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Development and characterization of a novel compensating wheat-Dasypyrum villosum interstitial translocation line Dv6-IT1 carrying the powdery mildew resistance gene PmV

Renhui Zhao
Yangzhou Academy of Agricultural Sciences
Wentao Wan
Yangzhou Academy of Agricultural Sciences
Man Xu
Nanjing Agricultural University
Tiantian Chen
Yangzhou Academy of Agricultural Sciences
Ling Wang
Yangzhou Academy of Agricultural Sciences
Ronglin Wu
Yangzhou Academy of Agricultural Sciences
Shujiang Zang
Yangzhou Academy of Agricultural Sciences
Zunjie Wang
Yangzhou Academy of Agricultural Sciences
Wei Jiang
Yangzhou Academy of Agricultural Sciences
Datong Liu
Yangzhou Academy of Agricultural Sciences
Yong Zhang
Yangzhou Academy of Agricultural Sciences
Xiue Wang
Nanjing Agricultural University
Haiyan Wang
Nanjing Agricultural University
Tongde Bie
bietd@126.com

Yangzhou Academy of Agricultural Sciences https://orcid.org/0000-0001-8402-8729

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Abstract

The powdery mildew resistance gene *PmV*, derived from the diploid *Dasypyrum villosum* accession No.1026, was previously introgressed into common wheat via the T6V#4S·6DL translocation. However, severely reduced transmission rates limited its breeding utility. To address this limitation, a recombinant translocation line T6VS#4S-6V#2S·6AL with improved transmission was previously created, then two smaller segmental translocations—Dv6T25 (T6VS-6AS·6AL, harboring 6VS 0–85 Mb) and Dv6T31 (T6AS-6VS·6AL, harboring 6VS 70 Mb–centromere), both carrying *PmV*—were generated using the *ph1b* induction system. In this study, homologous recombination within the overlapping region of Dv6T25 and Dv6T31 was exploited. A novel interstitial translocation line, Dv6-IT1, carrying an introgressed fragment of approximately 15 Mb, was identified. Molecular cytogenetic analysis confirmed that Dv6-IT1 is a compensating interstitial translocation with the configuration T6AS-6VS-6AS·6AL. Genetic analyses demonstrated that *PmV* in Dv6-IT1 follows normal Mendelian inheritance through both female and male gametes across diverse genetic backgrounds. The development of this small-segment interstitial translocation line provides a promising resistance source with minimal linkage drag for wheat powdery mildew breeding. This study not only addresses the transmission barrier of the original T6V#4S·6DL translocation but also establishes a model strategy for precision introgression of wild species-derived resistance genes into common wheat, paving the way for more efficient and sustainable wheat improvement in the face of evolving pathogen threats.

key Message

A novel compensating wheat-Dasypyrum villosum interstitial translocation carrying an approximately 15-Mb alien segment harboring PmV was created and demonstrated normal inheritance powdery mildew resistance.

Introduction

Wheat powdery mildew, caused by the obligate biotrophic fungus *Blumeria graminis* f. sp. *tritici* (*Bgt*), poses a persistent threat to global wheat (*Triticum aestivum* L.) production, reducing both yield and quality. The deployment of host genetic resistance remains the most economically and environmentally sustainable strategy for disease control. Wild relatives of wheat serve as invaluable reservoirs of disease resistance genes. Among them, *Dasypyrum villosum* (2*n* = 14, VV), a species from the tertiary gene pool of cultivated wheat, harbors exceptional genetic resources conferring resistance to multiple diseases, including powdery mildew, as well as tolerance to abiotic stresses and improved end-use quality (De Pace et al. 1988; Chen et al. 1995; Gradzielewska 2006; Zhang et al. 2016, 2018, 2021).

Notably, the short arm of chromosome 6V (6VS) in *D. villosum* carries potent powdery mildew resistance genes (Chen et al. 1995; Chen et al. 1997; Huang et al. 2023). *Pm21*, located on 6V#2S and originating from an accession maintained at the Cambridge Botanic Garden (UK) (Chen et al. 1995), and *PmV*, located on 6V#4S and derived from a former Soviet Union accession (Chen et al. 1997), share high sequence similarity but confer distinct resistance spectra (Wang et al. 2021). Notably, *PmV* confers stronger resistance at lower temperatures, making it particularly valuable for reducing early-season inoculum buildup (Wan et al. 2023). Through chromosome engineering, two whole-arm wheat-*D. villosum* translocation lines T6V#2S·6AL carrying *Pm21* (Chen et al. 1995) and T6V#4S·6DL carrying *PmV* (Li et al. 2005) were developed. The T6V#2S·6AL translocation, with robust resistance and satisfactory transmission, has been extensively utilized in breeding

programs across southwestern China and the middle-lower reaches of the Yangtze River in South China, contributing to nearly 100 commercial cultivars (Bie et al. 2015; Li et al. 2007a; Wu et al. 2021). However, the full-length 6V#2S arm introduces significant linkage drag, adversely affecting key agronomic traits, particularly reduced tiller number and increased plant height, which has limited its adaption in high-tillering and semi-dwarf wheat production zones like the Huang-Huai and Northern wheat regions in North China (Bie et al. 2015; Li et al. 2007b; Zhao et al. 2019).

