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Rapid development of wheat-Dasypyrum villosum compensating translocations resistant to powdery mildew via a tri-marker strategy conducted on a large ph1b-induced population

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Abstract

Wheat powdery mildew, caused by *Blumeria graminis* f. sp. *Tritici* (*Bgt*), is one of the most destructive diseases in the middle and lower reaches of Yangtze River, China. Currently, nearly all the resistant varieties released in this area are carrying *Pm21* conveyed by the wheat-*Dasypyrum villosum* T6V#2S.6AL translocation, which brings a potential risk of resistance loss. *PmV*, a homologue of *Pm21*, carried by the wheat-*D. villosum* T6V#4S.6DL translocation, is more stably resistant to *Bgt* but less transmittable and exploited in breeding. To utilize *PmV* more effectively, a new recombinant translocation T6V#4S-6V#2S.6AL carrying *PmV* with higher transmission rate was used as a basic material for inducing smaller alien translocations. A localized *ph1b*-carried line “Yangmai 23-*ph1b*” conferring semi dwarf and high yield was developed with the *ph1b* locus in the genetic background of the commercial variety Yangmai 23. A large *ph1b*-induced F_3 population was created based on the cross between Yangmai 23-*ph1b* and T6V#4S-6V#2S.6AL. A modified tri-marker strategy using three co-dominant markers, the distal marker *6VS-GX4* and paracentric marker *6VS-GX17* of 6VS as well as the functional marker *MBH1* of *PmV*, was conducted for screening new recombinants. Forty-eight recombinants including 22 *PmV*-carried translocations were identified from 6,300 F_3 individuals. Two translocation lines, Dv6T25 with the shortest distal segment carrying *PmV* and Dv6T31 with the shortest proximal segment carrying *PmV* were identified with high powdery mildew resistance and normal transmission rate. This work is significant for promoting *PmV* in wheat breeding and gives a model for rapid development of wheat-alien compensating translocations.

Key Message

Twenty-two wheat-*Dasypyrum villosum* compensating translocations carrying the powdery mildew resistance gene *PmV* were rapidly developed via a tri-marker strategy conducted on a large *ph1b*-induced population.

Introduction

Powdery mildew, caused by *Blumeria graminis* f. sp. *tritici* (*Bgt*), is a globally destructive wheat disease. Breeding of resistant varieties is an economical way to control this disease. The middle and lower reaches of Yangtze River (MLRYR) is the second largest wheat planting area of China, and also a pandemic area of powdery mildew. A series of powdery mildew resistant wheat varieties, such as Yangmai 10, Yangmai 11, Yangmai 19 and Yangmai 20 carrying *Pm4a*, Yangmai 12 and Yangmai 13 carrying *Pm2a*, had successively played great roles in wheat production in the MLRYR wheat region before 2015. However, *Pm4a* and *Pm2a* had been ineffective, mostly due to the large accumulated growing area of these resistant varieties (Bie et al. 2015a).

On this premise, the wheat-*Dasyprum villosum* translocation T6V#2S.6AL carrying *Pm21* with broad-spectrum resistance to nearly all known *Bgt* isolates was unprecedented welcomed by the breeders (Chen et al. 1995; Wu et al. 2021). Currently, nearly all the powdery mildew-resistant varieties released in the

MLRYR area are *Pm21*-carried lines. According to our investigation, in the past five years, 139 advanced lines carrying *Pm21* were documented in the national or provincial regional tests of MLRYR, accounting for 41.9% of the tested lines and 95.9% of the resistant lines, respectively. There obviously exits overuse of the sole resistance of *Pm21*, which might bring extremely strong pressure on *Bgt* and lead to a new recurrent risk of loss of resistance.

PmV, a homologue of *Pm21*, carried by the wheat-*D. villosum* translocation T6V#4S.6DL, is less exploited in wheat breeding (Chen et al. 1997; Li et al. 2005). Few varieties carrying *PmV* have been approved in China, for example, Yangmai 22. The genetic effects of the T6V#2S.6AL and T6V#4S.6DL translocations had been compared thoroughly (Zhao et al. 2019). The T6V#2S.6AL translocation can increase grain weight, which is a good effect, but tends to increase plant height and leaf length, which brings the risk of plant logging and population degradation. It also causes a reduction in panicle number and somewhat influences wheat final yield, which is a major reason that T6V#2S.6AL is seldom utilized in the Huanghuai wheat area, the largest wheat plant area of China, where are full of multi-panicle varieties. Comparatively, the T6V#4S.6DL translocation has less adverse effects on agronomic traits, but it is much less transmittable than T6V#2S.6AL, which is the bottleneck retarding the utilization of *PmV* in breeding (Bie et al. 2015a, Zhao et al. 2019). The good news is that T6V#4S.6DL is more stably resistant to *Bgt* than T6V#2S.6AL at a low temperature, which is very significant for reducing the accumulation of *Bgt* in the field in early spring.

