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Improvement of three commercial spring wheat varieties for powdery mildew resistance by marker-assisted selection

Xingguo Ye [a,*], Shuangxi Zhang [b,**], Shijin Li [a], Jing Wang [a], Haiqiang Chen [a], Ke Wang [a], Zhishan Lin [a], Yiqin Wei [b], Lipu Du [a], Yueming Yan [c,***]

[a] Institute of Crop Sciences, Chinese Academy of Agricultural Sciences, Beijing, 100081, PR China
[b] Crop Research Institute, Ningxia Academy of Agri-Forestry Sciences, Yinchuan, 750105, PR China
[c] College of Life Science, Capital Normal University, Beijing, 100048, PR China

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ABSTRACT

Powdery mildew (PM) caused by *Blumeria graminis* f. sp. *tritici* is one of the most economically important wheat diseases in China. Almost all commercially cultivated wheat varieties are susceptible to PM disease, especially the commercial spring wheat varieties grown in the Northwestern region of China. *Pm21* was identified as one of the most effective resistance gene to PM in China, and many wheat varieties carrying *Pm21* have been developed and released in different areas since 2010. In this study, 3 PM susceptible spring wheat varieties (Ningchun4, Ningchun47, and Ningchun50) were crossed with a PM resistant line CB037 (carrying *Pm21*) which is a wheat-*Dasypyrum villosum* T6AL·6V#2S translocation line, and were then backcrossed with the three spring varieties as recurrent parents, combining the disease test with molecular marker selection. After five times of backcrossing and four generations of selfing, nine advanced lines with strong PM resistance and good agronomic traits (plant height, grains per spike, one thousand kernels weight, and yield) were developed. These lines were further evaluated using the analyses of *Pm21*-specific markers and genomic *in situ* hybridization (GISH), and tested in a field trial for agronomic traits including grain yield. Results indicated that all the improved lines were similar to their corresponding recurrent varieties except an obvious difference in plant height. Lines NZ39, NZ42, and NZ46 showed an increase in grain yield (by 1.70–9.38%) and one thousand kernel weight (by 0.4–7.3 g). Compared to their respective recurrent parents, lines NZ41 and NZ46 showed improved bread-making quality, displaying optimal application potential in wheat production.

1. Introduction

Wheat (*Triticum aestivum* L.) is one of the most important cereal crops worldwide, and is a source of starch, protein, fiber, minerals, and vitamins for humans and animals (Shewry, 2009; Tester and Langridge, 2010). Globally, wheat is cultivated in around 220 million hectares with an annual production of 750 million tons (FAO, 2017). Wheat is mainly distributed in the north and the Yangtze River regions of China, occupying 24 million hectares with an annual production of 123 million tons (FAO, 2017). In China, winter wheat is mainly cultivated in the central and northwestern regions and spring or semi-spring wheat is mainly cultivated in the northwest and northeast (Zhou et al., 2007; He et al., 2011). Wheat production is significantly affected by powdery mildew (PM) caused by *Blumeria graminis* f. sp. tritici (*Bgt*) with up to 40% annual reduction in the years when the disease is prevalent (Chen et al., 1997; Te Beest et al., 2008; Lin et al., 2013; Li et al., 2017). This disease is becoming increasingly serious in spring wheat growing areas of China. Ningchun4, a spring wheat variety released 30 years ago, is the most predominant variety in the spring wheat belt, occupying 350,000 ha annually (Cao et al., 2015; Zhang et al., 2015). Spring wheat varieties Ningchun47 and Ningchun50 were varieties developed during the last decade, which have ideal agronomic traits and are well-adapted to the environment (Fan et al., 2014; Zhang et al., 2015). However, all three varieties are highly susceptible to PM and their production was reduced by *Bgt* infection (Zhang et al., 2015). Due to lack of commercial varieties with acceptable levels of resistance to PM, currently chemical control

* Corresponding author.
** Corresponding author.
*** Corresponding author.
E-mail addresses: yexingguo@caas.cn (X. Ye), shxzhang@163.com (S. Zhang), yanym@cnu.edu.cn (Y. Yan).

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with fungicides remains as an only option in controlling PM in wheat. It is essential to improve the PM resistance of these varieties for sustainable wheat production in practice.

