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**Molecular Cytogenetic Identification of a Wheat-Rye 1R
Addition Line with Multiple Spikelets and Resistance to
Powdery Mildew**

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Abstract Alien addition lines is important for transferring useful genes from alien species into common wheat. Rye is an important and valuable gene resource for improving wheat disease resistance, yield and environment adaptation. A new wheat-rye addition line N9436B was developed from the progeny of the cross of common wheat (*Triticum aestivum* L., $2n = 6x = 42$, AABBDD) cultivar Shaanmai 611 and rye (*Secale cereal* L., $2n = 2x = 14$, RR) accession Austrian rye. We characterized this new line by cytology, genomic *in situ* hybridization (GISH), Fluorescence *in situ* hybridization (FISH), molecular marker and disease resistance screening. N9436B was stable in morphology and cytology with chromosome composition of $2n = 42+2t = 22 II$. The GISH investigations showed this line contained two rye chromosomes. GISH, FISH and molecular makers identification suggested that the introduced R chromosome and the missing wheat chromosome arms were 1R chromosome and 2DL chromosome arm, respectively. N9436B exhibited 30 to 37 spikelets per spike and high level of resistance to powdery mildew (*Blumeria graminis* f. sp. *tritici*, *Bgt*) isolate E09 at the seedling stage. N9436B was cytological stable, had the trait of multiple spikelets and resistance to powdery mildew, and is expected to be useful in wheat improvement.

Key words: Wheat-rye addition line, Multiple spikelets, Powdery mildew resistance, GISH and FISH, molecular makers.

Introduction

Rye (*Secale cereale* L., $2n = 2x = 14$, RR), a species closely related to wheat (*Triticum aestivum* L., $2n = 6x = 42$, AABBDD), has been used extensively and successfully as a valuable and significant germplasm resources for wheat cultivar improvement in improving disease resistance, quality, yield, and environment adaptation (Friebe et al. 1996). Importing the exogenous gene of rye into wheat can lead the genetic variation to further expand, produce new materials, so both in genetic analysis, or in breeding new materials rye have important value.

The productivity of single spike's and yield showed significant positive correlation on the basis of certain spikes number (Song et al. 1996), so as the most important way to improve wheat yield, the improvement of single spike's productivity was wide-spread attention from international breeders (Smocek 1988; Li 1993; Ren 1995; Song et al. 1996). The multiple spikelets of rye, from 33 up to 40, are potential valuable source of genes for wheat yield improvement. Transferring its multiple spikelet characteristic to common wheat can cultivate wheat germplasms with multiple spikelets. To date, the multiple spikelets line 10-A with 30 to 37 spikelets per spike was developed by Yen (Yen et al. 1993). 10-A was derived from the cross an octaploid triticales / common wheat cultivar Avrora, and octaploid triticales was artificially synthesized from common wheat cultivar Yaanai No.10 and *S.*

cereale accession Qinling rye (Yen et al. 1993). Aurora was proved to be a 1B/1R translocation line (Zeller 1973) and 10-A was proved carried the 1RS/1BL wheat-rye translocation chromosome (Wei et al. 1999). Therefore, rye is a potential reservoir for the improvement of wheat yield.

Powdery mildew of wheat caused by *Blumeria graminis* f. sp. *tritici* (*Bgt*) is one of the most damaging diseases. The pathogen can attack all above-ground wheat parts including leaves, stems, and spikes, and is the most seriously wheat diseases effect the production safety of wheat in China and other parts of the world. Powdery mildew can cause significant yield losses in most of the wheat production areas. Powdery mildew made yield losses ranging from 17 to 34 % have been reported (Johnson et al. 1979; Leath and Bowen 1989). Therefore, the deployment of resistant cultivars is the most reliable, economical, and environmentally safe approach to cope with this disease (Bennett 1984). To date, about 80 formally designated *Pm* genes have been identified at 49 loci in wheat and its wild relatives (*Pm1*–*Pm54*, *Pm18* = *Pm1c*, *Pm22* = *Pm1e*, *Pm23* = *Pm4c*, *Pm31* = *Pm21*, *Pm8* is allelic to *Pm17*) with the loci *Pm1*, *Pm2*, *Pm3*, *Pm4*, *Pm5* and *Pm24* having 5, 3, 17, 4, 5 and 2 alleles, respectively (Hao et al. 2008; Hsam et al. 1998; McIntosh et al. 2013, 2014; Singrün et al. 2003; Xie et al. 2012; Ma et al. 2015; Hao et al. 2015; Xu et al. 2015). Among these genes or alleles, about 40 were derived from *T. aestivum*, whereas the others originated either from species closely related to