To upgrade transmissibility of *PmV*, we transferred *PmV* to the T6VS.6AL translocation with chromosomal form of T6V#4S-6V#2S.6AL, which had a higher transmission rate than the original T6V#4S.6DL (Zhao et al. 2019). However, based on near isogenic line (NIL) analysis, we found the T6V#4S-6V#2S.6AL translocation has the similar adverse effects on panicle number and plant height to T6V#2S.6AL (Table S1). Therefore, development of resistant translocations with smaller alien segment eliminating adverse linkage drags is necessary.

Chinese Spring *ph1b* mutant (CS *ph1b*) is an effective tool and widely used to induce homoeologous recombination between wheat and wild relatives (Bhullar et al. 2014; Lukaszewski et al. 2004; Rey et al. 2015; Sears 1976; Wan et al. 2020; Zhao et al. 2013). Nevertheless, in our previous study, interstitial chromosomal exchanges between *D. villosum* 6VS and wheat counterparts are extremely difficult to induce by *ph1b* (Li 2010; Ma et al. 2022). Obviously, construction of a large enough *ph1b*-induced population is required. Meanwhile, due to the poor agronomic performance of CS *ph1b*, especially its too high plant height, soft stem, and low yield, the wheat-alien translocations or introgressions produced by CS *ph1b* were hard to utilize in wheat breeding directly. Development of new *ph1b*-carried lines with genetic backgrounds adapted to the local ecology is desirable.

As we know, conventional cytogenetic tools such as chromosome banding, genomic in situ hybridization (GISH) or fluorescence in situ hybridization (FISH) is usually applied in identification of wheat-alien chromosomal translocations, but laborious, time-consuming, and inappropriate for identification of a large population. A high-throughput molecular marker strategy will facilitate to identify the translocations

efficiently. Excitedly, benefited from the fulfillment of the sequencing of *D.villosum* 6VS telomere and the success in the cloning of *Pm21* (He et al. 2018; Xing et al. 2018; Zhang et al. 2021), development of molecular markers spanning the 6VS arm becomes easier, and marker strategy for screening resistant compensating translocations could be optimized at a large degree.

In this research, a large *ph1b*-induced population was constructed based on the cross between the T6V#4S-6V#2S.6AL translocation line and Yangmai 23-*ph1b*, a newly developed *ph1b*-carried line adapted to the MLRYR ecology. Thereafter, we will demonstrate the rapid development and characterization of a series of wheat-*D.villosum* compensating translocations resistant to powdery mildew via a modified tri-marker strategy.

Materials And Methods

Plant materials

The CS *ph1b* mutant was provided by Cytogenetics Institute, Nanjing Agricultural University, which was firstly developed by Sears (1976). Yangmai 23, a semi-dwarf bread wheat variety with high lodging resistance, high yield, moderate resistance to *Fusarium* head blight, susceptibility to powdery mildew, and great adaptation to the MLRYR wheat area, was developed at Yangzhou Academy of Agricultural Sciences (YAAS). Yangmai 18 carrying the wheat-*D. villosum* T6V#2S.6AL translocation and Yangmai 22 carrying the T6V#4S.6DL translocation were both developed at YAAS, containing the powdery mildew resistance genes *Pm21* and *PmV*, respectively. RIL12401, a newly developed wheat-*D.villosum* whole arm translocation line with the chromosomal form of T6V#4S-6V#2S.6AL carrying *PmV* on the 6V#4S segment, was identified from recombinant inbred line (RIL) population of Yangmai 18 and Yangmai 22 (Zhao et al. 2019). RIL 12401 is also a semi-dwarf and high-yield advanced line.