To date, more than 100 PM resistance genes have been identified in wheat and its wild relatives, including *Pm1a*, *Pm1b*, *Pm1d*, *Pm2a-Pm2c*, *Pm3a-Pm3g*, *Pm4a*, *Pm4b*, *Pm4d*, *Pm5a-Pm5e*, *Pm6-Pm18* (*Pm1c*), *Pm19-Pm22* (*Pm1e*), *Pm23* (*Pm4c*), *Pm24a*, *Pm24b*, *Pm25-Pm30*, and *Pm32-Pm64* (Li et al., 2014; Xu et al., 2015; Zhang et al., 2016, 2019; Guo et al., 2017; Sun et al., 2018; Wu et al., 2019). Among them, *Pm21*, derived from chromosome 6V#2S of *Dasypyrum villosum* (VV, 2n = 14), confers a broad-spectrum resistance to most *Bgt* isolates (Chen et al., 1995, 2013; Qi et al., 1995; Cao et al., 2011). Some molecular markers that conveniently trace the resistance gene have been developed (Chen et al., 2006; Wang et al., 2007; Song et al., 2009; Qi et al., 1996; He et al., 2013; Lin et al., 2013; Bie et al., 2015; Li et al., 2017, 2019). To date, 12 wheat varieties containing *Pm21*, such as Shimai14, Xingmai2, Nannong9918, Neimai836, Yangmai18, Yangmai21, Lantian27, and Jinhe9123, have been developed and cultivated in China (Li et al., 2007; Bie et al., 2015; He et al., 2016). Eight years ago, the putative PM resistance gene *Stpk-V*, encoding serine/threonine protein kinase within the *Pm21* locus, was isolated (Cao et al., 2011). Recently, *Pm21* was cloned and characterized, which encodes a typical coiled coil-nucleotide binding site-leucine rich repeat (CC-NBS-LRR) protein and functions as a broad-spectrum *Bgt* resistance gene (He et al., 2018; Xing et al., 2018). *Pm21* has been characterized at the molecular level and can be used in backcross breeding programs to improve the resistance of commercial wheat varieties to PM. In fact, *Pm21* has been identified as one of the most effective resistance genes to PM both in China (Li et al., 2007; Bie et al., 2015). So, T6V#2S·6AL translocation line CB037 carrying *Pm21* could have a potential role for wheat breeding.

In this study, *Pm21* was gradually transferred into the commercial spring wheat varieties Ningchun4, Ningchun47, and Ningchun50 through marker assisted backcross selection (MABS). The improved lines were evaluated using genomic *in situ* hybridization and tested in a field trial for agronomic traits including yield performance. Further, the improved lines were analyzed for bread-making quality.

2. Materials and methods

2.1. Plant materials

The commercial spring wheat varieties, Ningchun4, Ningchun47, and Ningchun50, have desirable agronomic traits and high yield potential, but they are highly susceptible to PM. They were provided by the Crop Institute of Ningxia Academy of Agri-Forestry Sciences. Spring wheat line CB037 (being highly resistant to PM) is a wheat-*D. villosum* T6AL·6V#2S translocation line containing *Pm21*, which was developed through multiple-cross method by Prof. Xiao Chen at the Institute of Crop Sciences, Chinese Academy of Agricultural Sciences, China.

2.2. Development of improved wheat lines with PM resistance

Susceptible wheat varieties Ningchun4, Ningchun47, and Ningchun50 were used as the maternal parents and crossed with the PM resistant line CB037 as the paternal parent in a greenhouse. Briefly, 50–60 hybrid seeds were harvested from each cross. The F_1 progeny resistant to PM were backcrossed to their recurrent parents as a pollen donor in the field, in which five spikes from each F_1 hybrid were used for the backcrossing. From BC_1 to BC_5 generations, all the backcrossed plants were evaluated with primer specific for *D. villosum* chromosome 6VS and tested for PM resistance in greenhouse and field studies. Plants were inoculated with *Bgt* inoculum at the seedling stage. Ten resistant plants with desirable agronomic traits (plant height, spike number and uniformity, and spike length) and the presence of the *Pm21* gene were selected in each generation from each combination, and continuously backcrossed with their respective recurrent parents up to BC_5 generations. In BC_5 generation, twenty desirable plants with ideal agronomic traits containing *Pm21* gene from each combination were subjected to four generations of selfing, both in greenhouse and field. The stable PM resistant lines with ideal agronomic traits were comprehensively identified by molecular markers and genomic *in situ* hybridization (GISH). Data on yield, plant height, spike number and uniformity, spike length, and PM resistance were collected from field trials.

2.3. PM resistance test in early generations

Wheat plants at the tillering stage were inoculated with a *Bgt* inoculum collected from the infected wheat seedlings grown in artificial conditions (24 °C, 16 h light/8 h dark with 300 μmol m^{-2} s^{-1} light intensity, 45% humidity). Two weeks after inoculation, infection type was evaluated microscopically using a 0–4 scale: 0 = immune type without any lesion and *Bgt* sporulation; 0; = hypersensitive reaction with local necrotic spot on leaf but no *Bgt* sporulation; 1 = highly resistant with lesions smaller than 1 mm in diameter, and few *Bgt* sporulation (less than 1% in severity); 2 = moderately resistant with small lesions less than 1 mm in diameter, and some *Bgt* sporulation (5–10% in severity); 3 = moderately susceptible with many separated lesions bigger than 1 mm in diameter, and a thick covering of *Bgt* sporulation; 4 = high susceptible type with many continuous lesions bigger than 1 mm in diameter, and a thick covering of *Bgt* sporulation (Xiao et al., 2013).

