111 (Dt5VS), NAU122 (T5VL·W), NAU5VS-4 (T5VS·W·W), and NAU5VS-5 (T5VS·6AS·6AL). A comparative analysis of these introgression lines confirmed that a novel dominant gene for powdery mildew resistance, designated Pm55, was successfully introduced into common wheat. This gene was physically mapped in the chromosome region 5VS FL0.60–0.80.

Pm55, conferred growth-stage and tissue-specific dependent resistance. Thus, translocation lines with this gene were susceptible at seedling stage against the mixed Bgt races from Nanjing field and 24 single isolates, but showed high resistance after the 5-leaf stage. However, visual assessment showed that plenty of pathogen spores were present in glume and awn of NAU222 (T5VS·5DL) at late filling stage under Nanjing field condition, but no spores were observed in leaves and stems. In contrast, pathogen spores were not observed in glume and awn of NAU222 (T5VS·5DL) and NAU421 (T5VS·5AL) under Zhengzhou and Beijing field conditions, respectively. This may be due to the different isolates in the three places. Pm55 as a developmental-stage and tissue-specific powdery mildew resistance gene may have a different resistance mechanism from both all-growth stage and adult-plant resistance genes. A similar reaction has been reported for stripe rust resistance. Two cultivars, Otane and Advantage, with adult-plant resistance to stripe rust on the flag leaf, were susceptible in the spikes (Cromey 1989). We hypothesize that the spike and leaf of wheat may be involved in two host immunity systems. Wheat germplasm with spike infections due to presence of Pm55 might reduce the utility of this gene in wheat cultivar development. But, late growth stage epidemics of powdery mildew can result in significant yield loss and grain quality deterioration, and Pm55 could be pyramided with other slow-mildewing resistance gene(s) to develop durable resistance cultivars. Studies of field performance in adult plants in different genetic backgrounds are needed to ascertain the level of resistance conferred by Pm55.

To continue advances in wheat production while reducing the need for chemical control, new sources of disease resistance genes must be exploited. Pm55 will be of benefit to breeding programs working with powdery mildew disease. Molecular markers are powerful tools in breeding programs as they enable transfer of resistance genes without a need to perform disease tests. EST-PCR markers



Yellowish-green, 5V chromatin; red, wheat chromatin

‘+’, present; ‘-’, absent

R, resistant; S, susceptible

Fig. 4 Cytological structures, molecular analysis and five-leaf stage powdery mildew responses of wheat-*D. villosum* 5V introgression lines

that target coding regions can be developed by designing primers based on EST sequences. Coding regions are more highly conserved between species, making the EST-derived markers more likely to be transferrable across taxonomic boundaries (Wang et al. 2010). This transferability is especially useful for marker development in species where whole genome sequences are not yet available, such as *D. villosum*. Molecular marker 5EST-237 was developed from an EST sequence of wheat homoeologous group 5 (Zhang et al. 2012b). To determine if the 730-bp band for this marker was specific for chromosome 5VS, we identified chromosome arm 5VS in segregating populations by GISH and observed a complete association between chromosome 5VS and 5EST-237. In the case of NAU421 with T5VS-5AL, the 730-bp band substituted for 5AS-specific band (750 bp) and was associated with chromosome arm 5VS in NAU421/Yangmai 15 F₂ populations. Similar results were obtained in regard to the T5VS-5DL

translocation line where the 730-bp 5VS band replaced the 800-bp 5DS-specific band (Zhang et al. 2015). Therefore, the 730-bp band of 5EST-237 can be used to track chromosome 5VS in a wheat genetic background. In other words, the co-dominant marker 5EST-237 can be used for marker-assisted selection of Pm55 when either NAU421 (T5VS-5AL) or NAU421 (T5VS-5DL) is used as a donor parent.

Grain endosperm texture, i.e. hardness or softness, is an important trait that determines the end-use quality of wheat flour. It is mainly controlled by the completely linked Pin genes at the distal end of chromosome 5DS (Morris and Beecher 2012). Although orthologous puroindoline genes on chromosomes 5A and 5B are not present in cultivated wheat, expressed orthologs are present in the diploid donors of the A and B genomes (Li et al. 2008) as well as in chromosome arm 5VS of *D. villosum* where they were designated as Dina/Dinb (Zhang et al. 2010).