In contrast, the contemporaneously released T6V#4S·6DL translocation has achieved limited breeding success, with only two registered cultivars (Yangmai 22 and Yangfumai 9) to date (Wan et al. 2023). This disparity primarily stems from a critical limitation of T6V#4S·6DL: its severely reduced meiotic transmission rates, which result from diminished gametophytic competitiveness compared to T6V#2S·6AL (Bie et al. 2015; Ma et al. 2022; Zhao et al. 2019). Therefore, improving the transmission efficiency of *PmV* is essential for unlocking its full breeding potential.

Recent efforts have focused on enhancing *PmV* transmission. Ma et al. (2022) developed a wheat-*D. villosum* T6V#4S·6AL translocation line, likely resulting from a centric-division, exhibiting enhanced inheritance for powdery mildew resistance but retaining the entire 6VS arm. Zhao et al. (2019) identified a recombinant translocation line, RIL12401 (T6V#4S-6V#2S·6AL), from a Yangmai 18 (T6V#2S·6AL)/Yangmai 22 (T6V#4S·6DL) recombinant inbred line (RIL) population, which significantly improved the *PmV* transmission rate, similar to that of *Pm21*. However, it simultaneously introduced severe linkage drag, manifesting as significantly increased plant height and reduced tiller number, similar to the effects of T6V#2S·6AL (Wan et al. 2023). To minimize linkage drag, the *ph1b* mutant was employed to induce homoeologous recombination in combination with “tri-marker strategy”, which facilitated to identify two smaller segmental compensating translocations sharing the smallest overlapping region harboring *PmV*: Dv6T25 containing the distal 6VS segment (0–85 Mb) and Dv6T31 containing the proximal 6VS segment (70 Mb–centromere) (Wan et al. 2023).

This study aimed to exploit homologous recombination within the overlapping region between Dv6T25 and Dv6T31, to develop a novel, much smaller interstitial compensating translocation line carrying *PmV*. The newly developed line was rigorously characterized through physical markers and cytogenetic analyses to confirm its structural integrity and transmission efficiency. The ultimate goal is to provide the wheat breeding community with elite *PmV*-carrying germplasm exhibiting superior transmission rates and minimized genetic load, thereby facilitating the widespread utilization of this valuable resistance resource across diverse wheat-growing regions, and avoiding the risk of solo-utilization of *Pm21* in South China.

Materials and methods

Plant materials

Yangmai 18 is a powdery mildew-resistant cultivar developed by Yangzhou Academy of Agricultural Sciences (YAAS) using the T6V#2S·6AL translocation line carrying the *Pm21* gene on chromosome 6V#2S and released in 2008. Yangmai 22 is a resistant cultivar developed by YAAS using the T6V#4S·6DL translocation line Pm97033 carrying the *PmV* gene on chromosome 6V#4S and released in 2010 (Bie et al. 2015). Yangmai 23 and Yangmai 25 are two susceptible cultivars developed by YAAS and released in 2013 and 2015, respectively. Yangmai 39, Yangmai 56 and Yangmai 24M99 are three resistant cultivars carrying T6V#2S·6AL, developed by YAAS. The

above cultivars are adapted to the middle and lower reaches of the Yangtze River. Huaimai 33 is a high-yielding but powdery mildew-susceptible cultivar developed by Huai'an Academy of Agricultural Sciences (HAAS) and adapted to the Huang-Huai wheat region. Yangmai 23-*ph1b* is a *ph1b* mutant in the Yangmai 23 background. This line was developed by back-crossing Yangmai 23 (recurrent parent) with the Chinese Spring *ph1b* mutant (donor). It retains agronomic traits similar to Yangmai 23 but enables homoeologous recombination (Wan et al. 2023). The recombinant line RIL12401 carrying *PmV* (T6V#4S-6V#2S·6AL) was selected from the RIL population derived from the cross Yangmai 18/Yangmai 22 (Zhao et al. 2019). Two *PmV*-carrying translocation lines, the distal translocation Dv6T25 with putative homozygous T6VS-6AS·6AL and the proximal translocation Dv6T31 with putative homozygous T6AS-6VS·6AL, were selected from F_3 progeny of the cross between RIL12401 and Yangmai 23-*ph1b* (Wan et al. 2023).