2.4. DNA extraction and PCR detection

Leaf samples were collected from one-month-old seedlings of the three commercial wheat varieties, backcrossed plants from different generations, and the improved lines and stored in a −80 °C freezer. Genomic DNA was extracted from 1 g of the frozen leaves using a NuClean Plant Genomic DNA Kit (CW Bio Inc., China). The DNA pellet was resuspended in sterile water. Two *D. villosum* chromosome 6VS-specific molecular markers 6V-4 (5′-ACCATCCGTCTTGGCATATTCAGTC-3′, 5′-AGCAGTGAGCAGTTGTCTTCTTGTT-3′) (Li et al., 2019) and 6V-14 (5′-CCGTTCTACACAAGCATCACAACTACA-3′, 5′-TCTCAGACCA-GAGCAATCACCAAGT-3′) (data unpublished) were used to detect the target plants; these markers were developed from transcriptome data of *D. villosum#4*. PCR reactions mixture (15 μL) consisted of 100 ng of template DNA, 0.3 μL primer (10 μmol L^{-1}), and 7.5 μL 2 × Taq MasterMix (containing Mg^{2+} and dNTP, CW Bio Inc., China). Amplification of PCR products was performed in a Biometra Professional thermal cycler (Life Technologies, Germany) with an initial denaturation at 95 °C for 5 min, 35 cycles of 20 s at 95 °C, 30 s at 60 °C, 1 min at 72 °C, and a final extension of 8 min at 72 °C (Li et al., 2017). Amplicons were separated on a 2% agarose gel with a standard 1 × TAE buffer, and visualized by UV light after Genecolour II (Gene-Bio, Beijing, China) staining.

2.5. GISH identification

Wheat seeds of the PM improved lines were soaked in water for 24 h and transferred to moist filter paper in petri dishes. The root tips cut from the germinating seeds when they reached about 4–5 cm in length were put into a nitrous oxide container for 2 h, and then fixed in alcohol. Cell spreading preparations at mitosis were made as previously described (Han et al., 2004). The total genomic DNA of *D. villosum* was labeled with fuorescein-12-dUTP using the nick translation method according to the manufacturer's instructions. The labeled DNA was used as a probe. The total genomic DNA of wheat was fragmented by ultrasonication for 5 min and used as a blocker. GISH was performed as previously described (Han et al., 2004). Hybridization signals were observed under an Olympus BX-51 fluorescence microscope (Olympus Corporation, Japan) and photographed using a charge-coupled device system.

2.6. Trial test for yield and agronomic traits evaluation

Improved wheat lines and their recurrent parents were planted in a field trial in Ningxia, China, using a randomized completely block design with three replications, in a 12 m^2 plot (8 m × 1.5 m) consisting of 10 rows with a 15 cm row space. Field management including fertilization, irrigation, pest management and weed control followed local standards for wheat production. The seedling population was counted at the three-leaf- stage. The dates for jointing, booting, flowering, and maturity were recorded at the corresponding stages. PM disease incidence and severity without inoculum were evaluated at the tillering stage and booting stage. Before harvesting, spike number per m^2 was collected. Twenty plants were randomly picked from each plot at maturity to examine parameters including plant height, spike length, spikelets and grains per main spike, grain weight per spike, and one thousand kernel weight. The average value for the aforementioned agronomic traits was calculated. Grain yield in each plot was weighed after harvesting. All data were processed using statistical package for Microsoft Excel, and data processing software (DPS) (Tang and Zhang, 2013), and analyzed for difference significance by analysis of variance (ANOVA) test, followed by Tukey-Kramer at $P < 0.05$ and $P < 0.01$.

2.7. Bread-making quality analysis

To understand if the introgression of translocation chromosome carrying the PM resistance gene affects the bread-making quality of the improved wheat lines, Mixograph analysis and bread baking or Extensogram analysis of the improved wheat lines and their recurrent parents were conducted at the Wheat Quality Laboratory of Institute of Crop Sciences, Chinese Academy of Agricultural Sciences. Some main parameters related to wheat dough quality such as crude protein content, water absorption, formation time, stabilization time, and mixing tolerance were examined according to the procedure previously described by Yan et al. (2009). The baking procedure adopted the standard rapid-mix-test using 1 kg flour containing 14% moisture, and bread size and loaf score were investigated.

3. Results

3.1. Development of stable improved lines in the three genetic backgrounds

The detailed breeding process for improving the PM resistance of the three commercial spring wheat varieties, Ningchun4, Ningchun47, and Ningchun50, is shown in Table 1. All the F_1 plants showed resistance to PM after *Bgt* inoculation. After backcrossing with the corresponding recurrent parents for five times and selfing for four generations, nine stable improved lines with excellent agronomic characteristics and strong PM resistance in the BC_5F_4 generation were obtained, including three (NZ45, NZ46, and NZ47) with Ningchun4 genetic background, three (NZ37, NZ38, and NZ39) with Ningchun47 genetic background, and three (NZ41, NZ42, and NZ43) with Ningchun50 genetic background.