common wheat, such as *T. monococcum*, *T. turgidum*, *T. timopheevii*, or more different genera, such as *Secale*, *Aegilops*, *Haynaldia*, and *Elytrigia* (Xiao et al. 2013). Rye offers a rich reservoir of genes for enhancing useful genetic variability in wheat breeding. The powdery mildew resistance genes derived from rye are *Pm7*, *Pm8*, *Pm17* and *Pm20*, located on 2RL, 1RS, 1RS, and 6RL chromosomes, respectively. Some have already been successfully used in the commercial wheat production. The extensive utilization of these resistant genes may make them susceptible to new pathogen races because of co-evolution of host and pathogen, and the cultivars with these resistance genes lost resistance to pathogens. After widespread agricultural cultivation, the gene *Pm8* is now widely overcome by adapted mildew races (Lutz et al. 1992; Yang and Ren 1997). Therefore, it is important to identify and deploy of new resistance gene sources in other rye genotypes. It has been already reported that rye chromosomes 4R, 5R and 6R carry powdery mildew resistant genes (Friebe et al. 1994; An et al. 2013; Fu et al. 2010, 2011, 2014).

Distant hybridization can transfer the desirable traits from wild relatives into common wheat and promote the new alien germplasms with advantageous exogenous genes (Anamthawat-Jónsson 1995), including amphidiploids, addition, substitution, and translocation lines. Traditionally, addition line is not only a genetic material to research the origin and evolutionary of species, relationship between genome, interaction and

expression of gene, but also as an intermediate material play a bridging role for developing substitution lines and translocation lines and introgression lines in wheat breeding. To date, the complete set of wheat–rye addition lines include Holdfast–King II, Kharkov–Dakold, Chinese Spring (CS)–Imperial and CS–King II (Xue et al. 1993). In addition, other wheat–rye addition lines have been reported (O'Mara 1940; Hu and Wang 1990; Liu and Xin 1993; Fu et al. 2011). Other rye genotypes should be used to create different wheat–rye addition lines for potential utilization in wheat improvement.

Winter rye cultivar Austrian rye (*S. cereale* L.) is a valuable resistant resource for wheat improvement due to its superior and wide resistance to various isolates of powdery mildew pathogens prevalent in China. Common winter wheat cultivar Shaanmai 611 possesses the characters of high-yielding, dwarf and wide adaptation. A new wheat–rye 1R chromosome addition line, N9436B, derived from the cross of Shaanmai 611 and Austrian rye, has the property of multiple spikelets and showed a high level of resistance to powdery mildew. The objectives of this study were to determine the genomic composition of N9436B using molecular cytogenetic methods, characterize its resistance to powdery mildew, and evaluate its agronomic performance.

Materials and methods

Plant materials

A wheat–rye addition line, was produced by crossing winter wheat cultivar Shaanmai 611 with winter rye accession Austrian rye. Shaanmai 611 and Austrian rye were employed as controls in the agronomic trait assessment and in the DNA marker and electrophoretic analyses. Kavkaz with the gene *Pm8* and Amigo with the gene *Pm17* both derived from rye chromosome 1RS, and wheat cultivars Shaanyou 225 were conserved in the College of Agronomy, Northwest A & F University, Yangling, Shaanxi Province, China. They were used in this study as controls for testing resistance to powdery mildew. Total DNA extracted from rye cultivar Austrian rye was used as probe in genomic *in situ* hybridization (GISH) and Fluorescence *in situ* hybridization (FISH) detection. We used the total DNA extracted from 1R addition line of ‘CS × Imperial’ as control to detect the Austrian rye chromosome in N9436B by polymerase chain reaction (PCR) analysis. All plant materials were maintained by strict selfing in the field of Northwest A & F University.

Production of wheat–rye chromosome addition line N9436B

Wheat cultivar Shaanmai 611 crossed with Austrian rye was performed in 1994. We selected the plants from their F₁ hybrids of which has the multiple

spikelet property, then made these plants consecutive and strict selfing. Among the offspring, the plants with the trait of multiple spikelet and resistance to powdery mildew were selected. Finally, N9436B, with multiple spikelets, resistance to powdery mildew and genetically stable genotype with chromosome composition $2n = 42 + 2t = 22 \text{ II}$ had been obtained.

Evaluation of agronomic performance

The wheat-rye derivative N9436B and its parents Shaanmai 611 and Austrian rye were planted in early October and harvested in the middle of June next year. From seedling to maturity, we observed and recorded growth conditions of N9436B and their parents. Before harvested, 10 plants of each material were selected randomly, and measured and recorded the traits including plant type, plant height, spike length, spikelet number per spike, kernel number per spike, resistance to powdery mildew. After harvested, the characters of kernel and thousand-kernel weight (TKW) of each material were measured and recorded.