Genetic crosses and population development

The distal translocation Dv6T25 was crossed with the proximal translocation Dv6T31 to generate an F_2 segregation population. The newly developed interstitial translocation Dv6-IT1 (T6AS-6VS-6AS·6AL translocation carrying *PmV*) was crossed with six elite cultivars/lines: three susceptible cultivars (Yangmai 23, Yangmai 25 and Huaimai 33) and three resistant cultivars carrying T6V#2S·6AL (Yangmai 39, Yangmai 56 and Yangmai 24M99). The F_1 hybrids were subsequently self-crossed to generate six F_2 populations. To assess the transmission efficiency of *PmV* (harbored by the interstitial translocation Dv6-IT1) through both female and male gametes, F_1 hybrids from the crosses between Dv6-IT1 and susceptible cultivars (Yangmai 23, Yangmai 25 and Huaimai 33) were reciprocally backcrossed with their respective susceptible parents, respectively. Furthermore, to compare the transmission efficiencies of *PmV* (harbored by Dv6-IT1) and *Pm21* (harbored by the T6V#2S·6AL translocation) through the both gametes, F_1 hybrids from the crosses between Dv6-IT1 and resistant cultivars (Yangmai 39, Yangmai 56 and Yangmai 24M99) were reciprocally crossed with the susceptible cultivar Yangmai 25. All F_2 and backcross progeny were genotyped using the codominant marker *MBH1*. Chi-square (χ^2) tests were conducted to determine the goodness-of-fit of the experimental segregation ratios to the theoretical value.

Molecular marker analysis

DNA was extracted from fresh leaves at two-leaf stage using the modified cetyltrimethylammonium bromide (CTAB) method (Allen et al. 2006). A total of eleven molecular markers specific to the recombinant 6VS arm (6V#4S-6VS#2S) were recruited to genotype and detect the breakpoints and fragment sizes of the translocation chromosomes. Among them, *6VS-GX4* is a co-dominant marker tagging the distal regions of 6V#4S and 6AS, *6VS-GX17* is a co-dominant marker tagging paracentric regions of 6V#2S and 6AS (Wan et al. 2023), and *MBH1* was developed according to the sequence of the promoter of *Pm21* and able to distinguish the alleles on *D. villosum* 6V#2S and 6V#4S and wheat 6AS and 6DS chromosomes (Bie et al. 2015). The remained eight markers, including five Intron-Targeting (IT) markers and three simple sequence repeat (SSR) markers, were developed according to the genomic sequence of *D. villosum* (Wan et al. 2023, 2024). All primers were synthesized by Tsingke Biological Technology (Nanjing, China). The primers information was shown in Table S1.

Polymerase chain reaction (PCR) was performed according to Zhao et al. (2019). PCR products were separated in 8% non-denaturing polyacrylamide gels (Acr: Bis = 29:1 or 39:1), and the bands were visualized by silver nitrate

staining (Bassam and Gresshof 2007).

Cytogenetic analysis

Chromosome preparation of root-tips cells (RTC) at mitotic metaphase was performed according to Song et al (2020). The genomic/fluorescence *in situ* hybridization (GISH/FISH) analyses were conducted following established protocols (Dai et al. 2020; Zhang et al. 2004). For GISH, total genomic DNA of *D. villosum* was labeled with Fluorescein-12-dUTP by nick translation and used as a probe. The TAMRA (6-carboxy tetramethyl rhodamine)-labeled single-stranded oligos prepared from the chromosome 6A-specifc library were used as probes for FISH (Song et al. 2020). Fluorescence imaging was performed using an Olympus BX53 fluorescence microscope equipped with a SPOT CCD camera system (Olympus DP80) for signal capture and documentation.

Powdery mildew resistance assessment

Powdery mildew resistance was evaluated in a climate-controlled chamber. *Blumeria graminis* f. sp. *tritici* (*Bgt*) isolate YZ01 was dusted onto leaves of tested plants. Their powdery mildew responses were assessed 10 days post-inoculation. Yangmai 18 and Yangmai 22 were used as resistant controls, and Yangmai 23 was used as a susceptible control, respectively. Plants exhibiting no visible sporulation on leaves were classified as resistant (R), while those with spreading spores were classified as susceptible (S).

Results

Genotyping of an F_2 population from the cross Dv6T25/Dv6T31

A 130-plant F_2 population derived from the cross Dv6T25/Dv6T31 was genotyped using three codominant molecular markers: *6VS-GX4* (targeting the 6VS terminal region), *MBH1* (targeting the *PmV* resistance gene), and *6VS-GX17* (targeting the 6VS paracentric region) (Fig. 1). Five distinct genotypes were identified (Table S2).