3.2. PM resistance of stable improved lines in the greenhouse

The nine stable improved wheat lines were inoculated with *Bgt* inoculum at the tillering stage in the greenhouse, and evaluated for PM symptoms at the jointing stage, booting stage, and grain filling period. During the whole growth period, all stable improved wheat lines showed strong PM resistance, and no disease lesions or pathogen colony was observed under a microscope in the leaves or other organs and tissues in which the incidence and severity of the disease were all zero, with infection type 0 (Fig. 1A–J). The three recurrent parents were susceptible to the disease (Fig. 1A–J): Ningchun4 and Ningchun50 displayed moderate susceptibility to PM with infection type 3 and the disease incidence of 100% and severity of 30%, and Ninchun47 displayed high PM sensitivity with infection type 4 and the disease incidence of 100% and severity of 30%.

Table 1
Improved progress of the three commercial wheat varieties susceptible to PM.

Generation	Plants selected in each cross	PM-resistant donor parent or recurrent variety	Main traits for selection	Marker application
Ningchun4, Ningchun47, and Ningchun50	3	CB037	Agronomic traits	-
F_1	5	Ningchun4, Ningchun47, and Ningchun50	PM resistance	-
BC_1	10	Ningchun4, Ningchun47, and Ningchun50	PM resistance and agronomic traits	+
BC_2-BC_5	10	Ningchun4, Ningchun47, and Ningchun50	PM resistance and agronomic traits	+
BC_5F_1-BC_5F_4	20	-	PM resistance and agronomic traits	+

In addition, the improved lines were similar to their corresponding recurrent parents in growth and developmental process, and agronomic traits including booting date, flowering date, maturity date, plant type, spike shape and length (Fig. 1G–J), and grain color, with the exception of plant height in two lines (NZ39 and NZ42). These results indicated that the *Pm21* gene on *D. villosum* 6VS conferred PM resistance to the improved lines, and the improved lines maintained the phenotypic features and agronomic traits of their recurrent parents.

3.3. Identification of stable improved lines using molecular markers and GISH

Specific bands of 732 bp and 841 bp of 6V-4 and 6V-14 were observed in the nine stable improved lines and CB037, respectively, but no bands were amplified in Ningchun4, Ningchun47 and Ningchun50. This confirms that chromosome 6VS of *D. villosum* is present in the improved lines (Fig. 2). A pair of translocated chromosomes displaying the green signal of *D. villosum* was clearly observed in the improved lines (Fig. 3). This result indicates that the nine improved lines are genetically stable and harbored *D. villosum* chromosome 6VS carrying *Pm21*.

3.4. Test for yield and agronomic traits of stable improved lines in a field trial

The nine stable improved lines were tested for agronomic traits and yield production in Ningxia in 2016 and 2017 in a field trial with three replications (Fig. A. 1.). In both years, most of these improved lines were almost similar to their corresponding recurrent parents Ningchun4, Ningchun47, and Ningchun50 in growth duration, plant height, spike length, spikelet number per spike, grains per spike, and thousand-kernel weight (Table 2). Their grain color was all the same red to the recurrent parents. However, lines NZ39 and, NZ42 showed highly significant differences in plant height from Ningchun47 and Ningchun50 ($P < 0.01$), respectively (Table 2). Lines NZ45 and NZ47 were significantly different in thousand-kernel weight from Ningchun4 ($P < 0.01$). Lines NZ37 and NZ42 and NZ43 and NZ46 are also different in thousand-kernel weight from their corresponding parents in one of the two years evaluated. Lines NZ39 and NZ42 showed not only a slightly enhanced yield by 4.27–9.38% even though there was no significant difference,

Fig. 1. Response of the improved wheat lines and their recurrent parents to powdery mildew in the greenhouse. Disease symptoms were clearly observed in the leaves (left side of photos A-F) and the adult plants (left side of photos G-J) of the recurrent parents in the booting stage and late filling stage, while no pathogens or lesions were found in the leaves (right side of photos A-F) or the adult plants (right side of photos G-J) of the stable improved lines. A, G: NZ46 and its recurrent parent Ningchun4; B: NZ47 and its recurrent parent Ningchun4; C: NZ37 and its recurrent parent Ningchun47; D, H: NZ38 and its recurrent parent Ningchun47; E, I: NZ41 and its recurrent parent Ningchun50; F, J: NZ43 and its recurrent parent Ningchun50.