Identification of the Cytology

The chromosome number of the RTC (Root tip cell)

When the roots were 1.5–2.0 cm in length, the root tips were removed and pretreated with ice-water at 0–4 °C for 24 h, fixed in Carnoy's fixative fluid (a 3: 1 ethanol-acetic acid mixture) at 4 °C for at least 2 days. The root tips were stained with 1% (w/v) aceto-carmin solution overnight and then squashed in 45% (v/v) acetic acid. Finally, identified and photographed the number of chromosomes with an Olympus BX-43 microscope (Japan) equipped with a PhotometricsSenSys CCD camera. 30 or more cells were observed.

The configuration of PMC (Pollen Mother Cell) at MI (Metaphase I)

Young spikes were sampled at the appropriate stage in the morning 7:00 ~ 9:00, with the temperature 12°C ~ 17°C in early April 2014. The spikes were put into 6:3:1 ethanol-chloroform-acetic acid mixture for at least 48 h. Then the anthers were removed and squashed in 1% aceto-carmin solution. Finally, the cells at MI with a complete chromosome complement were photographed with an Olympus BX-43 microscope (Japan) equipped with a PhotometricsSenSys CCD camera. 30 or more cells were observed.

Identification of resistance to powdery mildew

Assessment at seedling stage

Powdery mildew reactions of the seedling stage of N9436B were assessed via inoculation with *Bgt* isolate E09 (kindly provided by Drs Xiayu Duan and Yilin Zhou, State Key Laboratory for Biology of Plant Disease and Insect Pests, Institute of Plant Protection, Chinese Academy of Agricultural Sciences, Beijing, China). We plant N9436B and its parents Shaanmai 611 and Austrian rye, Shaanyou 225, Kavkaz and Amigo in each pot with 20 seeds, Shaanyou 225 as the susceptible control. E09 was maintained on Shaanyou 225 until the leaf was fully expanded by conidia. Plants were inoculated by dusting conidia from sporulating seedlings of Shaanyou 225 at the two to three leaf stages, and then transferred to a temperature-controlled greenhouse in the College of Agronomy, Northwest A & F University, Yangling, Shaanxi, China. After inoculated about 15 days, when the pustules were fully developed on Shaanyou 225, then investigate and record the infection types (IT) of each material, again investigate and record after 3 days. IT was recorded based on a 0–4 level of each plant, of which 0 = immune, no visible symptoms and signs; 0₊ = almost immune, necrotic flecks without sporulation; 1 = high resistant, sparse aerial hypha and little sporulation, with diameter of colonies less than 1 mm; 2 = middle resistant, moderate aerial hypha and sporulation, with diameter of colonies less than 1 mm; 3 = middle susceptible, thick aerial

hypha and abundant sporulation, with diameter of colonies more than 1 mm; and 4 = high susceptible, abundant sporulation with more than 80 % of the leaf area covered with aerial hypha. Plants with an IT score of 0–2 were considered resistant, while those with an IT score of 3–4 were considered as susceptible (Si et al. 1992).

Assessment at adult stage

Resistance to powdery mildew at adult stage was tested on N9436B and its parents, Kavkaz and Amigo in the powdery mildew disease nursery in the College of Agronomy, Northwest A & F University, Yangling, Shaanxi, China, using a mixture of *Bgt* isolates prevalent in Guanzhong region of Shaanxi Province in China. Individual plants were spaced 10 cm apart within 1-m-long rows, with row spacings of 25 cm. As the susceptible control, Shaanyou 225 was planted around the nursery. The tests with the mixture of the isolates were conducted using the procedures described by Duan (1998). After the wheat heading, investigate and record the level of resistance powdery mildew of the testing material when the susceptible controls Shaanyou 225 were fully infected. Disease reaction was assessed on a 0–9 scale, which 0 = whole plant disease-free after heading; 1–2 = high resistant, plant disease extended to the top fourth leaf; 3–4 = middle resistant, the disease extended to the top third

leaf; 5–6 = middle susceptible, the disease extended to the second leaf; 7–8 high susceptible, flag leaf has disease; 9, the diseases extended to spike. The adult plant reactions test was repeated in the following year's growing season using the same procedure.

DNA Extraction

The genomic DNA of common wheat Shaanmai 611, Austrian rye and wheat – rye addition line were isolated from seedling leaves using a modified CTAB method (Doyle and Doyle 1987), with one additional purification step using chloroform to obtain high-quality DNA, which were used for GISH, FISH and molecular marker analysis.