T5VS:5AL translocation line NAU421 simultaneously has softness loci Pina-D1a/Pinb-D1a on 5DS and Dina-V1a/Dinb-V1a on 5VS, which provides a possibility of extending the range of grain textures to a super-soft category. Therefore, the negative side of these translocations if they can be successfully exploited is that their use will be restricted to soft wheat breeding programs. However, it should be possible to mutate one or other of the softness genes to achieve hard textured derivatives if future powdery mildew resistant derivatives are proven to have acceptable agronomic performance. Both Zhengmai 9405 intercalary translocation lines NAU5VS-4 and NAU5VS-5 carry Dina-V1a/Dinb-V1a and have soft grain texture (Zhang et al. 2012b), but produce differential powdery mildew reactions. Line NAU5VS-4 was resistant, whereas line NAU5VS-5 was susceptible, suggesting the softness locus is distal to Pm55.

In conclusion, a novel powdery mildew resistance gene Pm55 was identified and physically mapped to chromosome bin 5VS FL 0.60–0.80 of *D. villosum*. Pm55 becomes effective after the 5-leaf stage and is present in two Robertsonian T5VS:5AL and T5VS:5DL translocation lines. In these lines Pm55 is tightly linked to the softness locus Dina/Dinb, and currently has potential only for soft wheat improvement. A co-dominant molecular marker 5EST-237 for Pm55 was developed for marker-assisted selection in breeding programs.

Author contribution statement Conceived and designed the experiments: Ruiqi Zhang; Performed the cytogenetic experiments: Bingxiao Sun, Juan Chen and Yigao Feng; Evaluated the powdery mildew resistance: Aizhong Cao and Liping Xing; Wrote the paper: Ruiqi Zhang, Caixia Lan and Peidu Chen.

Acknowledgments This study was supported by National Natural Science Foundation of China (No. 30871519). We thank Prof. Bob McIntosh, Plant Breeding Institute, University of Sydney, Cobbitty, NSW, Australia, for comments on the manuscript.

Compliance with ethical standards

Conflict of interest All the authors have no conflicts of interest and agree with publication.

References

- Cao YP, Cao AZ, Wang XE, Chen PD (2009) Screening and application of EST-based PCR markers specific to individual chromosomes of *Haynaldia villosa*. *Acta Agron Sin* 35:1–10
- 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
- Cromey MG (1989) Infection and control of stripe rust in wheat spikes. *N Z J Crop Hortic Sci* 17:159–164
- De Pace C, Vaccino P, Cionini G, Pasquini M, Bizzarri M, Qualset CO (2011) *Dasypyrum*. In: Kole C (ed) *Wild crop relatives: genomic and breeding resources, cereals, vol 1, chapter 4*. Springer, Heidelberg, pp 185–292
- Gill BS, Friebe B, Endo TR (1991) Standard karyotype and nomenclature system for description of chromosome bands and structural aberrations in wheat (*Triticum aestivum*). *Genome* 34:830–839
- Gradzielewska A (2006) The genus *Dasypyrum*—part 2. *Dasypyrum villosum*—a wild species used in wheat improvement. *Euphytica* 152:441–454
- He RL, Chang ZJ, Yang ZJ, Yuan ZY, Liu JX, Zhan HX, Zhang XJ (2009) Inheritance and mapping of a powdery mildew resistance Pm43 introgressed from *Thinopyrum intermedium* into wheat. *Theor Appl Genet* 118:1173–1180
- Li GP, Chen PD, Zhang SZ, Wang XE, He ZH, Zhang Y, Zhao H, Huang HY, Zhou XC (2007) Effects of the 6VS.6AL translocation on agronomic traits and dough properties of wheat. *Euphytica* 155:305–313
- Li WL, Huang L, Gill BS (2008) Recurrent deletions of puroindoline genes at the grain hardness locus in four independent lineages of polyploid wheat. *Plant Physiol* 146:200–212
- Liu C, Qi LL, Liu WX, Zhao WC, Wilson J, Friebe B, Gill BS (2011) Development of a set of compensating *Triticum aestivum*–*Dasypyrum villosum* Robertsonian translocation lines. *Genome* 54:836–844
- Luo PG, Luo HY, Chang ZJ, Zhang HY, Zhang M, Ren ZL (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
- McIntosh RA, Dubcovsky J, Rogers J, Morris C, Appels R, Xia XC (2013) Catalogue of gene symbols for wheat. In: KOMUGI-integrated wheat science database at <http://www.shigen.nig.ac.jp/wheat/komugi/genes/download.jsp>
- Morris CF, Beecher BS (2012) The distal portion of the short arm of wheat (*Triticum aestivum* L.) chromosome 5D controls endosperm vitreosity and grain hardness. *Theor Appl Genet* 125:247–254
- Qi LL, Friebe B, Zhang P, Gill BS (2007) Homoeologous recombination, chromosome engineering and crop improvement. *Chromosome Res* 15:3–19
- Qi LL, Pumphrey MO, Friebe B, Zhang P, Qian C, Bowden RL, Rouse MN, Jin Y, Gill BS (2011) A novel Robertsonian translocation event leads to transfer of a stem rust resistance gene (Sr52) effective against race Ug99 from *Dasypyrum villosum* into bread wheat. *Theor Appl Genet* 123:159–167
- Shen XK, Ma LX, Zhong SF, Liu N, Zhang M, Chen WQ, Zhou YL, Li HJ, Chang ZJ, Li X, Bai GH, Zhang HY, Tan FQ, Ren ZL, Luo PG (2015) Identification and genetic mapping of the putative *Thinopyrum intermedium*-derived dominant powdery mildew resistance gene PmL962 on wheat chromosome arm 2BS. *Theor Appl Genet* 128:517–528
- Wang MJ, Zhang Y, Lin ZS, Ye XG, Yuan YP, Ma W, Xin ZY (2010) Development of EST-PCR markers for *Thinopyrum intermedium* chromosome 2Ai#2 and their application in characterization of novel wheat-grass recombinants. *Theor Appl Genet* 121:1369–1380
- Yildirim A, Jones SS, Murray TD (1998) Mapping a gene conferring resistance to *Pseudocercospora herpotrichoides* on chromosome 4V of *Dasypyrum villosum* in a wheat background. *Genome* 41:1–6
- Zhang QP, Li Q, Wang XE, Wang HY, Lang SP, Wang Y, Wang SL, Chen PD, Liu DJ (2005) Development and characterization of a *Triticum aestivum*–*Haynaldia villosa* translocation line