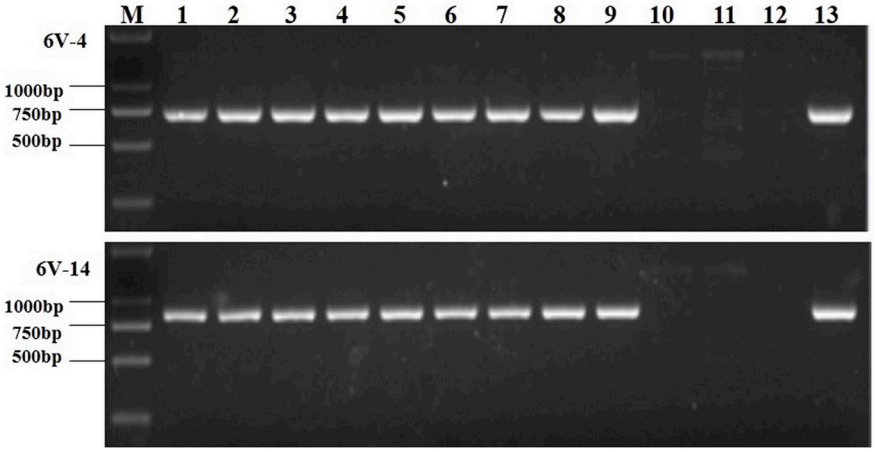

Fig. 2. Identification of the improved wheat lines and their recurrent parents by PCR using two markers, 6V-4 and 6V-14, specific to *D. villosum* chromosome 6VS. M: ladder; 1: NZ37; 2: NZ38; 3: NZ39; 4: NZ41; 5: NZ42; 6: NZ43; 7: NZ45; 8: NA46; 9: NZ47; 10: Ningchun47; 11: Ningchun50; 12: Ningchun4; 13: CB037.

but also decreased plant height by 16.4–22.9 cm compared to their corresponding recurrent parents (Table 2, Fig. 4). All improved lines showed strong resistance to PM in the trial conditions.

3.5. Test for bread-making quality of stable improved lines

Line NZ41 showed enhancement on most parameters including crude protein content (14.5%), water absorption (60.1), formation time (4.5 min), stabilization time (8.6 min), bread size (920 cm^3) and loaf score (90) compared to its recurrent parent Ningchun50 (Table 3, Fig. 5). NZ42 and NZ46 showed a slight increasing in bread size (910 and 925 cm^3) and loaf score (90.5 and 90.0) even though they had a similar or decreased value in other parameters in comparison with their recurrent parents Ningchun50 and Ningchun4, respectively (Table 3, Fig. 5). While NZ39 displayed a decrease in all tested parameters compared to its recurrent parent Ningchun47. Results suggests that the introgression of chromosome 6VS does not influence the bread-making quality of wheat, and lines NZ41 and NZ46 shows an obviously improved dough or bread-making quality.

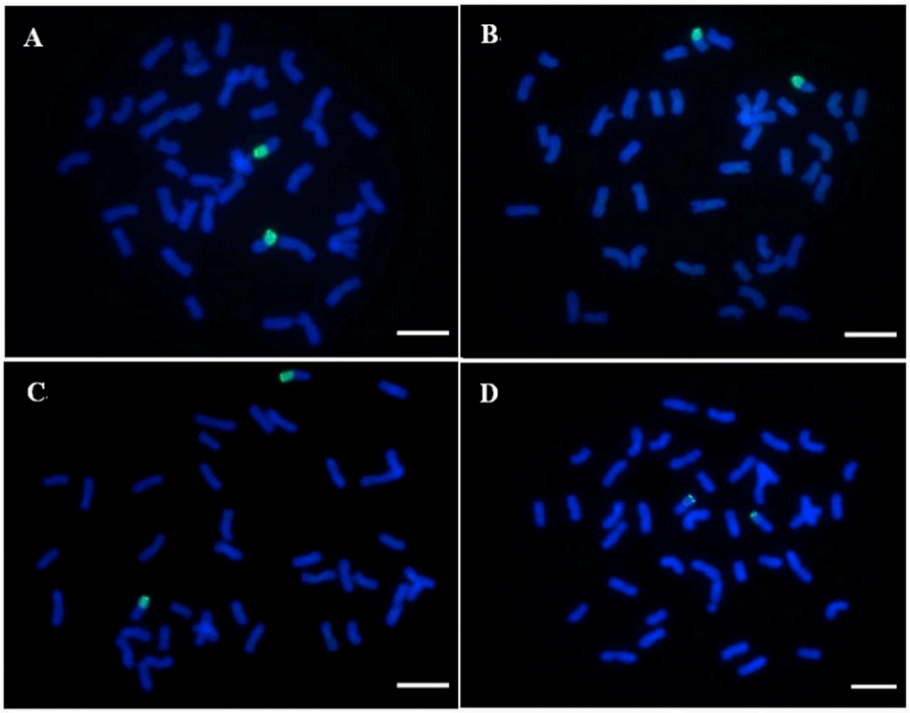

Fig. 3. GISH analysis of the improved wheat lines using the total genomic DNA of *D. villosum* as a probe. The *D. villosum* chromosome 6VS carrying *Pm21* is labeled in green and wheat chromosomes are labeled in blue. A pair of translocation chromosomes between wheat 6AL and *D. villosum* 6VS were observed in the improved wheat lines. A: NZ37; B: NZ39; C: NZ42; D: NZ46. Scale bar = 10 μm. (For interpretation of the references to color in this figure legend, the reader is referred to the Web version of this article.)