Molecular markers screening and electrophoretic analysis

Polymerase chain reaction (PCR) assays was used to detect the alien chromosome in wheat–rye addition line N9436B. The materials including Shaanmai 611, Austrian rye, N9436B, 1R addition line of 'CS × Imperial'.

In order to detect the alien chromosome in wheat-rye addition line N9436B, one SSR marker, *TSM716*, specific for rye chromosome arm 1RS, and two STS-PCR markers, *NOR-R1* and *NOR-I* (Koeber 1995), specific for rye

chromosome 1R and rye chromosome arm 1RS, respectively (Table 2). The primers were all synthesized by Beijing AuGCT DNA-SYN Biotechnology Co., Ltd. DNA amplification was conducted in 10µl reaction volume containing 6.54µl of double-distilled water, 1.0µl of 10 × PCR buffer, 0.8µl of dNTP mixture (Mg^{2+}) (2.5 mM), 0.06µl of *Taq* DNA polymerase (2.5 U/µl), 0.4µl of each primer, and 0.8µl of template DNA (100-150ng/µl). The PCR was performed by using a S1000TM Thermal Cycler (Bio-Rad, California, USA) for 1 cycle at 94 °C for 3 min, followed 35 cycles at 94 °C for 30s, 50-60°C (based on the primer information) for 30s, and 72 °C for 45s, with a final extension at 72 °C for 10 min before cooling to 4 °C. The PCR products were separated in 8 % nondenaturing polyacrylamide gel and then silver-stained (Tixier and Sourdille 1997) and photographed.

Genomic *in situ* hybridization (GISH) analysis

GISH analysis was conducted to detect alien chromosome in N9436B. Seeds were germinated on moistened filter paper in petri dishes, after seeds germination, put petri dishes into refrigerator with 4°C for 24 h, then in incubator with 23°C until the roots developed to 1.5-2.0 cm, put the roots into centrifuge tube filled with ice-water at 0–4 °C for 24 h, fixed in Carnoy's fixative fluid (a 3: 1 ethanol-acetic acid mixture) at 4 °C for at least 2 d. The

root tips were digested in 1% pectinase + 2% cellulase at 37 °C for 50-60 min (different materials were subjected to different digestion time), slides were prepared using the drop technique (Han et al. 2004). Genomic DNA of Austrian rye was labeled with DIG-Nick-Translation Mix and used as a probe (Roche, Germany). The GISH procedure was performed as described by Liu (2010) with minor modifications. Finally, detected and took photos, the images captured for each color channel was viewed and photographed with a PhotometricsSenSys CCD camera (Olympus BX53F, Japan).

Fluorescence *in situ* hybridization (FISH) and GISH analysis

Multicolor FISH and GISH analysis was conducted to detect alien chromosome and the missing wheat chromosome arms in N9436B using Oligo-pTa535 (red) and Oligo-pSc119.2 (green) as probes on root tip metaphase chromosomes of Austrian rye, Shaanmai 611 and N9436B, respectively. Chromosome spreads of materials prepared according to the methods previously described by Han (2004). Oligonucleotide probes, Oligo-pTa535 and Oligo-pSc119.2, were 5' end-labelled with 6-carboxyfluorescein (6-FAM) or 6-carboxytetramethylrhodamine (Tamra), synthesized by Shanghai Invitrogen Biotechnology Co. Ltd. (Shanghai, China), as described by Tang (2014b). The genomic DNA of Austrian rye was labeled with Alexa

Flour 488-5-dUTP (Invitrogen). Each rye chromosome can be discriminated by Oligo-pSc119.2 signals. Chromosomes were counterstained with DAPI (blue). Finally, detected and took photos, the images captured for each color channel was viewed and photographed with a PhotometricsSenSys CCD camera (Olympus BX53F, Japan).

Results

Cytological characterization of N9436B

The root tips and young spikes were sampled in the field at the appropriate time for the respective samplings. The results of the root tips and young spikes indicated that N9436B had chromosome number of $2n = 42+2t$ with four satellite chromosomes at mitotic metaphase cells (Fig. 1a) and its configuration was $2n = 22 \text{ II}$ at MI of PMC (Fig. 1b). Therefore, we confirmed that N9436B was cytogenetically stable.