Thirty-three plants (Type I) were homozygous for the T6VS-6AS·6AL translocation, identical to parent Dv6T25 (Fig. 1, lanes 6–10). Twenty-six plants (Type II) were homozygous for the T6AS-6VS·6AL translocation, identical to parent Dv6T31 (Fig. 1, lanes 11–15). Sixty-seven plants (Type III) were heterozygous, carrying both T6VS-6AS·6AL and T6AS-6VS·6AL chromosomes (Fig. 1, lanes 16–20). Three plants (Type IV) exhibited a heterozygous genotype characterized by T6VS-6AS·6AL and a novel chromosome predicted to be an interstitial translocation (T6AS-6VS-6AS·6AL) derived from overlapping recombination (Fig. 1, lanes 21–22). Only one plant (Type V) was heterozygous for T6AS-6VS·6AL and T6VS·6AL, with the latter likely resulting from recovered recombination (Fig. 1, lane 23). Schematic diagrams illustrating these five genotypes are presented in Fig. S1.

Molecular and cytogenetic identification of the predicted interstitial translocation

Type IV plants were self-pollinated to generate a segregation population. Genotyping with markers *6VS-GX4*, *MBH1*, and *6VS-GX17* identified plants homozygous for the predicted interstitial translocation (genotype BAB for these markers), which was designated as Dv6-IT1 with the translocation configuration T6AS-6VS-6AS·6AL.

Dual-color GISH/FISH analysis was performed on Dv6-IT1 simultaneously using *D. villosum* genomic DNA as a probe (green signals) and the 6A-specific oligos as a probe (red signals). The results confirmed the predicted structure: one pair of chromosomes exhibited short interstitial green signals embedded in large red signals (Fig. 2c), indicating the insertion of a small *D. villosum* segment into wheat chromosome 6A, consistent with the genotyping prediction.

Fine mapping using a series of molecular markers spanning the 6VS arm localized the recombination breakpoints. The distal breakpoint was flanked by markers *6VS-SSR129* (69.6 Mb) and *CINAU2592* (70.7 Mb), while the proximal breakpoint was flanked by markers *6VS-SSR62* (85.1 Mb) and *6VS-SSR63* (85.3 Mb) (Fig. 2d), defining an introgressed *D. villosum* segment about 14.4–15.7 Mb length. These results confirmed that the tertiary introgression resulted from homologous recombination, yielding a cytogenetically stable, compensating translocation. This configuration preserves the functional domain of the *PmV* gene while minimizing linkage drag from larger segments. Inoculation with *Bgt* isolate YZ01 confirmed that Dv6-IT1 is highly resistant to powdery mildew (Fig. S2).

Gamete transmission analyses of PmV in different wheat genetic backgrounds

Using the *MBH1* molecular marker, we genotyped plants from reciprocal backcrosses of F_1 hybrids (Dv6-IT1×Yangmai 23, Dv6-IT1×Yangmai 25, and Dv6-IT1×Huaimai 33) to their recurrent susceptible parents. Chi-square (χ^2) analysis of the segregation ratios (*PmV* vs. non-*PmV)* across all six resulting populations showed no significant deviation from the expected Mendelian 1:1 ratio (Table 1). This confirms that the *PmV* allele on the T6AS-6VS-6AS·6AL translocation transmits normally through both male and female gametes in the tested wheat lines.

Table 1

Analysis of gamete transmission efficiency of PmV conveyed by the T6AS-6VS-6AS·6AL translocation chromosome in different wheat genetic backgrounds

Cross combination	Transmission gamete type	Total tested plants	No. of plants carrying *PmV*	No. of plants without *PmV*	Transmission rate	$\chi^2_{1:1}$	*P*-value
(Dv6-IT1×Yangmai 23)×Yangmai 23	female	178	93	85	52.24%	$\chi^2 = 0.36 < \chi^2_{0.05,1}$	0.55
Yangmai 23×(Dv6-IT1×Yangmai 23)	male	148	71	71	47.97%	$\chi^2 = 0.24 < \chi^2_{0.05,1}$	0.62
(Dv6-IT1×Yangmai 25)×Yangmai 25	female	229	124	105	54.15%	$\chi^2 = 1.58 < \chi^2_{0.05,1}$	0.21
Yangmai 25×(Dv6-IT1×Yangmai 25)	male	132	61	71	46.21%	$\chi^2 = 0.76 < \chi^2_{0.05,1}$	0.38
(Dv6-IT1×Huaimai 33)×Huaimai 33	female	131	68	63	51.90%	$\chi^2 = 0.19 < \chi^2_{0.05,1}$	0.66
Huaimai 33×(Dv6-IT1×Huaimai 33)	male	102	42	60	41.17%	$\chi^2 = 3.18 < \chi^2_{0.05,1}$	0.07

