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Sequence evidence for intergeneric DNA introgression from *Haynaldia villosa* 6VS chromosome into wheat near-isogenic lines

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Abstract

Introgressive hybridization has played a crucial role in crop breeding. Powdery mildew-resistant gene 21 (*Pm21*), located on the short arm of chromosome 6V of *Haynaldia villosa* (*H. villosa*), is typically a representative element which is transferred to hexaploid wheat through introgressive hybridization. Determination of sequence evidence for *H. villosa* DNA introgression would help trace *Pm21* in breeding wheat that are resistant to powdery mildew. In this study, we generated a wheat near-isogenic line (NIL) that was resistant to *Blumeria graminis* f. sp. *tritici* (*Bgt*) by using the wheat cultivar, Jing411, as the recurrent parent and the wheat-*H. villosa* chromosome translocation line, T6VS.6AL, as the donor parent of the *Bgt* resistance gene. We inoculated Jing411, T6VS.6AL, NIL, and *H. villosa* with *Bgt* race15. A large number of valving appressoria, contorted germ tubes, and slender germ tubes were observed in the NIL. In the host cells, cytoplasm agglomeration, papillae, haloes and hypersensitive reactions were detected. These results indicated that the NIL inherited the resistance to *Blumeria graminis* from the T6VS.6AL. We screened Jing411, T6VS.6AL, NIL, and *H. villosa* by using the Amplified Fragment Length Polymorphism (AFLP) technique and identified 21 AFLP fragments on chromosome 6VS. Among them, EACG/M14-301 in the NIL showed 100% identity to the fragments in T6VS.6AL and *H. villosa*. This result indicated that EACG/M14-301 might be a sequence evidence for intergeneric DNA introgression from *H. villosa* into common wheat. Our work will contribute to future investigations into wheat resistance breeding.

Keywords: Wheat, Near-isogenic line, T6VS.6AL, *H. villosa*, AFLP.
Abbreviations: *Bgt*_*Blumeria graminis* f. sp. *Tritici*; NILs_Near-isogenic lines; AFLP_Amplified Fragment Length Polymorphism.

Introduction

Near-isogenic lines (NILs) are generated mostly by crossing a species carrying a candidate gene from the donor parent with a cultivated species having other potential properties (i.e., the recurrent parent; RP), and followed by back-crossing to the RP for the subsequent 6-7 generations, and then finally self-crossing. Therefore, the NILs are a pair of species which differ only in the presence or absence of the target gene as well as a small region of flanking DNA sequence (Young et al., 1988). Owing to the fact that the genetic background of the NILs is nearly identical to the RP – except for the introgressed gene – NILs have been widely used for studying different aspects of crop genetics and breeding, including investigation of gene functions and screening of molecular markers closely linked to candidate genes (Eichten et al., 2011; Obando et al., 2008; Zhang et al., 2010). However, development of NILs is a laborious and time-consuming process. Furthermore the success or failure of establishing the line can be significantly influenced by environmental conditions and the selected derivation procedure (Zhang et al., 2012; Zhou et al., 2005). Therefore, it is very important to characterize and assess the genetic backgrounds of NILs to achieve improved selection effects with respect to agronomic traits. Over the last decade, evaluation of genetic background is typically performed by using a backcrossing regimen, and agronomic traits were observed subsequently. Despite the fact that a considerable number of studies have been carried out to examine similarities between the genetic backgrounds of NILs and the RP, the target gene specific to NILs needs to be identified (Brevis et al., 2008; Martin et al., 1991; Stam and Zeven, 1981; Wang et al., 2008; Zhu et al., 2008). Towards this goal, molecular markers have been employed to trace the target gene during the backcrossing in NILs. Encouragingly, much progress has been made in that regard recently. Kaczorowski et al. (2008) identified 1,500 SFPS (single feature polymorphisms) in soybean NILs; more than 70 SFPS were co-presented in the soybean NILs and RP, and 22 SFPS markers were linked to the donor gene in the resistant soybean NILs. Martin et al. (1991) used a set of tomato *Pto* NILs to confer resistance to *Pseudomonas syringae* and found that the donor gene fragment was present in the NILs. In our previous work, we have also identified AFLP molecular markers, $P1_{268}$ and $P2_{227}$, that are closely linked to the target gene of the donor parent Brock in NILs (Wang et al., 2008). These results demonstrated that molecular markers are indeed effective tools for optimizing the development and selection of NILs.

H. villosa (2N=14, VV) is a species related to common wheat (*Triticum aestivum*, 2N=6x=42, AABBDD). Chen et al. (1995) developed a wheat-*H. villosa* chromosome translocation line, T6VS.6AL, by hybridization in association with radiation treatment. The *Pm21* gene, located on the short arm of chromosome 6V of *H. villosa*, is responsible for the

durable and broad-spectrum resistance (BSR) to *Blumeria graminis* f. sp. *tritici* (*Bgt*) worldwide. Recently, the serine/threonine kinase gene, *STPK-V*, a key member of powdery mildew resistance gene, *Pm21*, has been identified on the *Pm21* locus (Cao et al., 2011). In this study, we developed a pair of wheat NILs by using wheat cultivar, Jing411, as the recurrent parent and wheat-*H. villosa* translocation line, T6VS.6AL, as the donor parent of the gene responsible for resistance to *Bgt*. Furthermore, we examined the genetic backgrounds of NILs by using the AFLP method. Twenty-one AFLP fragments, which were co-presented in *H. villosa*, T6VS.6AL and NILs but were absent in cultivated wheat Jing411, were identified. A specific AFLP fragment, EACG/M14-301, that originated from the donor parent *H. villosa* 6VS, was found in the NIL. The fragment may well be an appropriate sequence evidence for intergeneric DNA introgression into common wheat.