Development of the advanced ph1b **-carried line Yangmai 23-** ph1b

The crossing program for transferring *ph1b* mutant locus from CS *ph1b* to commercial variety Yangmai 23 is summarized in Figure S1. CS *ph1b* was crossed to Yangmai 23, and the F_1 hybrids were backcrossed with Yangmai 23 to generate BC_1F_1 progeny. Ten BC_1F_1 individuals were randomly selected to backcross with Yangmai 23 and self-pollinated, simultaneously. The progeny of each BC_1F_1 individual were identified by the dominant PCR marker *ABC302.3* (Wang et al. 1998). Only the BC_1F_1 individuals that generated self-pollinated progeny maintaining part plants genotyped with homozygous *ph1b*, were selected for a next recurrent backcrossing. The same selection cycle was repeated three times. In each generation, the plants with similar traits to Yangmai 23 were selected preferentially. Then, self-pollination was conducted to produce BC_4F_2 progeny and BC_4F_3 family lines. Those lines with homozygous *ph1b* were selected for further agronomic evaluation. The line with the best comprehensive agronomic traits was maintained and designated Yangmai 23-*ph1b*.

Evaluation of morphological parameters of Yangmai 23- ph1b

Five major agronomic traits including plant height (PH), spike length (SL), spikelet number per spike (SNPS), grain number per spike (GNPS) and thousand grain weight (TGW) were compared among Yangmai 23, Yangmai 23-*ph1b* and CS-*ph1b*. The data representing the mean ± standard deviation of 50 individuals per material for morphological parameters were compared by *t*-test.

Construction of a large ph1b -induced population

Yangmai 23-*ph1b* was crossed with RIL12401 containing the T6V#4S-6V#2S.6AL translocation carrying *PmV*. The F_1 hybrids were planted in climate chamber. The matured F_2 seeds were harvested and planted as a F_2 population. The dominant marker *ABC302.3* was used for tagging homozygous *ph1b* in F_2 plants. *MBH1*, a co-dominant marker developed based on the polymorphism between the promoter sequences of *Pm21* and *PmV*, could distinguish the alleles from 6V#2S, 6V#4S, 6AS and 6DS (Bie et al. 2015a), which was used for tagging heterozygous *PmV* in F_2 plants. Those F_2 plants genotyped with homozygous *ph1b* and heterozygous *PmV* were maintained, well cultivated, and self-pollinated to create a large F_3 population with more than 5,000 individuals.

Molecular Markers Used For Identification Of Translocations Involving 6vs

In previous research, sixteen molecular markers were identified physically located from the distal end to the paracentric region of the new 6VS arm (6V#4S-6VA#2S) in RIL12401 (Table S2). Those markers were recruited for identification of the newly developed translocations involving 6VS. Among them, *6VS-GX4* is a co-dominant marker tagging the distal regions of 6V#4S and 6AS, and *6VS-GX17* is a co-dominant marker tagging paracentric regions of 6V#2S and 6AS.

Procedures of screening and identification of wheat- D.villosum compensating translocations

A modified tri-marker strategy was used to screening wheat-*D. villosum* compensating translocations carrying the powdery mildew gene *PmV*. The distal marker *6VS-GX4* and the paracentric marker *6VS-GX17* were firstly used to identify each individual in the *ph1b*-induced F_3 population. The plants identified with homozygous *6VS-GX4* and *6VS-GX17* simultaneously or heterozygous *6VS-GX4* and *6VS-GX17* simultaneously were believed having none structural changes. Those with nonequivalent for the two markers were considered as candidate compensating translocations. The functional marker *MBH1* was then applied to confirm whether they carry the gene *PmV*. The other 13 6VS-specific markers were further used to genotype all the recombinants, which helps to define the physical intervals of the alien translocated segments.

Evaluation Of Powdery Mildew Resistance

Evaluation of powdery mildew resistance was conducted in a greenhouse. A mixture of *Bgt* isolates was collected from Yangzhou, China. All newly developed translocations with the parents were planted in plastic pots and inoculated with *Bgt* isolates at seedling stage. Yangmai 22 and Yangmai 23 were used as the resistant and susceptible controls, respectively. When Yangmai 23 was severely infected, powdery mildew responses of the tested materials were evaluated and scored. Seedlings without visible spores on leaves were marked as resistant (R) while the ones covered with spores on leaves were marked as susceptible (S).