GISH analysis of N9436B

GISH analysis was done to determine the chromosome constitution of N9436B using genomic DNA of Austrian rye as a probe. The mitotic GISH of

somatic cells showed that N9436B had two chromosomes with bright yellow-green hybridization signals (Fig. 2*a*), the meiotic GISH of PMC MI showed that N9436B possessed a bivalent with bright yellow-green hybridization signal (Fig. 2*b*). Therefore, N9436B contained two chromosomes from Austrian rye and 40 complete chromosomes and two telosomes of wheat, and meanwhile the two alien chromosomes formed one paired bivalent during the meiotic stage. The GISH analysis also showed that other chromosomes displayed red signals counterstained with DAPI, indicating that these chromosomes originated from the wheat parent Shaanmai 611. So, N9436B was proved to be a wheat-rye addition line. In order to make the results more convincing, we have identified 35 plants of N9436B using the GISH analysis and 33 plants obtained same results, indicating that N9436B was a genetically stable wheat-rye addition line.

Multicolor FISH and GISH analysis of N9436B

FISH analysis was conducted to detect alien chromosome and the missing wheat chromosome arms in N9436B. Inspired by that FISH probes—tandem repeat sequence pTa-535 (red) and pSc119.2 (green) can identify wheat A-, B-, and D-genome chromosomes effectively (Tang et al. 2014b) and pSc119.2 could also discriminate R-genome chromosomes (McIntyre et al. 1990). FISH

karyotypes of Austrian rye, Shaanmai 611 and N9436B were primarily established by employing Oligo-pSc119.2 (green), Oligo-pTa-535 (red)/Oligo-pSc119.2 (green) and Oligo-pTa-535 (red)/Oligo-pSc119.2(green), respectively (Figs. 3*a*, 3*b*, 3*c*). Therefore, the two chromosomes from Austrian rye in N9436B is 1R chromosome. Then, chromosome constitutions of N9436B was successfully karyotyped by combining Oligo-pTa-535, Oligo-pSc119.2 and rye's genomic DNA (green) as probes (Fig. 3*d*) which further demonstrated the karyotype of N9436B. Therefore, the chromosome constitutions of N9436B is $2n = 42 + 2t = 40W + 2t_w + 2(1R)$ (W = wheat chromosome; t_w = wheat telosome chromosome; 1R = rye 1R chromosome).

The karyotype of Austrian rye, Shaanmai 611 and N9436B established according to Tang (2014b). The rye chromosome in N9436B is 1R chromosome by compared karyotype of Austrian rye with N9436B and it is losing an Oligo-pSc119.2 green signal on 1RL (Figs. 3*a*, 3*d*, 3*c*). The missing wheat chromosome arms in N9436B were 2DL by compared karyotype of N9436B with Shaanmai 611 (Figs. 3*b*, 3*d*, 3*c*). We also labeled the 4A chromosome in Shaanmai 611 and N9436B because of in whole chromosomes only chromosome 4A similar to chromosome 2D, the short arms of them are same. However, Oligo-pTa535 red signal contained on chromosome arm 2DL but not on chromosome arm 4AL. N9436B has complete 4A chromosome, so we confirmed that the missing wheat chromosome arms in N9436B were

2DL.

More interestingly, some chromosomes' FISH signal patterns of N9436B different from their parents' to some extent, especially the A-genome and B-genome's chromosomes, which indicate alterations of wheat chromosomes. Specifically, 5AL arm of N9436B displayed Oligo-pSc119.2 green signal loss and intercalary Oligo-pTa535 red signal gain. 1BS and 1BL, 7BS and 7BL arms of N9436B contained intercalary terminal Oligo-pTa535 red signal. In addition, 3AL of N9436B presented Oligo-pTa535 red signal enhancement than Shaanmai 611.

Therefore, N9436B was demonstrated to be a wheat-rye addition line carrying A-genome, B-genome, D-genome's chromosomes (missing chromosome arms 2DL) of wheat and 1R chromosome of Austrian rye. The plentiful structural alterations of wheat chromosomes were observed in N9436B.

Molecular markers screening and electrophoretic

In this study, markers *TSM716*, *NOR-RI* and *NOR-I* were used to detect the alien chromosome in wheat-rye addition line N9436B. DNA fragments range from 100 to 2000 bp, amplified from N9436B and Austria rye, indicating that N9436B contained the DNA region specific for chromosome 1R derived from

Austria rye. The corresponding diagnostic fragments were also detected in 1R addition line of 'CS×Imperial' (Fig. 4). The results showed that the alien chromosome in N9436B was chromosome 1R, which were consistent with FISH detection results of N9436B.

Agronomic performance and reaction to powdery mildew of N9436B

After more than 10 generations of selfing, no segregation was observed in wheat–rye addition line N9436B, neither in morphology nor in cytology. The plant type of N9436B was similar to common wheat and had a compact plant type but was higher than its parent Shaanmai 611 (Table 1; Fig. 5c). The spikes of N9436B were showed superior performance on spike length, spikelets per spike and kernels per spike (Table 1; Fig. 5b). The seeds of N9436B were red and similar to Shaanmai 611 in shape and size (Fig. 5a). The average TKW of N9436B was 30.06 g, which was higher than Austrian rye but less than Shaanmai 611 (Table 1).