Table 2
Main agronomic traits and grain yields of the improved lines and their recurrent parents.

Material	Year	Plant height (cm)	Spike length (cm)	Spikelet number	Grains per spike	Weight per spike (g)	One thousand kernels weight (g)	Spikes number (million/hectare)	Yield (ton/hectare)
Ningchun47	2016	79.3	9.3	18.7	36.2	1.70	47.3	4.41	7.86
	2017	84.0	8.4	16.9	31.3	1.43	46.9	5.53	8.97
NZ37	2016	80.3	10.7*	19.9	33.3	1.40	42.6**	4.19	6.85*
	2017	82.0	9.1	17.0	29.1	1.37	46.9	5.75	8.49
NZ38	2016	79.3	10.3	20.4	39.3	1.80	46.9	3.95	7.51
	2017	85.0	9.6	17.0	34.1	1.62	47.6	5.89	8.68
NZ39	2016	63.4**	10.2	20.4	39.3	1.80	46.8	5.93*	8.46
	2017	65.5**	9.4	19.1	38.3**	1.82	48.7	6.04	9.39
Ningchun50	2016	85.8	9.1	18.2	32.1	1.40	44.4	6.29	8.95
	2017	86.0	9.1	16.3	27.9	1.33	47.7	5.91	9.43
NZ41	2016	81.2*	9.4	16.9	30.6	1.50	47.5	6.04	8.90
	2017	84.0	9.7	16.6	30.5	1.57	51.4**	5.93	8.85
NZ42	2016	62.9**	9.4	17.5	36.0	1.70	46.7	6.55	9.79
	2017	64.0**	9.5	17.3	36.5**	1.76	48.2	6.69	9.83
NZ43	2016	81.8*	8.7	16.8	26.0*	1.30	48.6	6.35	8.36
	2017	85.0	9.1	16.6	29.1	1.49	51.2*	6.01	8.69
Ningchun4	2016	86.0	9.1	16.3	27.9	1.33	47.7	5.91	9.43
	2017	84.0	8.7	16.8	30.5	1.35	44.3	6.09	9.81
NZ45	2016	84.0	9.7	16.6	30.5	1.57	51.4**	5.93	8.85
	2017	82.5	8.4	17.2	26.4	1.25	47.4**	5.87	8.92
NZ46	2016	64**	9.5	17.3	36.5**	1.76	48.2	6.69	9.83
	2017	86.0	9.7	16.4	28.5	1.47	52.1**	6.06	9.97
NZ47	2016	85.0	9.1	16.6	29.1	1.49	51.2*	6.01	8.69
	2017	85.0	10.1	16.6	28.5	1.44	50.3**	6.0	9.24

* and ** represents significant differences at $P < 0.05$ and $P < 0.01$, respectively.

4. Discussion

The improved wheat lines developed in this study were translocations T6V#2S·6AL between wheat and *D. villosum*. Theoretically, translocation lines between wheat and its wild species contain some useful genes related to biotic and abiotic stresses, yield and quality improvement, and are very important materials for wheat breeding (Molnár-Láng and Dolezel, 2015). The most successful case in wheat improvement is the application of the T1BL·1RS translocation line developed from the cross between wheat and *Secale cereale* (RR, 2n = 14) (He et al., 2011). On average, 38% of Chinese wheat varieties released from 1980 to 2004 contained T1BL·1RS, even though the frequency differed in different ecological zones (Zhou et al., 2004). Moreover, the frequency of T1BL·1RS translocation varieties developed the in the Northern China winter wheat region from 1960 to 2000 was up to 42.6% (Zhou et al., 2007). However, the varieties with T1BL·1RS translocation have low dough elasticity (Zhou et al., 2004). A recent study demonstrated that T1BL·1RS translocation significantly affected Extensograph extensibility, maximum resistance, content of unextractable glutenin polymeric protein, quantity of gluten protein fractions and

Fig. 4. Two improved wheat lines NZ39 and NZ42 with short plant height and good agronomic traits. A: recurrent parent Ningchun47 (left) and NZ39 (right); B: recurrent parent Ningchun50 (left) and NZ42 (right).

their ratios in flour dough. High molecular weight glutenin subunits (HMW-GSs) may have some complementary effects on the quality defects caused by T1BL·1RS. For breeding varieties to obtain medium to high dough elasticity, T1BL·1RS parents are not recommended (Zhou et al., 2004).