Segregation analyses in diverse genetic backgrounds

The transmission efficiency of the T6AS-6VS-6AS·6AL chromosome was then assessed in F_2 populations derived from crosses between Dv6-IT1 and the above three susceptible wheat lines. Chi-square (χ^2) tests confirmed that the segregation ratios of homozygous (*PmV*/*PmV*), heterozygous (*PmV*/*pmV*), and non-alien (*pmV*/*pmV*) plants in all three F_2 populations adhered to the expected 1:2:1 Mendelian ratio (Table 2), as expected given the unbiased transmission of the interstitial translocation via both male and female gametes in the reciprocal backcrosses. All F_2 plants were inoculated with *Bgt* YZ01. Plants genotyped as homozygous or heterozygous for *PmV* were resistant, while non-alien plants were susceptible, confirming the complete association of the *PmV* gene with resistance.

Table 2

Segregation ratios of PmV conveyed by the T6AS-6VS-6AS·6AL translocation chromosome in different F_2 populations

Cross combination	Total tested F_2 plants	Homozygous (*PmV/PmV*)	Heterozygous (*PmV/pmV*)	Non-alien (*pmV/pmV*)	$\chi^2_{1:2:1}$	*P*-value
Dv6-IT1×Yangmai 23	244	59	128	57	$\chi^2 = 0.62 < \chi^2_{0.05, 2}$	0.73
Dv6-IT1×Yangmai 25	317	73	161	83	$\chi^2 = 0.71 < \chi^2_{0.05, 2}$	0.70
Dv6-IT1×Huaimai 33	482	127	227	128	$\chi^2 = 2.17 < \chi^2_{0.05, 2}$	0.44

Comparison of gamete transmission efficiency between PmV (T6AS-6VS-6AS·6AL) and Pm21 (T6V#2S·6AL)

To compare the transmission efficiency of the *PmV*-carrying T6AS-6VS-6AS·6AL translocation chromosome with that of the *Pm21*-carrying T6V#2S·6AL translocation chromosome through male and female gametes, F_1 hybrids derived from crosses between Dv6-IT1 and three *Pm21*-carrying resistant wheat lines (Yangmai 39, Yangmai 56 and Yangmai 24M99) were reciprocally testcrossed with the susceptible line Yangmai 25.

In all six testcross combinations, the segregation ratio of progeny inheriting the T6AS-6VS-6AS·6AL translocation (*PmV*) versus the T6V#2S·6AL translocation (*Pm21*) fit the expected 1:1 Mendelian ratio (Table 3). These results indicate that when both translocation chromosomes are present in a heterozygous state, *PmV* and *Pm21* are transmitted equally to progeny via both male and female gametes.

Table 3

Comparison of gamete transmission efficiency between PmV (T6AS-6VS-6AS·6AL) and Pm21 (T6V#2S·6AL) in different genetic backgrounds

Crosse combination	Type of transmission	Total tested plants	No. of plants carrying *PmV*	No. of plants carrying *Pm21*	$\chi^2_{1:1}$	*P*-value
(Dv6-IT1×Yangmai 56)×Yangmai 25	female gamete	162	81	81	$\chi^2 = 0.00 < \chi^2_{0.05,1}$	1.00
Yangmai 25×(Dv6-IT1×Yangmai 56)	male gamete	158	85	73	$\chi^2 = 0.91 < \chi^2_{0.05,1}$	0.34
(Dv6-IT1×Yangmai 39)×Yangmai 25	female gamete	272	128	144	$\chi^2 = 0.94 < \chi^2_{0.05,1}$	0.33
Yangmai 25×(Dv6-IT1×Yangmai 39)	male gamete	80	43	37	$\chi^2 = 0.45 < \chi^2_{0.05,1}$	0.50
(Dv6-IT1×Yangmai 24M99)×Yangmai 25	female gamete	119	50	69	$\chi^2 = 3.03 < \chi^2_{0.05,1}$	0.08
Yangmai 25×(Dv6-IT1×Yangmai 24M99)	male gamete	159	84	75	$\chi^2 = 0.51 < \chi^2_{0.05,1}$	0.48

Segregation analyses of PmV and Pm21 in F_2 populations

Chi-square (χ^2) tests were then performed on F_2 populations derived from crosses between Dv6-IT1 and the three *Pm21*-carrying resistant wheat lines (T6V#2S·6AL), which segregated for both translocations. As shown in Table 4, the segregation ratios of homozygous T6AS-6VS-6AS·6AL (*PmV*/*PmV*), heterozygous (*PmV*/*Pm21*), and homozygous T6V#2S·6AL (*Pm21*/*Pm21*) plants in the F_2 populations followed the expected 1:2:1 ratio, confirming the absence of segregation distortion and normal transmission through both male and female gametes.