- T4VS/4DL conferring resistance to wheat spindle streak mosaic virus. *Euphytica* 145:317–320
- Zhang RQ, Cao YP, Wang XE, Feng YG, Chen PD (2010) Development and characterization of a *Triticum aestivum*-*D. villosum* T5VS.5DL translocation line with soft grain texture. *J Cereal Sci* 51:220–225
- Zhang JJ, Yuan HX, Zhang RQ, Xing XP, Dai JL, Niu JS, Li HL, Chen PD (2012a) Analysis of resistance to *Heterodera filipjevi* in *Triticum aestivum*-*Dasyphyrum villosum* germplasm. *Acta Agron Sin* 38:1–8
- Zhang RQ, Wang XE, Chen PD (2012b) Molecular and cytogenetic characterization of a small alien-segment translocation line carrying the softness genes of *Haynaldia villosa*. *Genome* 55:639–646
- Zhang W, Zhang RQ, Feng YG, Bie TD, Chen PD (2013) Distribution of highly repeated DNA sequences in *Haynaldia villosa* and its application in the identification of alien chromatin. *Chin Sci Bull* 58:890–897
- Zhang H, Li GR, Zhang XJ, Li X, Guo HJ, Gong WP, Jia JQ, Qiao LY, Ren YK, Yang ZJ, Chang ZJ (2014a) Chromosomal location and comparative genomics analysis of powdery mildew resistance gene Pm51 in a putative wheat-*Thinopyrum ponticum* introgression line. *PLoS One* 9(11):e113455. doi:[10.1371/journal.pone.0113455](https://doi.org/10.1371/journal.pone.0113455)
- Zhang RQ, Zhang MY, Wang XE, Chen PD (2014b) Introduction of chromosome segment carrying the seed storage protein genes from chromosome 1V of *Dasyphyrum villosum* showed positive effect on bread-making quality of common wheat. *Theor Appl Genet* 127:523–533
- Zhang RQ, Hou F, Feng YG, Zhang W, Zhang MY, Chen PD (2015) Characterization of a *Triticum aestivum*-*Dasyphyrum villosum* T2VS.2DL translocation line expressing a longer spike and more kernels traits. *Theor Appl Genet* 128:2415–2425
- Zhou YL, Duan XY, Chen G, Sheng BQ, Zhang Y (2002) Analyses of resistance genes of 40 wheat cultivars or lines to wheat powdery mildew. *Acta Phytopathol Sin* 32:301–305