Except *S. cereal*, *D. villosum* is another important wild species for wheat improvement. To use the available traits in *D. villosum*, addition lines including DA2V#2 to DA7V#2, substitution lines such as 6V(6A) between wheat and *D. villosum#2*, and an amphidiploid TH3 between *T. durum* and *D. villosum#4* were developed (Chen and Liu, 1986; Chen and Conner, 1996; Liu et al., 1988; Chen et al., 1995; Li et al., 2008). Continuously, whole arm translocation lines including T1V#2S·1BL, T1V#2L·1DL, T2V#2S·2DL, T2V#2L·2DS, T4V#2S·4DL, T5V#2S·5DL, T6V#2S·6AL, T6V#2L·6AS, and T6V#4S·6DL were obtained. Among them, translocation lines T6V#2S·6AL and T6V#4S·6DL showed strong PM resistance (Chen et al., 1995; Zhang et al., 2005, 2010,; Li et al., 2008). Further, T6V#2S·6AL translocation was introduced into 19 wheat varieties and advanced lines, and no significant differences were found between T6V#2S·6AL lines and their recurrent parents in agronomic, mixograph, and starch pasting traits, including grain yield, grains/spike, grain weight/spike, mixing time, and peak viscosity (Li et al., 2007). However, T6V#2S·6AL lines displayed significant positive effects on thousand-kernel weight and plant height, and small negative effects on grain test weight, and flour yield and color (Li et al., 2007). Thereby, the T6V#2S·6AL translocation is suggested to be used in wheat breeding programs as a PM resistant resource because it has no obviously negative effects on agronomic and quality traits (Li et al., 2007).

Currently, more than ten winter wheat varieties have been developed in China using the T6V#2S·6AL translocation carrying *Pm21*. However, no spring wheat variety has been bred and released in

Table 3
Bread-making related parameters of the improved lines and their recurrent parents[a].

Materials	Crude protein content (%)	Water absorption (500fu)	Formation time (min)	Stabilization time (min)	Mixing tolerance	Bread size (cm^3)	Loaf score
Ningchun47	14.6	57.9	5.0	8.3	45	980	89.5
NZ39	12.8	56.6	1.8	2.9	75	925	88.0
Ningchun50	13.9	57.3	2.1	5.8	45	900	87.5
NZ41	14.5	60.1	4.5	8.6	40	920	90.0
NZ42	12.8	57.3	1.3	4.5	70	910	90.5
Ningchun4	14.2	57.9	5.2	7.5	45	900	88.5
NZ46	14.2	59.0	3.1	7.0	45	925	90.0

[a] The values presented in this table are one data point.

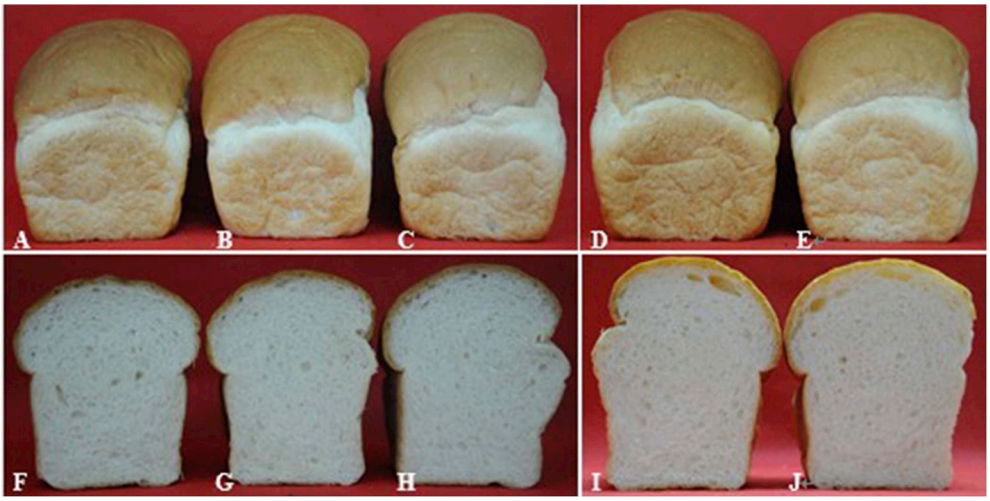

Fig. 5. Bread sizes (A to E) and slices (F to J) made from the three improved lines and their recurrent parents. A and F: Ningchun50; B and G: NZ41; C and H: NZ42; D and I: Ningchun4; E and J: NZ46. The bread size processed from NZ41, NZ42, and NZ46 was larger than that from the recurrent parents Ningchun50 and Ningchun4.

production using T6V#2S·6AL translocation. PM disease is becoming more serious in the north-western spring wheat region of China. In order to efficiently transfer *Pm21* gene and other PM resistance genes in different chromosomes of *D. villosum* into wheat, recently we developed 101 specific molecular markers to each chromosome arm of *D. villosum#4* (Li et al., 2017, 2019), and further identified 6 types of alien chromosome lines including translocation line between wheat and *D. villosum#4* using these specific PCR markers (Li et al., 2019).

