Theor Appl Genet (2013) 126:2921–2930
DOI 10.1007/s00122-013-2181-y

ORIGINAL PAPER

Induction of 4VS chromosome recombinants using the CS *ph1b* mutant and mapping of the wheat yellow mosaic virus resistance gene from *Haynaldia villosa*

Renhui Zhao · Haiyan Wang · Jin Xiao · Tongde Bie · Shunhe Cheng · Qi Jia · Chunxia Yuan · Ruiqi Zhang · Aizhong Cao · Peidu Chen · Xiue Wang

Received: 25 February 2013 / Accepted: 12 August 2013 / Published online: 29 August 2013

Abstract The wheat spindle streak mosaic virus (WSSMV) or wheat yellow mosaic virus (WYMV) resistance gene, *Wss1*, from *Haynaldia villosa*, was previously mapped to the chromosome arm 4VS by the development of 4V (4D) substitution and T4DL·4VS translocation lines. For better utilization and more accurate mapping of the *Wss1*, in this research, the CS *ph1b* mutant was used to induce new translocations with shortened 4VS chromosome fragments. Thirty-five homozygous translocations with different alien fragment sizes and breakpoints of 4VS were identified by GISH and molecular marker analysis. By field test, it was found that all the identified terminal translocations characterized as having smaller 4VS chromosome segments in the chromosome 4DS were highly resistant to WYMV, while all the interstitial translocations with 4VS inserted into the 4DS were WYMV susceptible. Marker analysis using 32 4VS-specific markers showed that both the terminal and interstitial translocations had different alien fragment sizes. Five specific markers could be detected in the WYMV-resistant terminal translocation line NAU421 with the shortest introduced 4VS fragment, indicating they can be used for marker-assisted selection in wheat breeding. Based on the resistance evaluation, GISH and molecular marker analysis of the available translocations, the gene(s) conferring the WYMV resistance on 4VS could be further cytologically mapped to the distal region of 4VS, immersed in the bin of FL 0.78–1.00. The newly developed small fragment translocations with WYMV resistance and 4VS specific markers have laid solid groundwork for the utilization in wheat breeding for WYMV resistance as well as further cloning of *Wss1*.

Communicated by I. D. Godwin.

R. Zhao and H. Wang contributed equally to this article.

Electronic supplementary material The online version of this article (doi:10.1007/s00122-013-2181-y) contains supplementary material, which is available to authorized users.

R. Zhao · H. Wang · J. Xiao · Q. Jia · C. Yuan · R. Zhang · A. Cao · P. Chen · X. Wang (✉)
State Key Laboratory of Crop Genetics and Germplasm Enhancement, Cytogenetics Institute, Nanjing Agricultural University, Nanjing 210095, Jiangsu, China
e-mail: xiuew@njau.edu.cn

T. Bie · S. Cheng
Department of Wheat Breeding, Agricultural Sciences Institute of the Lixiahe District, Yangzhou 225007, Jiangsu, China

Introduction

Wheat yellow mosaic disease, which is caused by wheat yellow mosaic bymovirus (WYMV), has been growing as one of the most serious diseases threatening wheat production in China. The epidemic area of WYMV is widely distributed in the winter wheat growing regions including Shanxi, Sichuan, Hubei, Shandong, Henan, Anhui, Jiangsu and Zhejiang provinces. It has been reported that grain yield losses caused by WYMV ranges from 20 to 70 % (Liu et al. 2004, 2005a). The resting spores of *Polymyxa graminis*, the fungal vector of WYMV, are extremely environment tolerant and can survive in plants residues for as long as 10 years (Chen 2005). This makes the chemical control of the disease extremely difficult, and thus it is widely accepted that the most effective and sustainable strategy for WYMV control is the use of varieties with WYMV resistance.

Wild relatives of wheat contain a large number of favorable genes for crop production and can be used for wheat improvement. *Haynaldia villosa* (L.) Schur (syn. *Dasypyrum villosum* L. Candargy, $2n = 14$, VV), a wild

relative of wheat, has been identified as an important source of useful genes for wheat improvement, such as resistances to diseases including powdery mildew, rusts, take-all, eyespot, wheat spindle streak mosaic virus (WSSMV), as well as tolerances to drought and frost, good tiller ability, and high grain protein content (Hyde 1953; Chen and Liu 1982; Blanco et al. 1987; Murray et al. 1994; Chen et al. 1995; Uslu et al. 1998; Zhang et al. 2005; Grądzielewska 2006). Previous studies showed that *Triticum aestivum –H. villosa* disomic substitution line DS4V(4D) and translocation line T4DL·4VS were highly resistant to WYMV, and the WYMV resistance gene was located on 4VS (Zhang et al. 2005). However, the presence of the whole short arm of chromosome 4V in the T4DL·4VS translocation line may simultaneously introduce some unfavorable genes for agronomic and end-use quality traits caused by redundant alien chromatin, or wild 'linkage drag'.

Alien genes can be introduced into wheat by wide hybridization followed by the production of alien chromosome lines, especially translocation lines. Jiang et al. (1994) has summarized the procedures for translocation production. Among them, the induction of meiotic recombination between the alien chromatin and its homoeologous (i.e., partially homologous) region of wheat chromosome resulted in the compensative translocation and is considered to be most favorable. In wheat, homologous chromosome pairing is ensured by the presence of the *Ph1* gene on chromosome 5BL (Riley and Chapman 1958). The absence of *Ph1* will significantly elevate the frequency of homoeologous chromosome pairing and homoeologous recombination except in those cases where it is hindered by structural rearrangements (Qi et al. 2007). The *Ph1*-deficient genetic stocks such as nullisomy for chromosome 5B and mutation (e.g., *ph1b* and *ph1c)* have been widely used in transferring useful genes from wild relatives into wheat for the improvement of resistances or tolerances to biotic and abiotic stresses (Friebe et al. 1996; Xin et al. 2001; Qi et al. 2008; Mullan et al. 2009).

The aim of this study was to induce recombination between chromosome 4VS from *H. villosa* with homoeologous group 4 chromosomes in bread wheat, identify recombinants involving different regions of 4VS, develop new translocations and physical map the *Wss1* to specific chromosome regions.

Materials and methods

Plant materials

T. durum–H. villosa amphiploid (AABBVV), *T. aestivum–H. villosa* disomic substitution line DS4V(4D), *T. aestivum–H. villosa* translocation line T4DL·4VS, *T. aestivum–H. villosa* translocation line T5DL·4VL were developed by the Cytogenetics Institute, Nanjing Agricultural University (CINAU, hereafter). The common wheat variety 'Chinese Spring' (CS) is maintained at CINAU. Chinese Spring *ph1b* mutant (CS *ph1b*) was kindly provided by the Wheat Genetics Resource Centre at Kansas State University, Manhattan, USA, and was used to induce meiotic recombination between the chromosome arm 4VS and its homoeologous group 4 chromosomes of wheat. The *H. villosa* (Accession No. 91C43, the donor of *Wss1*) was introduced from Cambridge Botanical Garden, UK.