For testing the powdery mildew reaction at the seedling stage, N9436B, Austrian rye, Kavkaz, Amigo and susceptible controls Shaan you 225 were inoculated with the *Bgt* isolate E09. Austrian rye and N9436B showed immunity to E09 isolate with an IT score of 0. In contrast, the susceptible control Shaanyou 225 was highly susceptible to E09 isolate with IT score of 4,

Shaanmai 611 was middle susceptible to E09 isolate with IT score of 3 and Kavkaz and Amigo were susceptible to E09 isolate (Fig. 6). For adult plant tests with powdery mildew in the field, the plants of N9436B, Austrian rye, Shaanmai 611, Kavkaz, Amigo and the susceptible control Shaanyou 225, were inoculated with the mixture of *Bgt* isolates prevalent in Guanzhong region of Shaanxi Province in China in two consecutive wheat growing seasons. Shaanmai 611 and the susceptible control Shaanyou 225 were covered by *Bgt* spores and all showed highly susceptible with the disease reaction from 7 to 8 scale, Kavkaz with the gene *Pm8* and Amigo with the gene *Pm17* showed susceptible with the disease reaction on 8 and 6 scale, respectively. Whereas Austrian rye and N9436B showed immune to the mixture of *Bgt* isolates with a disease reaction on 0 scale (Fig. 6). Therefore, N9436B was immune to powdery mildew at seedling stage and adult stage. The powdery mildew resistant gene(s) in N9436B should be from 1R chromosome of Austrian rye and the gene (s) could be a new gene in 1R for resistance or new alleles of *Pm8* and *Pm17*.

Discussion

There are three factors affecting the yield of wheat, spikes per acre, kernels per spike and KTW. Therefore, the improvement of wheat spike traits is one

way to get high yield. Rye a species closely related to wheat, per spike have the characteristic of the multiple spikelets about 30 generally, up to 40. Transferring its property to common wheat can cultivate wheat germplasm with multiple spikelets. To date, the multiple spikelets line 10–A with 30 to 37 spikelets per spike was developed by Yen (Yen et al. 1993), and has been proved carried wheat–rye 1RS/1BL translocation chromosome (Wei et al. 1999). In the present study, N9436B, with 31–37 spikelets per spike, was proved to be a wheat–rye 1R addition line. Both 10–A and N9436B have the same rye chromosome 1R, so the gene(s) controlling the trait of multiple spikelets may be related to rye chromosome 1R. However, other wheat–rye 1R addition, substitution, or translocation lines were not be reported with the characteristic of multiple spikelets (Xue et al. 1993). The gene loci located on wheat group 2 chromosomes were already shown to be involved in the control of number of the spikelets and kernel number per spike in wheat (Sears, 1954; Klingworth et al. 1990; Peng et al. 1998; Dobrovolskaya et al. 2009; Li et al. 2012; Zhang et al. 2013). The gene on chromosome 2D has the strongest effect on the expression of the multiple spikelets character (Peng et al. 1998), and genes governing spike branching and supernumerary spikelets located on chromosome arm 2DS (Dobrovolskaya et al. 2009). Sears (1954) found that hexaploid wheat nullisomic for the chromosome 2A or 2D might generate multiple spikelets trait of which the gene inhibiting this trait located on

chromosome 2DS and 2AL. chromosome 2D of common wheat carried a strong inhibitor of multiple spikelets expression (Klingworth et al. 1990). Therefore, the missing 2DL arms also may have caused significant spikelet number increase. These results suggested the possible influence of the genotype of rye and wheat or the missing 2DL arms on the appearance of multiple spikelets. In addition, N9436B has the shortcomings of late maturity, the higher plant height and low KTW (30.6 g), which need to be further improved. Therefore, the wheat-rye addition N9436B should be a useful bridge material to produce wheat-rye substitution line and translocation line with multiple spikelets.