In this study, nine spring wheat lines with strong PM resistance were developed by crossing between CB037 and Ningchun4, Ningchun47, Ningchun50, respectively, along with marker-assisted selection. Field tests showed that lines NZ39, NZ42, and NZ46 increased grain yield and thousand-kernel weight compared to their corresponding recurrent parents. The improved lines can be sustainably applied in wheat production to avoid the grain loss risk caused by PM even though they did not show a yield increasing significantly. In particular, lines NZ39 and NZ42 displayed a decreased plant height (Fig. 4). The translocated chromosome T6V#2S·6AL proved to have a positive effect on thousand-kernel weight and grain yield in a previous study (Li et al., 2007). However, it did not increase plant height (Table 2), and the dwarfing phenomenon occurred in our present study (Fig. 4), which was not consistent with the results in a previous report (Li et al., 2007). The two dwarfing lines NZ39 and NZ42 only had a plant height of 63–65 cm while their recurrent parents Ningchun47 and Ningchun50 and the *Pm21* donor line CB037 all had a plant height of 80–85 cm. The reason for dwarfing in lines NZ39 and NZ42 needs to be investigated further.

Additionally, the improved lines containing T6V#2S·6AL translocation or *Pm21* gene were evaluated for bread-making quality. Some lines showed an increased value in dough and loaf parameters, while others showed a decreased value in these parameters compared to their recurrent parents (Table 3, Fig. 5). It is suggested that the introgression of chromosome 6VS of *D. villosum* didn't influence the bread-making quality and at least had no obvious negative effects on quality traits of wheat, which is consistent with the results reported by Li et al. (2007). Variation of the bread-making quality for the newly developed lines should be associated with the combination and interaction of the genes for dough quality in their parents. To our knowledge, the genes encoding HMW-GSs and low molecular weight glutenin subunits (LMW-GSs) which greatly affect the bread-making quality are located on the group 1 chromosome of wheat and its wild relatives (Payne, 1987; Payne et al., 1988; Wrigley, 1996; Rasheed et al., 2014). Thereby, T1BL·1RS translocation has a close relationship with the bread-making quality of wheat flour (Zhou et al., 2004, 2007; Zhao et al., 2015), and T6V#2S·6AL translocation involving wheat chromosome 6A and *D. villosum* chromosome 6V doesn't influence its bread-making quality (Li et al., 2007). Considering the excellent performs of T6V#2S·6AL translocation in PM resistance and yield potential as well as its independence in bread-making quality based on previous study (Li et al., 2007) and the present results, we believe that both translocations T6V#2S·6AL and T6V#4S·6DL will be widely used in wheat breeding and production as the contribution of T1BL·1RS translocation to this crop. In fact, three of the new lines developed in this study have been identified in regional trial approved by local government.

5. Conclusions

Nine wheat lines with strong PM resistance and good agronomic traits were developed using the PM susceptible commercial spring wheat varieties, Ningchun4, Ningchun47, and Ningchun50, as the recurrent parents and a PM resistance line, CB037, as the donor parent. The improved lines were further confirmed by specific markers for the T6V#2S·6AL translocation or *Pm21* gene and GISH analysis. Tests in a field trial demonstrated that all the improved lines had similar agronomic traits to their corresponding recurrent parents except for plant height; three lines, NZ39, NZ42, and NZ46, showed increased grain yield and thousand-kernel weight. Bread-making quality test of some improved lines showed that NZ41 and NZ46 had slight increase in dough parameters and loaf size. The developed new lines will be continuously evaluated in field trials in more places for possible application in wheat production.

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Appendix

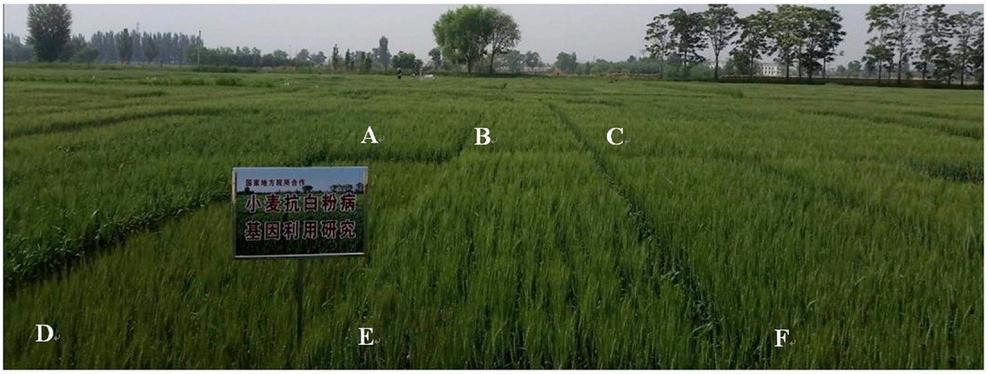

Fig. A1. Field test of stable improved wheat lines for agronomic and yield traits. A: NZ43; B: Ningchun4; C: NZ45; D: NZ39; E: Ningchun50; F: NZ41.

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