Chromosome manipulation

To induce meiotic recombination between chromosomes 4D and T4DL·4VS, the translocation line T4DL·4VS was crossed to the homozygous CS *ph1b* mutant. The F_1 plants were backcrossed to CS *ph1b* and the derived BC_1F_1 were screened by molecular markers to identify individuals with genotype as *ph1bph1b* and heterozygous for chromosomes T4DL·4VS and 4D. GISH on PMCs at meiotic metaphase I (PMC MI) of the identified genotype was further used to observe the presence of chromosome pairing between chromosome T4DL·4VS and group 4 chromosomes from wheat. Those BC_1F_1 plants having homoeologous chromosome pairing between wheat and alien chromosomes were self-crossed, and the derived progenies were screened by GISH and molecular markers to identify recombinants. The procedure is shown in Fig. 1.

Molecular marker analysis

A total of 607 EST-PCR primer pairs were designed based on the sequences of ESTs that were physically mapped to homoeologous group 4 chromosomes of wheat (http://wheat.pw.usda.gov/cgi-bin/westsql/map_locus.cgi). In addition, 82 SSR

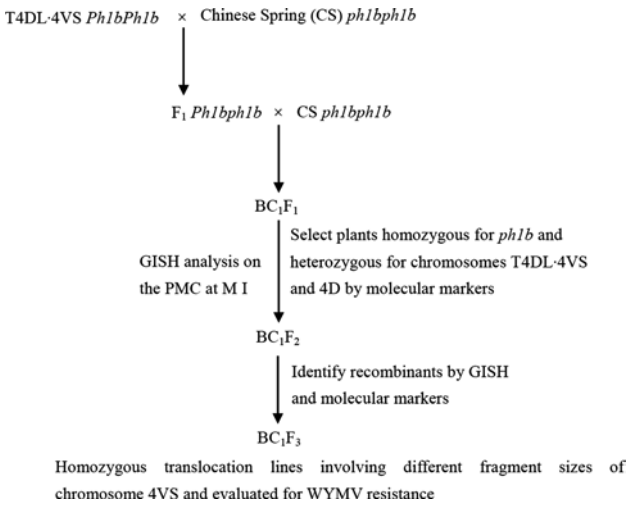

Fig. 1 Procedure for producing *T. aestivum–H. villosa* translocation chromosomes using the CS *ph1b* mutant

primer pairs were synthesized based on the sequence information reported by Somers et al. (2004). All these primers were used to analyze *H. villosa*, Chinese Spring and *T. aestivum–H. villosa* alien chromosome lines to identify molecular markers specific for chromosome 4V of *H. villosa*.

One molecular marker ABC302.3 designed by Wang et al. (1998) was used to screen the BC_1F_1 to identify individuals homozygous for *ph1b*. All primers were synthesized by Invitrogen Life Technologies (Shanghai, China).

DNA extraction and PCR

Genomic DNA was extracted from 2-g fresh leaves at three-leaf stage with SDS–phenol–chloroform method described by Sharp et al. (1989) and Devos et al. (1992) and purified for further elimination of RNA, amylase and other unwanted components. The purity and concentration of DNA was assessed by comparison with standard DNA samples in 0.8 % agarose gel. The DNA was finally diluted to approximately 50 ng/μl each and stored at −20 °C until use.

PCR amplification was carried out in a 10 μl reaction containing 40 ng genomic DNA, 2 μM each of the primer pairs, 2.5 mM each dNTPs, 2.5 mM $MgCl_2$, 1× PCR buffer (10 mM Tris–HCl, pH 8.5, 50 mM KCl), and 0.5 U *Taq* DNA polymerase with a PTC-200 thermal cycler (Bio-Rad, Hercules, CA, USA). Amplification was conducted at 94 °C for 3 min, followed by 35 cycles at 94 °C for 30 s, annealing of different primers at 50, 55, or 60 °C for 50 s at a ramp rate of 0.5 °C/s, 72 °C for 1 min 10 s, and a final extension at 72 °C for 10 min. PCR products were resolved in 8 % non-denaturing poly-acrylamide gels (Acr:Bis = 19:1 or 39:1) and the band patterns were visualized with silver staining (Bassam and Gresshoff 2007).

Cytogenetic analysis

Chromosome preparations of pollen mother cells (PMC) at meiotic metaphase I and root tip cells (RTC) at mitotic metaphase followed that of Chen et al. (1995). The techniques of GISH and FISH followed that of Zhang et al. (2004). Total genomic DNA of *H. villosa* was labeled with fluorescein-12-dUTP by Nick Translation method and used as a probe for GISH. The repetitive sequence clones *pSc119.2* and *pAs1* (Rayburn and Gill 1986; McIntyre et al. 1990), labeled with biotin-16-dUTP and digoxigenin-11-dUTP, respectively, were used as probes for Multi-color FISH (mc-FISH). Hybridization signals were observed using BX51 fluorescent microscope. Photographs were taken with SPOT CCD camera.

Evaluation of WYMV resistance

Homozygous translocation lines with different fragment sizes of chromosome 4VS, the parental lines, the resistant and susceptible wheat varieties were grown in the natural WYMV nursery of Institute of Agricultural Sciences in Lixiahe District of Jiangsu Province. The field trials were organized in a randomized block design with three replications. Twenty-five seeds per plot were planted for each material in a 1.5-m row and spaced 0.25 m apart. All the materials were planted in late October each year, and managed following the agronomic practices commonly adopted in the area and harvested in next June.

Infection types (ITs) were rated using a 0–5 scale, the grading standards were carried out according to Zhu et al. (2012). For the wheat–*H. villosa* translocations and their parental lines, all individual plants for each line were scored twice in year 2012 (each on 26 February and 24 March). For each line in each block, a disease index (DI) was calculated using the formula: $DI\ (\%) = \sum(DS \times Ni) \times 100/(5 \times N)$, where DS was a disease scale which represented an IT, *Ni* was the number of plants of the relevant DS, and *N* was the total number of plants observed per line. Then the mean of the DI for each line was calculated.