Table 4

Segregation ratios of PmV (T6AS-6VS-6AS·6AL) and Pm21 (T6V#2S·6AL) in different F_2 populations

Cross combination	Total tested F_2 plants	Homozygous (*PmV/PmV*)	Heterozygous (*PmV/Pm21*)	Homozygous (*Pm21/Pm21*)	$\chi^2_{1:2:1}$	*P*-value
Dv6-IT1×Yangmai 56	345	81	187	77	$\chi^2 = 2.53 < \chi^2_{0.05, 2}$	0.28
Dv6-IT1×Yangmai 39	279	72	138	69	$\chi^2 = 0.10 < \chi^2_{0.05, 2}$	0.95
Dv6-IT1×Yangmai 24M99	345	83	179	83	$\chi^2 = 0.49 < \chi^2_{0.05, 2}$	0.78

Discussion

The introgression of valuable traits from the tertiary gene pool of wheat, particularly distant relatives like *D. villosum*, remains challenging due to suppressed homoeologous recombination (Cao et al. 2011). Historically, exploiting *D. villosum* germplasm required disruptive cytogenetic interventions like radiation mutagenesis or gametocidal systems (Bie et al. 2007; Cao et al. 2009; Chen et al. 2002, 2008a; Chen et al. 2008b; Chen et al. 2013). These approaches, however, typically yield non-compensating translocations burdened by excessive linkage drag, severely limiting their breeding value. For instance, radiation-induced small-segment translocations carrying *Pm21* (e.g., T1AS·1AL-6VS and T4BS·4BL-6VS-4BL) often suffered from transmission instability (Chen et al. 2013; Wang et al. 2015) and detrimental agronomic penalties.

Recent efforts have focused on developing compensating interstitial translocations for *Pm21* via *ph1b*-mediated homoeologous recombination (Lukaszewski and Cowger 2017; Zhang et al. 2023a), demonstrating the potential for target introgression. However, these segments remained substantial (36.9–39.1 Mb) (Zhang et al. 2023a) or unknown length (Lukaszewski and Cowger 2017). Progress with the highly valuable *PmV* gene has been limited due to its severe transmission instability in the original T6V#4S·6DL translocation (Bie et al. 2015; Ma et al. 2022; Zhao et al. 2019). To overcome this bottleneck for *PmV* utilization is therefore of great importance.

This study presents a breakthrough: the development of Dv6-IT1, a compensating interstitial translocation line (T6AS-6VS-6AS·6AL) carrying *PmV* with a drastically reduced alien segment of only ~ 15 Mb, retaining merely ~ 6% of the original 6VS arm. Genetic analyses demonstrated that Dv6-IT1 exhibits normal Mendelian inheritance in both backcross and F_2 populations across diverse genetic backgrounds, resolving the severe transmission distortion associated with the original T6V#4S·6DL translocation, which is unequivocally attributed to the full cytogenetic compensation achieved by the interstitial design. Furthermore, comparative analysis confirmed equivalent transmission rates between *PmV* (Dv6-IT1) and *Pm21* (T6V#2S·6AL) in the same genetic background, unlike previously reported distortions (Bie et al. 2015; Zhao et al. 2019). This work addressed the transmission barrier that hindered *PmV* deployment for nearly three decades, and unlocked the full potential of this broad-spectrum resistance gene for practical breeding.

This breakthrough establishes a novel framework for the precise engineering of tertiary gene pool resources. Although Sears (1981) proposed an overlap-recombination strategy for targeted introgression, its application requires isolating distal and proximal translocation lines that share overlapping segments containing the target gene—a process that involves large-scale screening and poses a major bottleneck, especially for distant relatives such as *D. villosum*. To address this, we developed an innovative “tri-marker strategy”—using codominant distal, proximal, and functional markers of the target gene—leveraging the completed genome sequence of *D. villosum* (Zhang et al., 2023b) to enable high-throughput screening of recombination events within a large *ph1b*-induced population (Wan et al., 2023). Candidate secondary translocations were subsequently characterized through precise physical mapping, which facilitated the identification of distal and proximal lines with minimal overlapping segments. This allowed the development of a novel tertiary interstitial translocation. By integrating tri-marker screening, advanced physical mapping, and targeted overlap-recombination, this strategy offers a highly efficient and precise method for generating compensating interstitial translocations with minimal linkage drag, significantly improving on traditional approaches dependent on random homoeologous recombination.