Results

Characterization of the advanced ph1b -carried line Yangmai 23- ph1b

The dominant marker *ABC302.3* was used to verify the homozygous *ph1b* deletion in Yangmai 23-*ph1b*. The result showed that Yangmai 23-*ph1b* could amplify the specific band of 5A, but missed the ~ 920 bp band specific to 5B, the same as CS *ph1b*, while Yangmai 23 could amplify the both the specific bands (Fig. 1b), indicating that Yangmai 23-*ph1b* is deemed to be homozygous for *ph1b*. Major agronomic parameters including plant height, spike length, spikelet number per spike, grain number per spike and thousand grain weight were statistically analyzed among Yangmai 23, Yangmai 23-*ph1b* and CS *ph1b*. The results showed that the comprehensive traits of Yangmai 23-*ph1b* are very similar to Yangmai 23, and much better than CS *ph1b* (Table 1, Fig. 1a, 1c-e). Especially, the plant height of Yangmai 23-*ph1b* was reduced at a large degree. In addition, the leaf wither disappeared.

Marker-assisted construction of a large ph1b -induced population

The F_2 plants derived from the cross between Yangmai 23-*ph1b* and RIL12401 with the T6V#4S-6V#2S.6AL translocation carrying *PmV* were genotyped with the molecular markers *ABC302.3* and *MBH1* for screening of individuals with homozygous *ph1b* and heterozygous *PmV.* A total of 105 F_2 plants were screened out with the genotype *ph1bph1b*/*PmVpmV* (Fig. 2), which were further well cultivated and self-crossed to produce a large F_3 population.

Screening For Compensating Translocations Via The Modified Tri-marker Strategy

The modified tri-marker strategy using three co-dominant markers, the 6VS distal marker *6VS-GX4*, the paracentric marker *6VS-GX17* as well as the functional marker *MBH1* for *PmV*, was conducted following the procedure in the method. Recombinants and recombination types were rapidly identified through amplification of the three markers simultaneously (Fig. S2). Plants with the markers *6VS-GX4* and *6VS-GX17* amplification equivalent homozygous or heterozygous simultaneously were considered having none recombination (Fig. 3, lanes 5 and 6). Plants with none 6VS specific amplifications were considered having none alien chromatin (Fig. 3, lanes 7 and 8). Plants with the markers *6VS-GX4* and *6VS-GX17* showing nonequivalent were considered as candidate compensating translocations. The plants with

single positive *6VS-GX4* were considered as translocations maintaining the distal region of 6VS (Fig. 3, lanes 9 to 17). The plants with single positive *6VS-GX17* were considered as translocations involving paracentric region of 6VS (Fig. 3, lanes 18 to 26). Based on analysis with *MBH1*, translocations with *PmV* were further confirmed (Fig. 3, lanes 16 to 24). A total of 48 candidate interstitial recombinants were identified from 6,300 F_3 plants, with a frequency of 0.76%. Among them, 28 distal translocations including four *PmV*-carried ones and 20 proximal translocations including 18 *PmV*-carried ones were identified.

Physical identification of the newly developed wheat- D. villosum **compensating translocations**

Thirteen markers spanning the 6VS chromosome were recruited to identify the physical intervals and breakpoints of the alien segments in the 48 newly developed wheat-*D. villosum* compensating translocations (Fig. 4), which could be grouped into 13 haplotypes involving nine distal and four proximal translocation types. The physical length of the smallest alien segment in the 28 distal translocations is less than 35.1 Mb and the longest one is less than 147.8 Mb. The physical length of the smallest alien segment in the 20 proximal translocations is less than 150.4 Mb and the longest one is less than 223.7 Mb. Among the translocations, 17 distal and 12 proximal translocations had crossovers within the 6 ~ 35 Mb interval, with the proportion of 60.4%, obviously, which is a hot spot region of recombination (Table 2). The closest recombinant breakpoint to the centromere is located within 128.0-147.8 Mb interval, indicating the remained 35.7% of the proximal segment of 6VS has none recombination.

Among the 22 *PmV*-carried lines, the distal translocation line Dv6T25 has the shortest distal segment of 6VS less than 89 Mb with the recombinant breakpoint at the interval flanked by *MBH1* and *CINAU2593*. The proximal translocation line Dv6T31 has shortest proximal segment of 6VS less than 166 Mb with the recombinant breakpoint at the interval flanked by *CINAU2702* and *CINAU2592*.