Results

Screening of molecular markers specific for chromosome arms 4VS and 4VL

A total of 607 EST-derived and 82 SSR primer pairs were used for amplification using the genomic DNA of CS, *H. villosa*, *T. durum–H. villosa* amphiploid, DS4V(4D), T4DL·4VS and T5DL·4VL as templates. Those markers which only amplified common specific bands in lines containing the 4V were chromosome 4V-specific markers. Among them, 32 were specific for 4VS and 26 were specific for 4VL, shown by their specific amplification only in T4DL·4VS or T5DL·4VL. The information of the 4V-specific markers is shown in the supplementary data (Table S1).

Identification of individuals with single chromosome T4DL·4VS and homozygous for *ph1b*

To induce meiotic recombination between chromosome T4DL·4VS and its homoeologous group 4 chromosomes of wheat, the translocation line T4DL·4VS was crossed with the CS *ph1b* mutant. Thirty-one F_1 plants were backcrossed to CS *ph1b* and the derived 693 BC_1F_1 progenies were screened by molecular marker analysis.

Two 4VS-specific EST-PCR markers (CINAU66 and CINAU295) were used to identify the individuals having a single chromosome T4DL·4VS (Fig. 2a, b), and 324 of the 693 plants were recognized as containing the translocation chromosome. The transmission frequency of chromosome T4DL·4VS was 46.8 %.

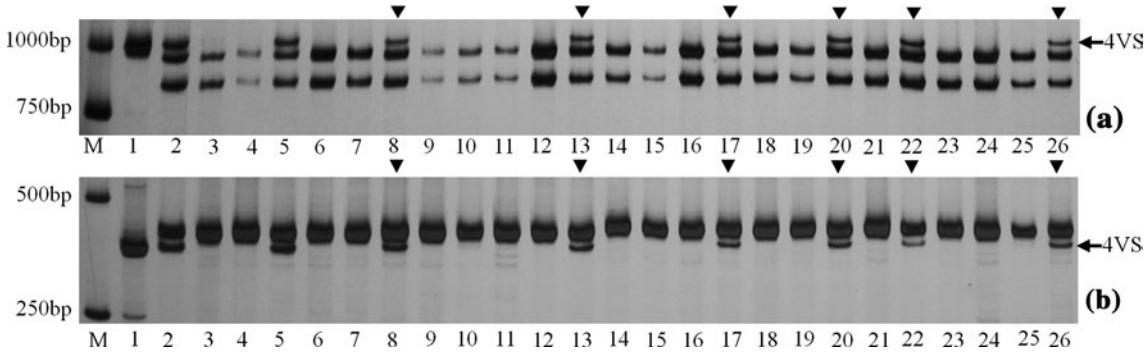

Fig. 2 Electrophoresis patterns of 4VS-specific EST-PCR markers CINAU66 (**a**) and CINAU295 (**b**) in part of plants of BC_1F_1 progenies derived from T4DL·4VS/CS *ph1b*//CS *ph1b*. Black arrows show specific bands of 4VS; *downward triangle* show BC_1F_1 plants containing T4DL·4VS. *M*, DL2000; *1*, *H. villosa*; *2*, *T. durum–H. villosa* amphiploid; *3*, *T. durum*; *4*, Chinese Spring; *5*, T4DL·4VS translocation line; *6*, T5DL·4VL translocation line; *7–26,* part of plants in the BC_1F_1 population

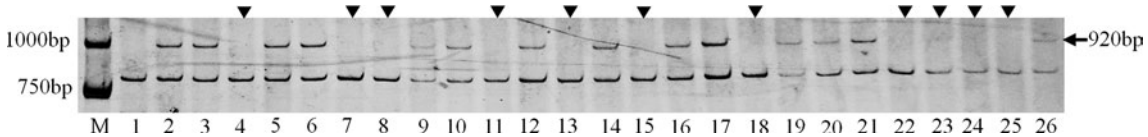

Fig. 3 Identification of homozygous *ph1b* plants in BC_1F_1 progeny derived from T4DL·4VS/CS *ph1b*//CS *ph1b*. *Downward triangles* show BC_1F_1 plants homozygous for *ph1b*. *M*, DL2000; *1*, CS *ph1b*; *2*, CS; *3–26*, part of plants from the BC_1F_1 population

ABC302.3, a specific STS-PCR marker for barley chromosome 5H, was previously developed and mapped within the *Ph1* deletion region. The diagnostic ABC302.3 fragments being about 920 bp is missing in the CS *ph1b* mutant and present in CS, allowing the identification of homozygous *ph1b* genotypes (Wang et al. 1998). ABC302.3 was used to screen the BC_1F_1 progeny to identify individuals with the genotype as *ph1bph1b*. Among the 324 plants with single chromosome T4DL·4VS, 159 were homozygous *ph1bph1b*. The ratio of plants homozygous for *ph1b* and having the chromosome T4DL·4VS is about 50 % (Fig. 3).

Homoeologous chromosome pairing frequency in the plants with homozygous *ph1bph1b* genotype

Thirty-five BC_1F_1 individuals with single chromosome T4DL·4VS and homozygous for *ph1b* were used for GISH analysis to observe the presence of homoeologous chromosome pairing between chromosome T4DL·4VS and its wheat group 4 chromosomes at PMC MI. In most of the observed cells, the chromosome T4DL·4VS and wheat group 4 chromosomes formed rod bivalents (Fig. 4a). Eight ring bivalents formed by wheat group 4 chromosomes and T4DL·4VS were observed in 8 of the 366 PMCs analyzed (Fig. 4b), which indicated that the 4VS and homoeologous group 4 chromosomes recombinants could be expected at a frequency of more than 2.19 %.

Terminal and interstitial wheat–*H. villosa* translocations involving 4VS

The eight BC_1F_1 plants identified having ring bivalents formed by wheat group 4 chromosomes and T4DL·4VS were self-crossed and a total of 790 derived BC_1F_2 plants were analyzed by GISH to identify the induced wheat–*H. villosa* recombinants. It was found that 43 plants were recombinants, including 26 terminal translocation lines and 17 interstitial translocation lines. By GISH of the derived BC_1F_3 plants, 35 homozygous translocation lines were identified, in which, 22 were terminal translocation lines and 13 were interstitial translocation lines.