As the tertiary gene pool for wheat, rye plays an important role in the genetic improvement of wheat, and as a cross-pollinated crop, rye offers significant and abundant genetic diversity within and between cultivars. The powdery mildew resistance genes derived from rye are *Pm7*, *Pm8*, *Pm17* and *Pm20*. They located on 2RL, 1RS, 1RS, and 6RL chromosomes, respectively, and they have already been successfully used in the commercial wheat production. *Pm8*, derived from rye cultivar Petkus (Hsam and Zeller 1997) and *Pm17*, from rye cultivar Insave (Heunet et al. 1990) were proved to be allelic genes and widely used in wheat breeding and improvement programs as translocations T1BL·1RS and T1AL·1RS lines, respectively (Rabinovich 1998). In China, there was about 38% of wheat cultivars with T1BL·1RS

translocation (Zhou et al. 2004). However, because of the co-evolution of pathogen and host, led to the new virulent pathogen isolates emergence rapidly (McDonald and Linde 2002). The cultivars with 1RS translocation successively lost the resistance to powdery mildew, *Pm8* and *Pm17* were not resistant to powdery mildew (Zhuang and Li 1993; Zhuang 2003). In addition, there were many new wheat-rye germplasms and powdery mildew resistance genes from rye were identified and reported. These *Pm* genes were located on 1R, 2R, 4R and 6R chromosomes of rye, and their reaction patterns were different from the four known *Pm* genes *Pm7*, *Pm8*, *Pm17*, and *Pm20* derived from rye (Li et al. 2004; Hysing et al. 2007; Tang et al. 2008; Ren et al. 2009; Fu et al. 2010, 2011, 2014; Wang et al. 2009, 2010; Zhuang et al. 2011; An et al. 2006, 2013;). In the present study, wheat cultivar Shaanmai 611 displayed highly susceptible to powdery mildew, wheat-rye addition line N9436B that contained 1R chromosome of Austrian rye showed immunity to powdery mildew. In this study, Kavkaz and Amigo, with the gene *Pm8* and *Pm17* located on rye chromosome 1R, respectively, were also employed, and displayed susceptible to powdery mildew. Therefore, the powdery mildew resistant gene in N9436B should be from 1R chromosome of Austrian rye and it could be a new gene in 1R for resistance or new alleles of *Pm8* and *Pm17*. Wheat-rye addition, substitutions as well as translocations have been successfully used in wheat breeding and improvement programs. To further

use the powdery mildew resistance of the 1R addition line N9436B in wheat improvement, we are developing small segmental translocation and introgression lines of chromosome 1R by the $^{60}\text{Co}\gamma$ radiation, *ph1b*-induced homoeologous recombination, gametocidal chromosome originating from *Aegilops* and backcrossing strategy with commercialized cultivars. Small segmental translocations would be identified by 1R-specific markers, GISH, mc-FISH and mc-GISH approaches. Finally, the translocation lines involving small fragment with resistance gene (s) will be obtained and used in wheat resistance breeding for powdery mildew.

Wide hybridization is one of the stresses that may cause reorganization of the parental genomes (McClintock 1978). Wide hybridization between wheat and rye is an important tool in wheat breeding and for development of more highly engineered introgression lines for wheat improvement programs. Wheat-rye derivatives include amphiploid, chromosome addition, substitution and translocation lines. The introduction of rye chromatin into common wheat could result in changes of chromosome structure of common wheat (Ren 1991). Chromosome instability and genome rearrangements in wheat-rye disomic addition lines were also reported (Szakacs and Molnar-Lang 2010; Bento et al. 2010). A single 1R chromosome added to wheat might cause abnormal mitotic behaviour of both wheat and rye chromosomes and different genetic variations might occur among the sibling 1R monosomic addition

lines (Fu et al. 2014). One 2D chromosome was broken and three 4A chromosomes were observed in one of the selfed progeny of 7R monosomic addition line. The elimination of 1A and 4B chromosomes, the structural variation and abnormal mitotic behaviour of 3D chromosome were detected in the selfed progeny of 6R monosomic addition line (Fu et al. 2013). The breakage and deletion of wheat chromosomes 7B, 3B and 4D were observed in the selfed progenies of 5R monosomic addition line (Ge et al. 2014). The alterations of wheat chromosomes including 5A, 6A, 1B, 2B, 6B, 7B, 1D, 3D and 7D were observed in the progeny of wheat-rye hybrids (Tang et al. 2014a). The results of preferential elimination of D-genome chromosomes, and alterations of wheat and rye chromosomes were reported in the derivatives of synthetic hexaploid wheat and Qinling rye (Hao et al. 2013). Complete elimination of D-genome, altered 5A, 5B, 7A chromosomes and restructured 2A chromosome were detected in two hexaploid triticales N9116H and N9116M derived from the cross of common wheat cultivar and Austrian rye (Li et al. 2015). In the present study, the deletion of 2DL chromosome arms and the altered 3A, 5A, 1B and 7B chromosome were detected in N9436B according to FISH karyotypes of N9436B and its parent Shaanmai 611. These phenomena and results suggested that rye chromosome added to wheat might result in structure alterations of wheat chromosome and this variation randomly occur.

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Table 1. Agronomic traits of wheat - rye addition line N9436B and its parents Shaanmai 611, Austrian rye.