Powdery mildew resistance evaluation of the newly developed wheat- D. villosum **translocations**

Powdery mildew reaction of all the identified recombinant translocations were evaluated in greenhouse using a mixture *Bgt* isolates dusted on leaves at seedling stage. The result showed that the 22 translocation lines identified with the positive functional marker *MBH1* were highly resistant, same to the resistant control Yangmai 22, and the remained 25 translocations with negative *MBH1* were highly susceptible, same to susceptible control Yangmai 23.

Transmission Analyses Of Translocation Lines Dv6t25 And Dv6t31

The translocation lines Dv6T25 with the shortest distal segment and Dv6T31 with the shortest proximal segment of 6VS were self-crossed to obtain homozugous offspring, respectively. Then, they were crossed with Yangmai 23 to generate two independently F_2 populations. By *MBH1* analysis, in the Yangmai 23/Dv6T25 F_2 population, 124 plants were identified carrying *PmV* and 49 ones without it, in according with the theoretical segregation ratio of 3:1 ($\chi^2_{3:1} = 0.85$, $P > 0.05$). In the Yangmai 23/Dv6T31 F_2

population, 160 plants were identified with *PmV* while 50 ones without it, also in according with the theoretical segregation ratio of 3:1 ($\chi^2_{3:1} = 0.1016$, $P > 0.05$).

Discussion

Status of powdery mildew resistance breeding in the MLRYR wheat area

The uniformity of powdery mildew resistance in the MLRYR wheat area has been a key problem in wheat breeding. Around 2010 ~ 2015, loss of resistance of two major utilized genes *Pm2a* and *Pm4a* resulted in a shortage of resistant varieties in this area. Thereafter, *Pm21*, a powdery mildew resistance gene from *D. villosum* with broad-spectrum resistance to *Bgt*, became nearly a sole resistance resource in breeding of this area. At present, nearly all the resistant varieties are *Pm21*-carried lines, which might bring a new risk of resistance loss. In this case, *PmV*, a homologue of *Pm21*, expressing different *Bgt* reaction (Wang et al. 2021), could be a good choice in resistance breeding. So far, only two varieties carrying *PmV* (Yangmai 22 and Yangfumai 17) were documented in the MLRYR area. The low transmission rate of *PmV* carried by T6V#4S.6DL significantly retards its utilization in wheat breeding. Modification of the *PmV*-carried translocation chromosomal form might be an alternative way to improve its breeding efficiency. In our previous research, a RIL population has been constructed from the cross between Yangmai 18 carrying the T6V#2S.6AL translocation and Yangmai 22 carrying the T6V#4S.6DL translocation. By genotype analysis of the RIL population with the functional marker *MBH1* for *Pm21* and *PmV*, a new recombinant line, RIL12401 with the chromosomal form T6V#4S-6V#2S.6AL carrying *PmV* was identified fortunately and proved having a higher transmission rate (Zhao et al. 2019). Further investigation of its genetic effects on agronomic traits revealed that T6V#4S-6V#2S.6AL still accompanied by increased plant height and reduced panicle number, similar to T6V#2S.6AL. Thus, development of smaller alien compensating translocations carrying *PmV* maintaining normal transmission rate and eliminating adverse drags is crucial.

In this research, via *ph1b* induction, some compensating translocation lines with smaller alien segments carrying *PmV* were identified and proved with normal transmission rate, which will promote the utilization of *PmV* in wheat resistance breeding greatly.

Marker strategies for screening wheat-alien translocations involving D. villosum **chromosome 6V or 6VS**

Bie et al. (2015b) had demonstrated a double-distal-marker strategy for efficient screening of structural abberations involving *D. villosum* chromosome 6V. Twenty structural abberations were rapidly screened out from two independent irradiation-treated populations involving whole 6V chromosomal lines by using the 6VS-distal marker *6VS381* and 6VL-distal marker *6VL358*. Except 12 whole-arm translocations deriving from centric misdivision, the others with interstitial breakpoints were all non-compensating, and improper for wheat breeding. For absence of functional markers of the resistance gene, under this

strategy, *Bgt* inoculation and resistance evaluation was still needed for identification of the resistant translocations.

Compared with the lines carrying the whole 6V chromosome like substitution or addition lines, wheat-*D. villosum* translocations carrying only the 6VS arm are undoubtedly more targetable for inducing intercalary translocations resistant to powdery mildew. Chen et al. (2008) had induced many intercalary translocations carrying the powdery mildew gene *Pm21* via female-gamete irradiation, however, most of which were non-compensating and restrained in breeding (Chen et al. 2013). As we had found, *D. villosum* 6VS arm is extremely to induce homoeologous recombination with wheat counterparts using CS *ph1b* (Li 2010). A large *ph1b*-induced population as well as an efficient marker strategy are obviously required.