Fragment sizes of 4VS in the newly developed translocations were different revealed by molecular marker analysis

Thirty-two molecular markers, which have been mapped to the short arm of chromosome 4V, were used to identify the fragment sizes and breakpoints of the alien chromosomes in the newly developed translocation lines. Combined the results of PCR analysis, GISH and FISH (Fig. 5), it was found that 22 terminal translocation lines could be grouped into 11 types (NAU421–NAU431), and 13 interstitial translocation lines could be grouped into 7 types (NAU432–NAU438). For those terminal translocation lines, NAU421 has the smallest 4VS terminal fragment, while the NAU431

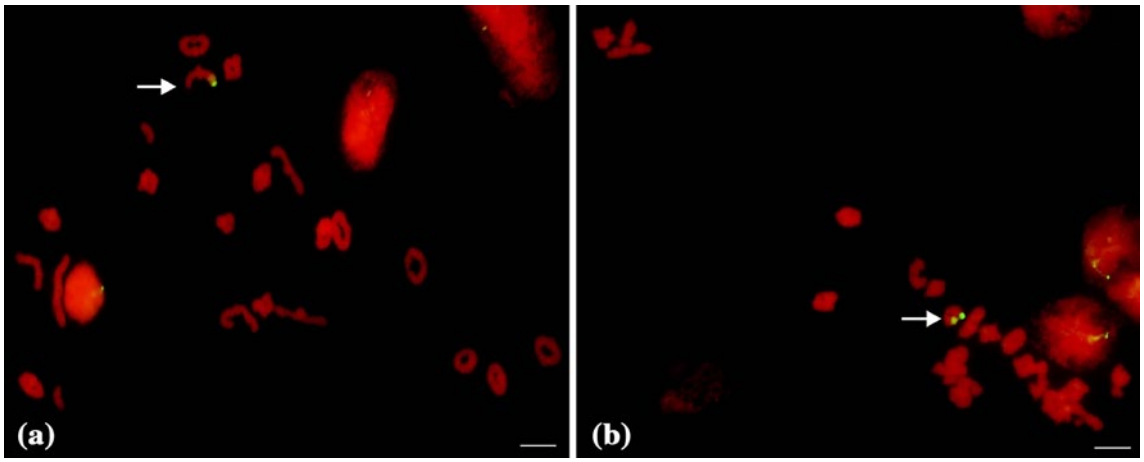

Fig. 4 GISH patterns of PMC at meiotic metaphase I of BC_1F_1 plants heterozygous for 4D and T4DL·4VS and homozygous for *ph1b*. **a** *Arrow* shows a rod bivalent formed between wheat group 4 chromosomes and T4DL·4VS. **b** Homoeologous pairing occurred between wheat and alien chromosome shown by the formation of a ring bivalent. *Arrow* shows a ring bivalent formed between wheat group 4 chromosomes and T4DL·4VS. *Scale bar* = 100 μm

has the largest. Among those interstitial translocation lines, line NAU432 has the smallest 4VS fragment inserted, while NAU 438 has the largest.

The chromosomal region localization of a marker was determined by comparison of the PCR results of two recombinant chromosomes with the closest breakpoints. When the 4VS-specific marker amplicons were present in one line while absent in the other line, the marker could be allocated to the different chromosome region resulted from the difference of recombination points of the two translocation chromosomes in the two lines. By this, the 32 4VS-specific markers could be assigned to 13 regions of 4VS, however, the order of markers within the same region is still unknown (Fig. 5)

Evaluation of WYMV resistance and cytological mapping of the WYMV resistance locus *Wss1*

All the developed homozygous translocation lines involving different fragment sizes of chromosome 4VS were evaluated for WYMV resistance, using the parental lines and the resistant and susceptible wheat varieties as controls. The plants were classified as highly resistant or susceptible (Fig. 5). General statistics for the DI of these plants are summarized in Table 1. WYMV differences represented by the DI between the two parents (T4DL·4VS and CS *ph1b*) were significant at the $P = 0.01$ level. The plants without the terminal fragment of chromosome 4VS were all highly susceptible to WYMV (Fig. 6g–j), with the means of the DI ranged from 64.55 to 75.27 %, which was very similar to the susceptible parent, CS *ph1b*. However, the terminal translocation lines were all highly resistant to WYMV (Fig. 6b–e). WYMV differences represented by the DI between these terminal translocations and the original resistant whole arm translocation T4DL·4VS were not significant at the $P = 0.01$ level.

Based on the above results from WYMV resistance evaluation, GISH and molecular marker analysis, the WYMV resistance locus from *H. villosa* could be further cytologically mapped to the distal region of 4VS.

The NAU421, a WYMV-resistant terminal translocation with the shortest 4VS fragment, has chromosome number of $2n = 42$ (Fig. 7a), the translocation chromosomes paired and formed a ring bivalent in PMCs at meiotic MI (Fig. 7c). FISH showed that in the translocation chromosome, there existed an interstitial *pAs1* signal in the short arm, four interstitial *pAs1* signals in the long arm, and a terminal *pSc119.2* signal at the end of the short arm (Fig. 7b, d). The *pAs1* signals are diagnostic for wheat chromosome 4D (Mukai et al. 1993), and the *pSc119.2* signals are diagnostic for *H. villosa* chromosome 4VS (Zhang et al. 2013). It appears that a small *H. villosa* fragment was transferred to the end of the short arm of wheat chromosome 4D, and thus this translocation chromosome could be designated as T4DL·4DS-4VS. Molecular marker analysis showed that the 4VS-specific amplicons of the primer CINAU66, CINAU70, CINAU74, CINAU77, CINAU301 were all present in line NAU421, indicating that these DNA markers were located in the distal segment of the chromosome arm 4VS (Fig. 5). NAU421 was highly resistant to WYMV (Table 1; Fig. 6b), thus the WYMV resistance locus could be physically mapped to the terminal region of the chromosome 4VS, with the Fragment Length (FL) being 0.78–1.00.