In conclusion, Dv6-IT1 represents a major advance in alien gene introgression. By integrating precise chromosome engineering—using a “tri-marker strategy” and “targeted overlap-recombination”—with new genomic tools, we have overcome the traditional trade-off between transmission stability and linkage drag in *Dasypyrum villosum* utilization. This interstitial translocation of approximately 15 Mb confers strong powdery mildew resistance with complete cytological compensation and stable Mendelian inheritance across genetic backgrounds. This breakthrough renews the value of *PmV* for breeding and provides a replicable model for exploiting wheat’s tertiary gene pool against evolving diseases. Moving forward, efforts should prioritize transferring *PmV* via Dv6-IT1 into elite wheat varieties across different agro-ecological zones to assess agronomic performance and enrich genetic diversity for powdery mildew resistance.

Declarations

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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Author contribution

statement TDB, RHZ and HYW conceived and designed the experiments. WTW designed the markers. MX and HYW performed the cytogenetic analysis of the translocation lines. RHZ, WTW, TTC and LW performed genotyping of the population and physical identification of the candidate translocations. RLW, SJZ, ZJW, WJ, DTL and YZ assisted breeding work. HYW and XEW supported genomic information and assisted sequence analysis. RHZ and TDB 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.

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Figures

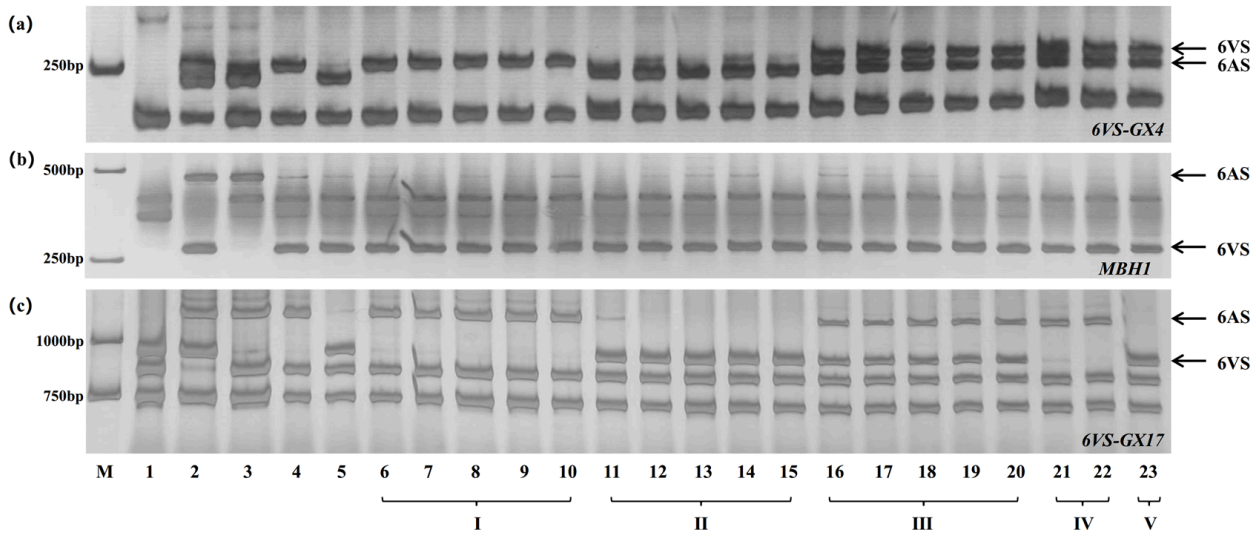


Figure 1

Genotypes identification in the F_2 population from the cross Dv6T25/Dv6T31 using the triple markers *6VS-GX4* (a), *MBH1* (b), and *6VS-GX17* (c). Left to right, M: DL2000 DNA marker; 1: Yangmai 18; 2: Yangmai 22; 3: Yangmai 23; 4: Dv6T25; 5: Dv6T31; 6-10: Type I plants homozygous for the T6VS-6AS·6AL chromosomes; 11-15: Type II plants homozygous for the T6AS-6VS·6AL chromosomes; 16-20: Type III plants heterozygous for T6VS-6AS·6AL and T6AS-6VS·6AL chromosomes; 21-22: Type IV plants heterozygous for T6VS-6AS·6AL and T6AS-6VS-6AS·6AL; 23: Type V plant heterozygous for T6AS-6VS·6AL and T6VS·6AL. Arrows indicate 6AS and 6VS, respectively.