In previous research, we had cloned the resistance gene *Pm21* and developed the efficient functional marker *MBH1* (Bie et al. 2015a; He et al. 2018). Meanwhile, genomic sequencing of *D. villosum* 6VS telomere was also fulfilled (Zhang et al. 2021). Benefited from the above work, we successfully developed the modified tri-marker strategy based on three co-dominant markers including the 6VS distal marker *6VS-GX4*, the paracentric marker *6VS-GX17*, and *MBH1*, the functional marker of *PmV*, which helped us to detect compensating translocations with various alien segments from a large *ph1b*-induced population, efficiently. Forty-eight putative compensating translocations including 22 *PmV*-carried translocations were thereafter identified from 6,300 segregation plants.

Compared with conventional cytogenetic techniques like chromosomal banding, GISH, and FISH, the tri-marker strategy used in this research is undoubtedly more efficient and precisely in screening of translocations carrying target genes, which gives a model for rapid development of wheat-alien compensating translocations.

Localization of ph1b **background upgrades breeding value of newly created germplasms**

Irradiation, gamete-cide effect, and CS *ph1b* are common tools for inducing wheat-alien translocations. Irradiation and gamete-cide effect have higher efficiencies in inducing interstitial breakpoints (Bie et al. 2007; Chen et al. 2007) than CS *ph1b*, but tend to create non-compensating translocations that are difficult to utilize in wheat breeding.

CS *ph1b* is a widely used tool for transferring alien useful genes from wild species to common wheat by inducing wheat-alien compensating translocations (Sears 1976; Martín et al. 2018). However, it is too high, less lodging resistant and low yielding. Translocation lines induced by CS *ph1b* are usually hard to utilize in breeding directly. Therefore, developing *ph1b*-carried lines adapted to local ecology has been a consensus (Devi et al. 2019; Friebe et al. 2012; Grewal et al. 2018; Grewal et al. 2020; Li et al. 2020). In this research, *ph1b* mutant was transferred from CS *ph1b* to Yangmai 23 background by continuous backcrossing, endowing the new *ph1b*-carried line Yangmai 23-*ph1b* with semi-dwarf, high yield, and broad adaptation to ecological conditions of the MLRYR wheat region. As we anticipated, most of the translocation lines induced by Yangmai 23-*ph1b* in this research have high lodging resistance and proper

plant heights, totally avoided the major adverse traits of CS *ph1b*, which will promote the utilization of the newly created germplasms in breeding procedure.

Values Of The Developed Translocations In Genetic Research And Wheat Breeding

So far, the genetic mechanism of adverse linkage drags brought by the *D. villosum* 6VS chromosome is unknown. In this research, forty-eight compensating translocations were identified with various alien segments of 6VS, which will be helpful for mapping loci related to key agronomic traits such as kernel weight, plant height, and spike number. Thereafter, creation of translocation haplotypes related to semi-dwarf, high kernel weight and multiple spike, and powdery mildew resistance could be realized, theoretically.

However, most of the recombinant events (87.6%) focused in the distal region (0 ~ 80 Mb) to *PmV*, which retarded freely proximal exchanges. The maldistributions of *ph1b*-induced breakpoints were also observed in other researches (Dai et al. 2020; Zhang et al. 2018).

Fortunately, the resistant translocation lines Dv6T25 with the shortest distal 6VS segment and Dv6T31 with the shortest proximal 6VS segment were developed, both expressed normal transmission rate and could be primarily used in breeding procedure. It's worth looking forward to developing much smaller intercalary translocations with powdery mildew resistance via two ways. Firstly, screening of secondary translocations carrying *PmV* in offspring of Dv6T25, in which the alien segment lengths are uncertain. Secondly, a putative intercalary translocation could be developed based on homologous recombination of the alien overlapping region in Dv6T25 and Dv6T31 followed the strategy of Sears (1981). In this case, the alien segment carrying *PmV* is constrained the same as the overlapping region (< 20 Mb), which helps to eliminate adverse linkage drags greatly and is more valuable in wheat resistance breeding.