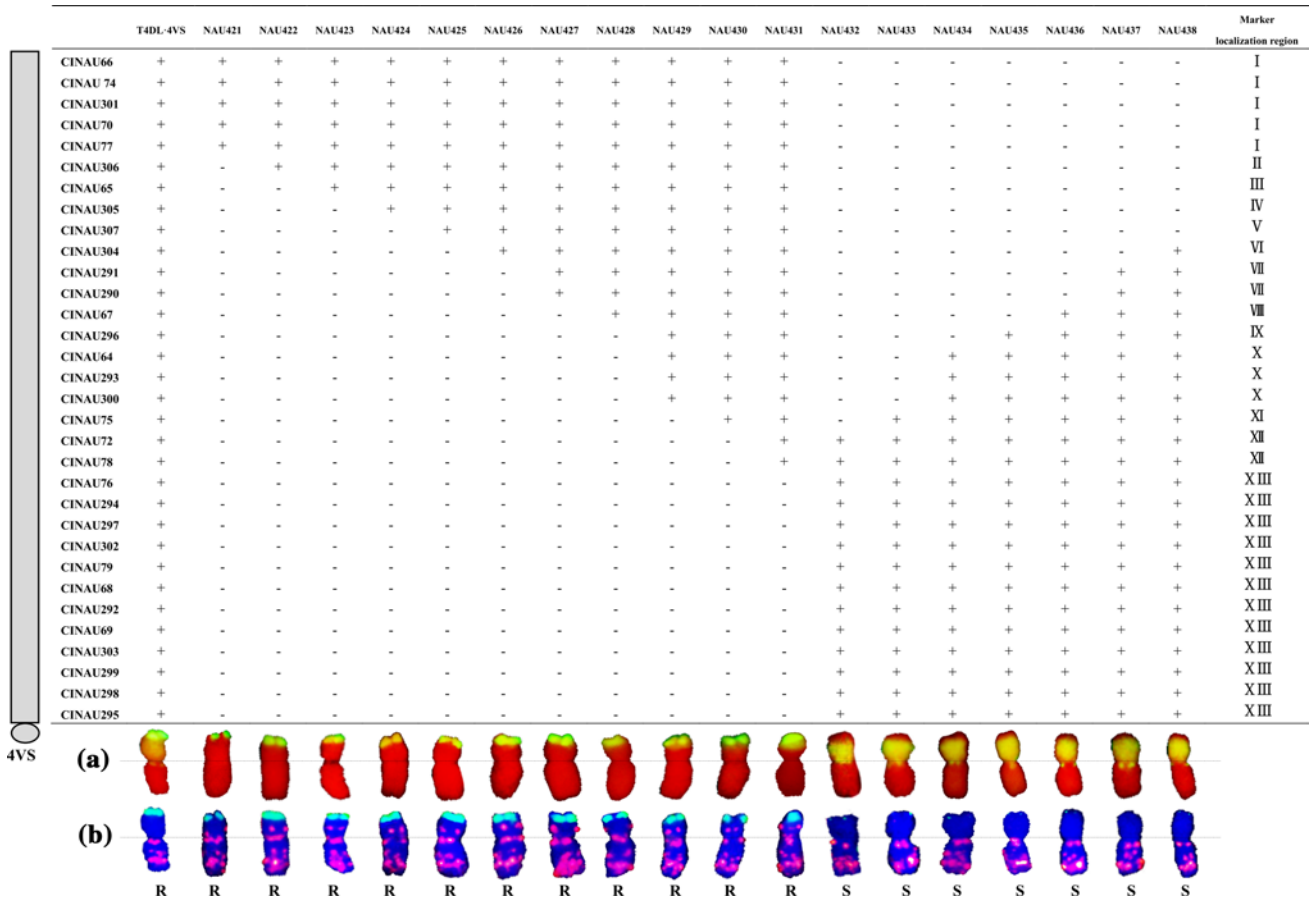

Fig. 5 GISH and FISH patterns, marker analysis using 4VS-specific markers of wheat–*H. villosa* translocation lines. **a** GISH patterns of wheat–*H. villosa* recombinant chromosomes. Total genomic DNA of *H. villosa* was labeled with fluorescein-12-dUTP, visualized with green fluorescence. Chromosomes were counterstained with propidium iodide (PI) and visualized with red fluorescence; **b** FISH patterns of wheat–*H. villosa* recombinant chromosomes. The repetitive sequence clone *pSc119.2* was labeled with biotin-16-dUTP and visualized with green fluorescence, the *pAs1* was labeled with digoxigenin-11-dUTP and visualized with red fluorescence. Chromosomes were counterstained with 4′,6-diamidino-2-phenylindole (DAPI) and visualized with blue fluorescence. "+" indicates the presence of the 4VS-specific marker loci, while "–" indicates the absence of the 4VS-specific marker loci. "I-XIII" indicates the localization region of markers on chromosome 4VS, the order of markers within the same region is unknown

Discussion

The transfer of useful genes from the tertiary gene pool is an effective way for broadening the genetic diversity in the genetic improvement of cultivated wheat. In comparison to the induction of chromosome breakage by ionizing radiation and the use of gametocidal gene effect, the induction of chromosome pairing and recombination between homoeologous chromosomes results in the production of compensatory translocations, which have a much higher chance of being agronomically desirable (Qi et al. 2008; Liu et al. 2011). The manipulation of the *Ph* genetic control system (e.g., nullisomy for 5B, a deletion mutant CS *ph1b* or CS Ph^I stock) (Chen et al. 1994; Li et al. 2011) has been successful in inducing homoeologous recombination and introgressing useful genes from wild relatives into wheat (Friebe et al. 1996; Wang et al. 2003). By using the CS *ph1b* mutant, many translocations between wheat and rye, barely, *Thinopyrum intermedium*, *Lophopyrum elongatum*, and *Aegilops speltoides* have been developed (Lukaszewski 2000; Lukaszewski et al. 2004; Taketa et al. 2005; Qi et al. 2007; Mullan et al. 2009; Niu et al. 2011). In this study, using the CS *ph1b* mutant, all recombinants identified were either terminal or interstitial translocations (Fig. 5), and all were compensatory translocations involving chromosomes 4V and 4D. They could be defined as T4DL·4DS-4VS or T4DS-4VS·4DL, respectively (Fig. 5, S1, S2), indicating that translocation chromosomes were the results of homoeologous recombination between chromosomes T4DL·4VS and 4D. Combined the results of GISH/FISH, we confirmed the bivalents in PMC at MI were formed by chromosomes T4DL·4VS and 4D. Although the compensate translocations showed normal seed set rate, most of them are agronomically poor

Table 1 General statistics for the DI of 18 wheat–*H. villosa* recombinant lines and their parental lines

Entry name	DI (%)			1 % Variance
	26 February, 2012	18 March, 2012	Mean	
T4DL·4VS	3.83	9.26	6.55	B
CS *ph1b*	77.57	74.41	75.99	A
NAU421	20.95	9.83	15.39	B
NAU422	22.29	7.21	14.75	B
NAU423	12.96	17.98	15.47	B
NAU424	19.67	11.96	15.81	B
NAU425	6.02	6.99	6.50	B
NAU426	3.70	5.90	4.80	B
NAU427	1.82	10.90	6.36	B
NAU428	3.50	9.02	6.26	B
NAU429	1.43	4.76	3.10	B
NAU430	12.79	11.56	12.17	B
NAU431	0.35	6.87	3.61	B
NAU432	71.93	66.00	68.97	A
NAU433	73.05	70.44	71.75	A
NAU434	53.36	75.85	64.61	A
NAU435	80.95	69.58	75.27	A
NAU436	69.14	59.95	64.55	A
NAU437	66.23	63.03	64.63	A
NAU438	69.84	68.57	69.21	A

A and B represent that differences between these plants were significant at the $P = 0.01$ level