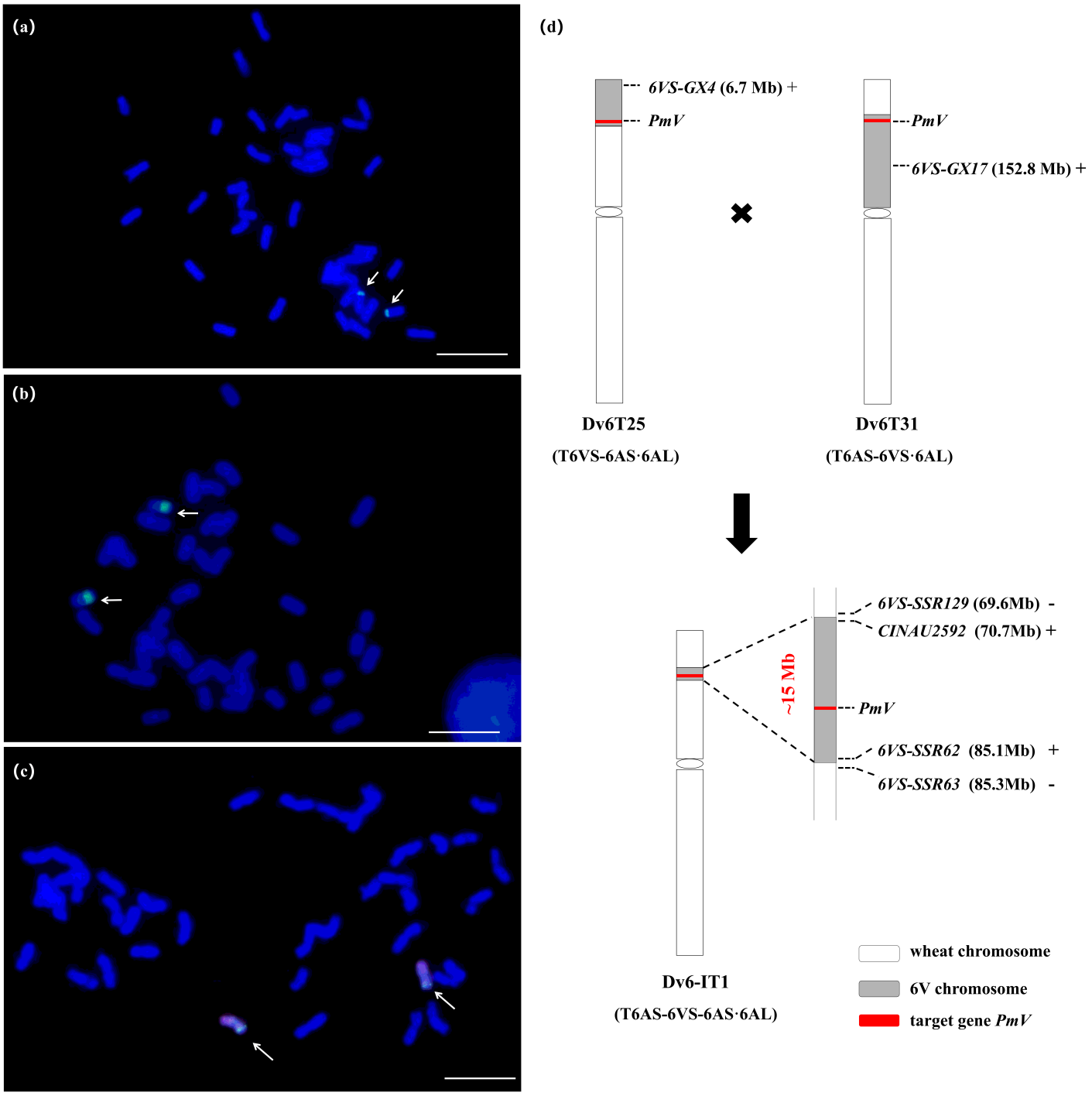


Figure 2

Cytogenetic identification and molecular analysis of wheat-*D.villosum* translocation lines Dv6T25, Dv6T31 and Dv6-IT1. a-b GISH patterns of chromosomes on root tip cells (RTCs) at mitotic metaphase of Dv6T25 (2*n* = 42) and Dv6T31 (2*n* = 42), respectively. Total genomic DNA of *D. villosum* was labeled with fluorescein-12-dUTP and visualized with green fluorescence. **c** Dual-color GISH/FISH pattern of chromosomes on RTC at mitotic metaphase of Dv6-IT1 (2*n* = 42). Green fluorescence represents GISH signals from genomic DNA of *D. villosum*, and the red fluorescence represents the FISH signals from 6A chromosome. Chromosomes were counterstained with 4',6-diamidino-2-phenylindole (DAPI) and visualized with blue fluorescence. White arrows

indicate the translocation chromosomes. Bar = 20 μm. **d** Schematic diagram of the development strategy and physical marker identification of the interstitial translocation Dv6-IT1. The white columns indicate the wheat chromosome 6A segment, the gray columns indicate the *D. villosum* chromosome 6V segment, and the red bars indicate the target gene *PmV*. The 6VS-specific markers are shown to the right of the schematic chromosome. “+” indicates the presence of the 6VS-specific marker loci, while “–” indicates the absence of the 6VS-specific marker loci.

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