Declarations

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Author contribution statement T.B., W.W., R.Z. conceived and designed the experiments. R.Z., T.C., and L.W. performed genotyping of the population. W.W., R.Z., and H.L. performed physical identification of the candidate translocations. X.Z. and X.W. supported genomic information and assisted sequence analysis. W.W., R.Z., and T.B. wrote the manuscript. All authors read and approved the final manuscript.

Availability of data and materials All the data generated or analyzed in this study are available in the manuscript and the supplementary information.

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

Ethical approval The authors declare that the experiments comply with the current laws of the country in which they were performed.

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Tables

Table 1 to 2 are available in the Supplementary Files section.

Figures

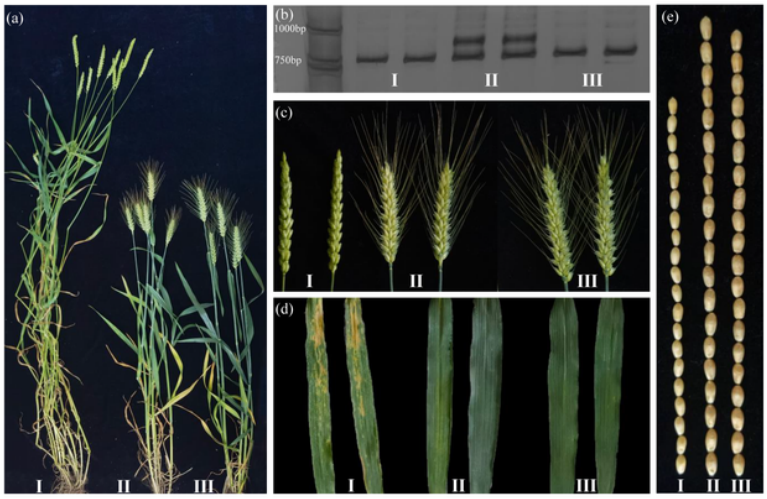


Figure 1

Morphological and genotype analysis among CS-*ph1b*, Yangmai23 and Yangmai23-*ph1b*. (a) Morphology of plant agricultural; (b) Genotype of *Ph1b* loci; (c) Morphology of spike; (d) Morphology of leaves at adult stage; (e) Morphology of seeds. Characters , and represent CS *ph1b*, Yangmai 23 and Yangmai 23-*ph1b*, respectively.

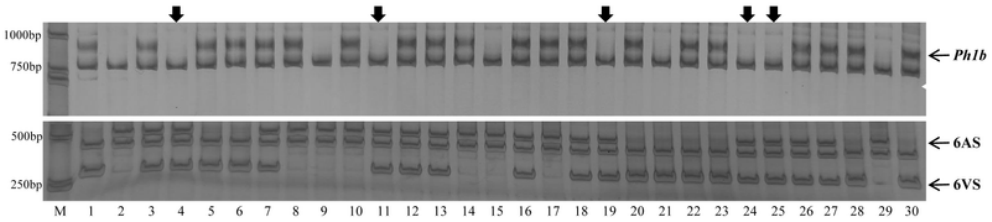


Figure 2

Screening of individuals with genotype of *ph1bph1b/PmVpmV* in F_2 population. Downward triangles show F_2 plants homozygous for *ph1b* and heterozygous for *PmV*. M: DNA ladder; 1: RIL12401; 2: Yangmai 23-*ph1b*; 3 to 30: part of individuals from the F_2 population.

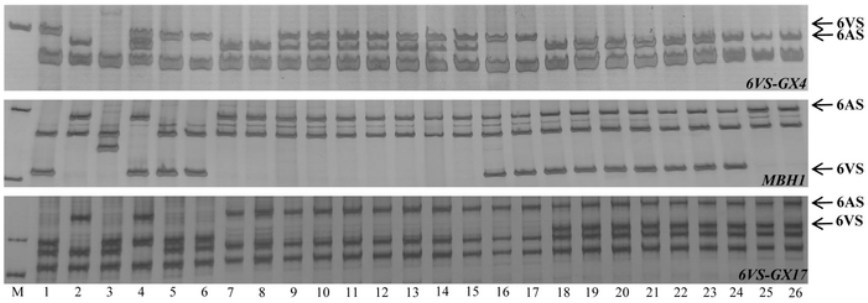


Figure 3

Screening of compensating translocations via the modified tri-marker strategy. M: DNA ladder; 1: RIL12401; 2: Yangmai 23-*ph1b*; 3: Yangmai18; 4: Yangmai22; 5-26: partial individuals from F_3 population divided from the cross between RIL12401 and Yangmai 23-*ph1b.*