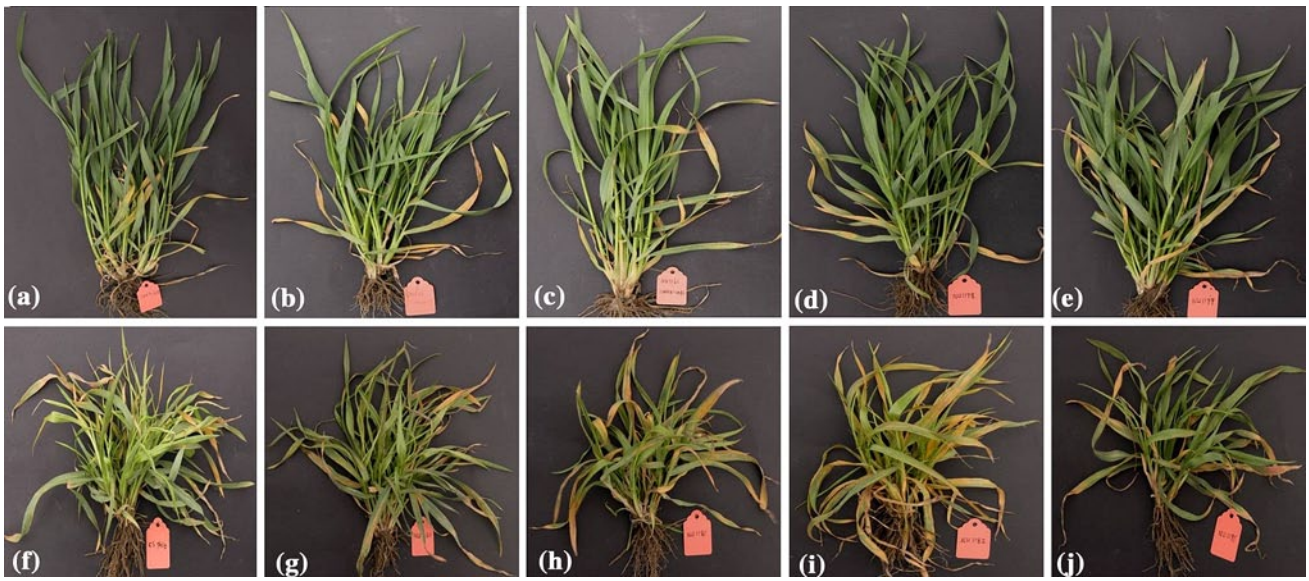

Fig. 6 Evaluation of WYMV resistance of the newly developed wheat–*H. villosa* translocation lines involving 4VS and their parents. The terminal translocations were highly resistant to WYMV, while the interstitial translocation lines were susceptible. **a** T4DL·4VS, **b** NAU421, **c** NAU423, **d** NAU429, **e** NAU431, **f** CS *ph1b*, **g** NAU432, **h** NAU433, **i** NAU434, **j** NAU438

because of the genetic background of Chinese Spring. For further utilization in wheat breeding, their agronomic traits need to be improved by backcrossing with the adapted elite lines or wheat varieties.

It has been reported that homoeologous recombination between chromosomes from alien species and wheat was non-random. Qi et al. (2007) found that all recombination events were restricted to the distal 18 % of the

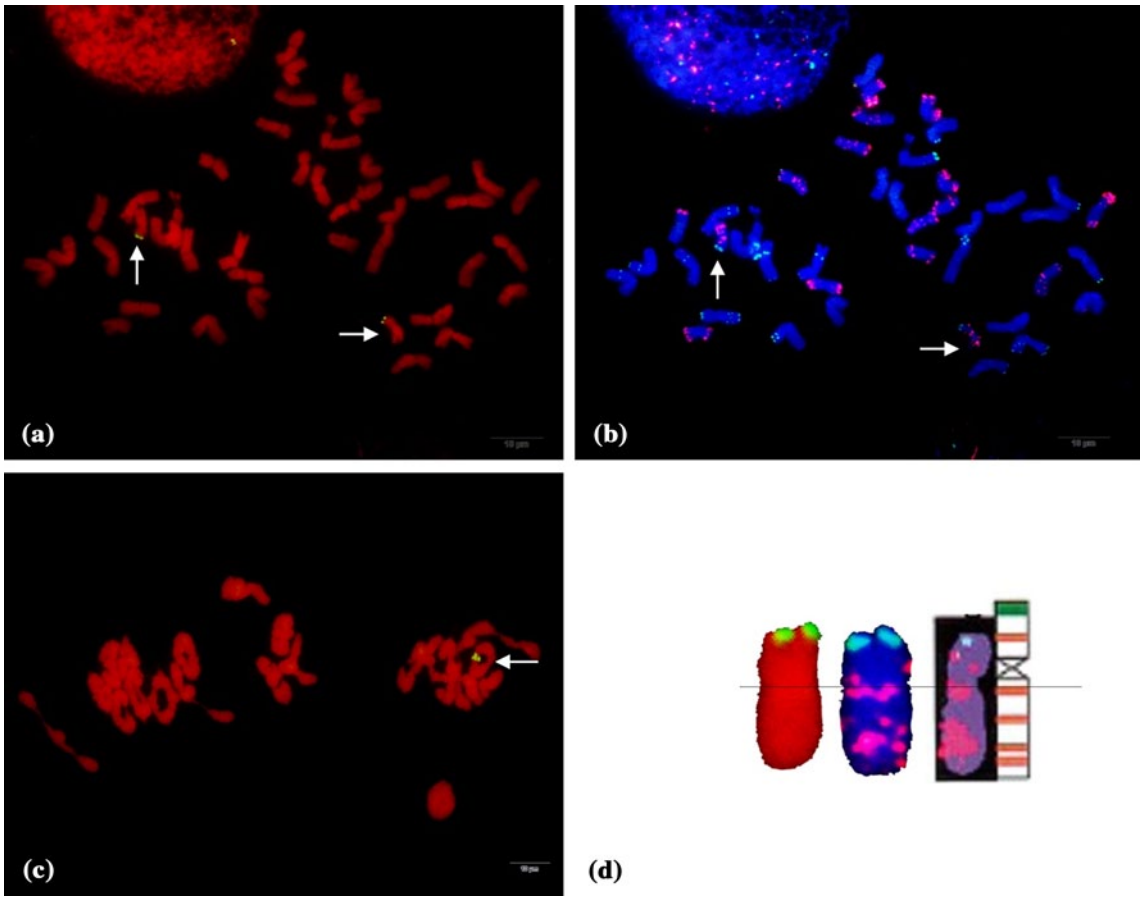

Fig. 7 GISH and FISH patterns of wheat–*H. villosa* translocation line NAU421. **a** GISH pattern of chromosomes on RTC at mitotic metaphase of NAU421 ($2n = 42$). **b** FISH pattern of chromosomes on RTC at mitotic metaphase of NAU421 ($2n = 42$). **c** GISH pattern of chromosomes of PMC at meiotic MI of NAU421; the ring bivalent formed by a pair of translocation chromosomes is indicated with an arrow. **d** From the left to right: GISH and FISH patterns of the translocation chromosome, FISH pattern of chromosome 4D (Mukai et al. 1993). The probes are described in Fig. 5. *Scale bar* = 10 μm