Materials	Plant height (cm)	Spike length (cm)	Spikelets /spike	Kernels /spike	Thousand kernel weight (g)	Awedness
Shaanmai 611	81 ± 3	10.0 ± 0.2	21 ± 3	45 ± 4	34.3 ± 0.5	Long
Austrian rye	172 ± 5	14.5 ± 0.4	43 ± 2	86 ± 3	26.1 ± 0.4	Long
N9436B	105 ± 3	13.5 ± 0.3	34 ± 3	82 ± 6	30.6 ± 0.3	Short

Table 2. SSR, EST-STS polymorphic markers applied to analysis introduced 1R chromosome of Austrian rye.

Marker	Type	Primer(5'-3')	Location	Annealing temperature (°C)
<i>TSM716</i>	SSR	F GTGCTCGTCCCACTTGATTC R GCATGGAGAGGACGTTTGAC	1RS	60
<i>NOR-1</i>	STS-PCR	F GCATGTAGCGACTAACTCATCG R CCCAGTTTTCCATGTCGC	1RS	55
<i>NOR-RI</i>	STS-PCR	F GACTGTAGCGACTAACTCATC R CCCAGTTTTCCATGTCGC	1R	55

Fig. 1. Chromosome characteristics of wheat-rye addition line N9436B in mitotic metaphase (a) and meiotic metaphase I (b). (a) $2n = 42+2t$, and (b) $2n = 22 \text{ II}$. The arrows show the two telosomes and four satellite chromosomes in (a) and the arrow show a bivalent from two telosomes in (b).

Fig. 2. GISH results of wheat-rye addition line N9436B at mitotic metaphase (a), meiotic metaphase I (b) using Austrian rye total genomic DNA labeled via nick translation with anti-digoxigenin-fluorescein Fab fragments (green) as a probe. (a) Mitotic metaphase GISH results of wheat-rye addition line N9436B showing two chromosomes with yellow-green hybridization signal, the arrows show the two telosomes, and (b) GISH results of wheat-rye addition line N9436B during meiotic metaphase I, showing a bivalent with yellow-green hybridization signal. (a) $2n=42+2t=40W+2t_w+2R$.

Fig. 3. Fluorescence *in situ* hybridization (FISH), FISH and genomic *in situ* hybridization (GISH) analysis of Austrian rye, Shaanmai 611 and wheat - rye addition line N9436B. FISH analysis using Oligo-pSc119.2 (green) as probe on root tip metaphase chromosomes of Austrian rye (a), Oligo-pTa535 (red) and Oligo-pSc119.2 (green) as probes on root tip metaphase chromosomes of Shaanmai 611 (b) and wheat-rye addition line N9436B (c). FISH and GISH analyses using Oligo-pSc119.2 (green), Oligo-pTa535 (red), and rye genomic DNA (green) as probes on root tip metaphase chromosomes of wheat-rye addition line N9436B (d). Chromosomes were counterstained with DAPI (blue). The white arrows show two chromosome 4A in (b), (c) and (d), the red arrows show two chromosome 2D in (b) and two telosomes 2DS in (c) and (d), and the yellow arrows show two chromosome 1R in (c) and (d). (a) $2n=14$, (b) $2n=42$, (c) $2n=42+2t=40W+2t_w+2(1R)$, (d) $2n=42+2t$

$$=40W+2t_w+2(1R).$$

Fig. 4. 8% non-denaturing polyacrylamide gel electrophoretic analysis of the introduced R chromosome. M, DL2000; 1, Shaanmai 611; 2, Austrian rye; 3 and 4, Wheat-rye addition line N9436B; 5, 1R addition line of 'CS×Imperial'. (a) *NOR-RI*, (b) *TSM716*, (c) *NOR-1*. The arrows show the target bands.

Fig. 5. Morphologic traits of wheat-rye addition line N9436B and its parents Shaanmai 611, Austrian rye. (a) Kernels of Shaanmai 611, Austrian rye and wheat-rye addition line N9436B, (b) Spikes of Shaanmai 611, Austrian rye and wheat-rye addition line N9436B, (c) Plant of Shaanmai 611, Austrian rye and wheat-rye addition line N9436B. 1–3 represent Austrian rye, Shaanmai 611 and wheat-rye addition line N9436B, respectively.

Fig. 6. Resistance of Shaanyou 225 (1), Shaanmai 611 (2), Austrian rye (3), wheat-rye addition line N9436B (4), Kavkaz (5) and Amigo (6) for powdery mildew at seedling stage (the above) and adult stage (the below).

Fig. 1

Draft

Fig. 2

Fig. 4

Fig. 5

Fig. 6