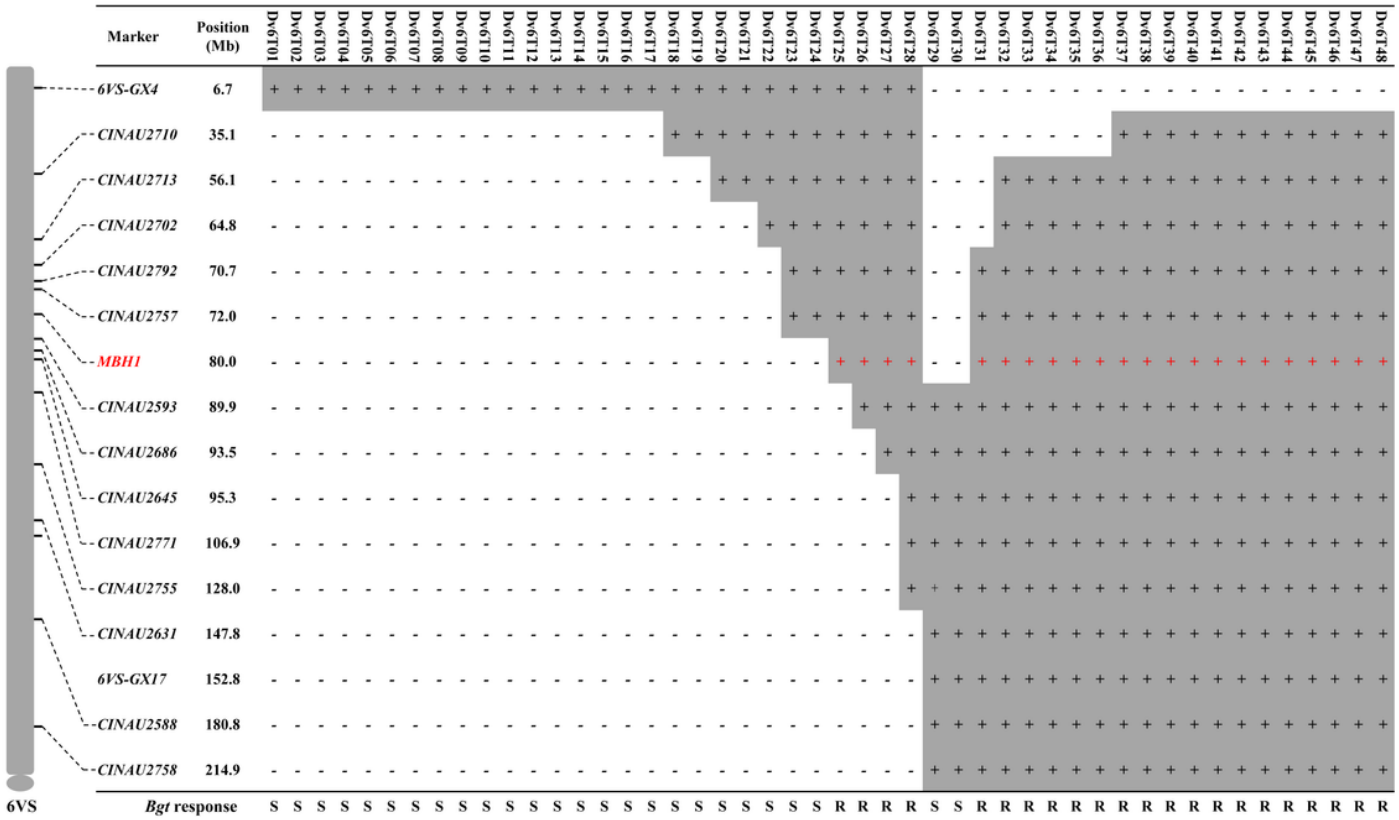


Figure 4

Physical identification and *Bgt* response of the newly developed wheat-*D. villosum* compensating translocations. Left: The schema graph of chromosome 6VS; the dotted lines represent the distribution of markers on chromosome 6VS. Right: The amplification of 6VS specific markers among translocation lines; “+” represents the positive amplification of markers in translocations; “-” represents the negative amplification of markers in translocations; the characters marked in red represent resistant gene *PmV*; R and S represent resistant and susceptible to powdery mildew, respectively.

Supplementary Files

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- Table1.png
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