chromosome arm 4Ai#S of *Th. intermedium*. Lukaszewski et al. (2004) discovered that recombination 2RL–2BL was confined to the terminal 25 % of the arm's length. We found that almost all the 18 independent recombinants and their breakpoints concentrated in the distal region of the chromosome arm 4VS (Fig. 5). This phenomenon is mainly due to positive chiasma interference operated in homoeologous recombination (Lukaszewski 1995). Chromosome pairing is initiated at the chromosome ends, first-order chiasmata occur at the chromosome ends, as is well known in wheat (Lukaszewski and Curtis 1993). The second- and third-order chiasmata occur in progressively proximal regions. Apparently, extreme positive interference between homoeologous chromosomes either eliminates chiasmata associations, hence recombination is entirely or very largely restricted to first-order chiasmata (Qi et al. 2007). Hence, it may be difficult to produce recombination and create the desired translocations by the manipulation of the *Ph* system if the potentially transferred target gene was not located in the hot spot region of chromosomal recombination.

Although a large amount of alien chromatin carrying the target genes has been introduced into common wheat, the successful cloning of these useful genes is rarely reported. The low frequency of pairing and recombination between chromosomes from the wild species and cultivated wheat largely limited the effort for cloning alien genes by map-based strategy. However, by using a GeneChip microarray combined with genetic mapping using a series of alien deletion and translocation lines, Cao et al. (2011) has cloned the *Stpk-V* gene from *H. villosa*, and proved it was the key member of the *Pm21* locus which confers high- and broad-spectrum powdery mildew resistance, providing a good example for cloning of the *Wss1* gene in our future study.

Although a number of wheat varieties with both high yield potential and high level of resistance to WYMV have been developed and released, the major genes were either

mapped to homoeologous group 2 (i.e., 2A and 2DL) (Liu et al. 2005a, 2005b; Nishio et al. 2010) or 5AL (Zhu et al. 2012), indicating a relatively narrow genetic diversity of WYMV resistance source. Zhang et al. (2005) first reported the presence of a WYMV resistance gene on 4VS of *H. villosa*, providing a new wild genetic resource for WYMV resistance. It has been found that resistance genes are often present as a gene cluster in a specific chromosome region (Dilbirligi et al. 2004; Krattinger et al. 2009; Zhu et al. 2012). Compared with single gene transfer, development of translocation lines can incorporate more than one useful gene simultaneously into common wheat. Moreover, translocation lines are genetically stable and their resistance is more durable compared with single gene transfer. The wheat-rye T1BL·1RS translocation line is particularly attractive to breeders, one of the important reason is that several useful genes especially disease resistance genes are located in 1RS, such as powdery mildew resistance gene, rust resistance genes, wheat streak mosaic virus resistance gene (Singh et al. 1990; Li et al. 2007). The development of translocation lines with small alien chromosome segments, especially interstitial translocations with multiple useful genes, appears promising in modern wheat breeding. The terminal translocation lines developed in this research are highly resistant to WYMV, whether other useful genes also exist in the terminal region of chromosome 4VS or not remains to be studied.

Acknowledgments This research was supported by National High Technology Research Program ('863' Program) of China (grant no. 2011AA100101, 2011AA10010201), the National Natural Science Foundation of China (grant no. 31201204), Natural Science Foundation of Jiangsu Province (grant no. BK2010448), Technology Support Program of Jiangsu Province (grant no. BE2012306), the Program of Introducing Talents of Discipline to Universities (No. B08025) and A Project Funded by the Priority Academic Program Development of Jiangsu Higher Education Institutions (PAPD).

References

Bassam BJ, Gresshoff PM (2007) Silver staining DNA in polyacrylamide gels. Nat Protoc 2:2649–2654

Blanco A, Simeone R, Resta P (1987) The addition of *Dasypyrum villosum* (L.) Candargy chromosomes to *durum* wheat (*Triticum durum* Desf.). Theor Appl Genet 74:328–333

Cao AZ, Xing LP, Wang XY, Yang XM, Wang W, Sun YL et al (2011) Serine/threonine kinase gene *Stpk-V*, a key member of powdery mildew resistance gene *Pm21*, confers powdery mildew resistance in wheat. Proc Natl Acad Sci USA 108:7727–7732

Chen JP (2005) Research status and prospect of cereal viruses transmitted by *Polymyxa graminis* in China. Proc Nat Sci 15:524–533

Chen PD, Liu DJ (1982) Cytogenetic studies of hybrid progenies between *Triticum aestivum* and *Haynaldia villosa*. J Nanjing Agric Univ 4:1–15

Chen PD, Tsujimoto H, Gill BS (1994) Transfer of *Ph*^{I} genes promoting homoeologous pairing from *Triticum speltoides* to common wheat. Theor Appl Genet 88:97–101

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

Devos KM, Atkinson MD, Chinoy CN, Liu CJ, Gale D (1992) RFLP-based genetic map of the homoeologous group 3 chromosomes of wheat and rye. Theor Appl Genet 83:931–939

Dilbirligi M, Erayman M, Sandhu D, Sidhu D, Gill KS (2004) Identification of wheat chromosomal regions containing expressed resistance genes. Genetics 166:461–481

Friebe B, Jiang J, Raupp WJ, McIntosh RA, Gill BS (1996) Characterization of wheat-alien translocations conferring resistance to diseases and pests: current status. Euphytica 91:59–87

Grądzielewska A (2006) The genus *Dasypyrum*—part 2. *Dasypyrum villosum*—a wild species used in wheat improvement. Euphytica 152:441–454

Hyde BB (1953) Addition of individual *Haynaldia villosa* chromosomes to hexaploid wheat. Am J Botany 40:174–182

Jiang JM, Friebe B, Gill BS (1994) Recent advances in alien gene transfer in wheat. Euphytica 73:199–212

Krattinger SG, Lagudah ES, Spielmeyer W, Singh RP, Huerta-Espino J, McFadden H et al (2009) A putative ABC transporter confers durable resistance to multiple fungal pathogens in wheat. Science 323:1360–1363

Li HJ, Conner RL, Liu ZY, Li YW, Chen Y, Zhou YL et al (2007) Characterization of wheat-triticale lines resistant to powdery mildew, stem rust, stripe rust, wheat curl mite, and limitation on spread of WSMV. Plant Dis 91:368–374

Li HF, Gill BS, Wang XE, Chen PD (2011) A Tal-Ph^{I} wheat genetic stock facilitates efficient alien introgression. Genet Resour Crop Evol 58:667–678

Liu WH, He ZT, Geng B, Hou MS, Zhang M, Nie H et al (2004) Identification of resistance to yellow mosaic disease of wheat and analysis for its inheritance of some varieties. Acta Phytopathol Sin 34:542–547

Liu WH, Nie H, Wang SB, Li X, He ZT, Han CG et al (2005a) Mapping a resistance gene in wheat cultivar Yangfu 9311 to yellow mosaic virus, using microsatellite markers. Theor Appl Genet 111:651–657

Liu WH, Nie H, He ZT, Chen XL, Han YP, Wang JR et al (2005b) Mapping of a wheat resistance gene to yellow mosaic disease by amplified fragment length polymorphism and simple sequence repeat markers. J Integr Plant Biol 47:1133–1139

Liu WX, Rouse M, Friebe B, Jin Y, Gill B, Pumphrey MO (2011) Discovery and molecular mapping of a new gene conferring resistance to stem rust, *Sr53*, derived from *Aegilops geniculata* and characterization of spontaneous translocation stocks with reduced alien chromatin. Chromosome Res 19:669–682

Lukaszewski AJ (1995) Physical distribution of translocation breakpoints in homoeologous recombinants induced by the absence of the *Ph1* gene in wheat and triticale. Theor Appl Genet 90:714–719

Lukaszewski AJ (2000) Manipulation of the 1RS·1BL translocation in wheat by induced homoeologous recombination. Crop Sci 40:216–225

Lukaszewski AJ, Curtis CA (1993) Physical distribution of recombination in B-genome chromosomes of tetraploid wheat. Theor Appl Genet 86:121–127

Lukaszewski AJ, Rybka K, Korzun V, Malyshev SV, Lapinski B, Whitkus R (2004) Genetic and physical mapping of homoeologous recombination points involving wheat chromosome 2B and rye chromosome 2R. Genome 47:36–45

McIntyre CL, Pereira S, Moran LB, Appels R (1990) New *Secale cereale* (rye) DNA derivatives for the detection of rye chromosome segments in wheat. Genome 33:635–640

Mukai Y, Nakahara Y, Yamamoto M (1993) Simultaneous discrimination of the three genomes in hexaploid wheat by multicolor

fluorescence in situ hybridization using total genomic and highly repeated DNA probes. Genome 36:489–494

Mullan DJ, Mirzaghaderi G, Walker E, Colmer TD, Francki MG (2009) Development of wheat–*Lophopyrum elongatum* recombinant lines for enhanced sodium 'exclusion'during salinity stress. Theor Appl Genet 119:1313–1323

Murray TD, Pena RC, Yildirim A, Jones SS (1994) A new source of resistance to *Pseudocercosporella herpotrichoides*, cause of eyespot disease of wheat, located on chromosome 4 V of *Dasypyrum villosum*. Plant Breed 113:281–286

Nishio Z, Kojima H, Hayata A, Iriki N, Tabiki T, Ito M et al (2010) Mapping a gene conferring resistance to *Wheat yellow mosaic virus* in European winter wheat cultivar 'Ibis'(*Triticum aestivum* L.). Euphytica 176:223–229

Niu ZX, Klindworth DL, Friesen TL, Chao SM, Jin Y, Cai XW et al (2011) Targeted introgression of a wheat stem rust resistance gene by DNA marker-assisted chromosome engineering. Genetics 187:1011–1021

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, Chen PD, Gill BS (2008) Molecular cytogenetic characterization of alien introgressions with gene *Fhb3* for resistance to *Fusarium* head blight disease of wheat. Theor Appl Genet 117:1155–1166

Rayburn AL, Gill BS (1986) Isolation of a D-genome specific repeated DNA sequence from *Aegilops squarrosa*. Plant Mol Biol Rep 4:102–109

Riley R, Chapman V (1958) Genetic control of the cytologically diploid behaviour of hexaploid wheat. Nature 182:713–715

Sharp PJ, Chao S, Desai S, Gale MD (1989) The isolation, characterization and application in the Triticeae of a set of wheat RFLP probes identifying each homoeologous chromosome arm. Theor Appl Genet 78:342–348

Singh NK, Shepherd KW, McIntosh RA (1990) Linkage mapping of genes for resistance to leaf, stem and stripe rusts and ω-secalins on the short arm of rye chromosome 1R. Theor Appl Genet 80:609–616

Somers DJ, Isaac P, Edwards K (2004) A high-density microsatellite consensus map for bread wheat (*Triticum aestivum* L.). Theor Appl Genet 109:1105–1114

Taketa S, Awayama T, Ichii M, Sunakawa M, Kawahara T, Murai K (2005) Molecular cytogenetic identification of nullisomy 5B induced homoeologous recombination between wheat chromosome 5D and barley chromosome 5H. Genome 48:115–124

Uslu E, Miller TE, Rezanoor NH, Nicholson P (1998) Resistance of *Dasypyrum villosum* to the cereal eyespot pathogens, *Tapesia yallundae* and *Tapesia acuformis*[J]. Euphytica 103:203–209

Wang XW, Lai JR, Chen LH, Liu GT (1998) Molecular identification for Chinese Spring *ph1b* mutant. Scientia Agric Sincia 31:31–34

Wang RRC, Li XM, Hu ZM, Zhang JY, Larson SR, Zhang XY et al (2003) Development of salinity-tolerant wheat recombinant lines from a wheat disomic addition line carrying a Thinopyrum junceum chromosome. Int J Plant Sci 164:25–33

Xin ZY, Zhang ZY, Chen X, Lin ZS, Ma YZ, Xu HJ et al (2001) Development and characterization of common wheat-*Thinopyrum intermedium* translocation lines with resistance to barley yellow dwarf virus. Euphytica 119:161–165

Zhang P, Li WL, Friebe B, Gill BS (2004) Simultaneous painting of three genomes in hexaploid wheat by BAC-FISH. Genome 47:979–987

Zhang QP, Li Q, Wang XE, Wang HY, Lang SP, Wang YN et al (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 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. Chinese Sci Bull 58:890–897

Zhu XB, Wang HY, Guo J, Wu ZZ, Cao AZ, Bie TD et al (2012) Mapping and validation of quantitative trait loci associated with wheat yellow mosaic bymovirus resistance in bread wheat. Theor Appl Genet 124:177–188