Results

Resistance response of NIL to Blumeria graminis f. sp. *tritici*

The *Bgt* race 15 is a virulent wheat powdery mildew strain originally found in northern China. In order to determine the resistance response of NIL to *Bgt*, we inoculated recurrent parent Jing411, donor of the resistance gene wheat-*H. villosa* translocation line (T6VS.6AL), and their NIL with the *Bgt* race 15 for 10 days before scoring. The plants manifested different resistance responses when exposed to powdery mildew (Fig 1). Jing411 was highly susceptible to *Bgt*. In Jing411, *Bgt* developed quickly, and a large number of spores were found – some of which even formed conidiophores; hyphae and conidiophores covered the leaf surface. *H. villosa* and T6VS.6AL were highly resistant to *Bgt* and no colonies, nor necrotic symptoms were observed when inoculated with *Bgt*. The NIL exhibited high levels of resistance to *Bgt*, although the morphological traits were nearly identical to the recurrent parent Jing411 (Fig 1, Table1). These results indicated that NIL inherited the T6VS.6AL resistance trait and maintained this resistance against powdery mildew after backcrossing with Jing411 for 7 generations.

Development of Bgt on the leaves of NIL and Jing411

To further examine the powdery mildew infection phenotypes of NIL and Jing411, we observed the propagation of *Bgt* in the different host cells. In the susceptible line Jing411, *Bgt* developed normally. Spores started to germinate after 0.5h of infection and the spores with 2-4 primary germ tubes were observed after 0.5h-4h into the infection process. *Bgt* formed rostriform appresora (RAs) after 12h of infection, and developed the penetration pegs (PP) after 16h of infection. Haustorial mother cells appeared after 20h of infection, and the host cells were covered with hyphae and conidiospores after 48h of infection (Fig 2). We observed the development of *Bgt* infection in T6VS.6AL and the NIL. The development of *Bgt* stopped after appresorium germ tube (AGT) formation, while large numbers of valving AGT (VA), contorted germ tubes (CG), and slender germ tubes (SA) were observed (Fig 3). In response to *Bgt* infection, in T6VS.6AL, *H. villos*a and NIL, cytoplasm agglomeration (CA) was detected, and papillae (P), haloes (H), and hypersensitive reactions (HR) were observed in the host cells post-infection (Fig 4). Although we observed the formation of the papillae and halo in the susceptible Jing411, there were demonstrable differences in response to *Bgt* infection between the resistant T6VS.6AL and the NIL, and the susceptible Jing411. In the susceptible plants, papillae formation following pathogen infection occurred at an earlier time point compared with the resistance plants.

Identification of DNA markers linked to powdery mildew resistance gene in NIL originated from 6VS

By using the AFLP technique, we identified DNA molecular markers linked to a powdery mildew resistance gene in NIL that originated from 6VS. The AFLP analysis results for *H. villosa*, T6VS.6AL, the NIL, and Jing411 are summarized in Figure 5. A total of 15 types of AFLP patterns were observed in this study (Supplementary Table 1).

Among those AFLP fragments, 3.8% of AFLP fragments were present in *H. villosa*, T6VS.6AL, NIL, and Jing411 (Type I). This result suggested that they were not correlated with resistance to *Bgt*. Type II AFLP fragments were present in T6VS.6AL, NIL, and Jing411, but did not appear in *H. villosa*. Therefore, it was shown that the Type II AFLP fragments were specific to the common wheat and did not originate from 6VS, even though 6VS has introgressed from *H. villosa* into T6VS.6AL and NIL. Type III AFLP fragments were only found in *H. villosa* and were not present in T6VS.6AL and NIL, which contained 6VS. This indicated that these AFLP fragments were not related to 6VS. In Type VIII, 2.3% of AFLP fragments can be amplified from *H. villosa*, T6VS.6AL, and NIL, while not from common wheat Jing411, implying that these AFLP fragments were located on the short arm of chromosome 6VS, and that these AFLP fragments were 6VS-specific. It is significant to note that 28.1% of AFLP band patterns were categorized as exceptional in this study. For instance, Type XV AFLP fragments can only be amplified in NIL, but were not detected in *H. villosa* and T6VS.6AL. Type VI AFLP fragments can be amplified in *H. villosa* and T6VS.6AL, but were not detected in the NIL. These results indicated that Types XV and VI lacked specific bands for 6VS. Therefore, it can be concluded that the powdery mildew gene of the NIL was derived from 6VS. To verify the molecular evidence for DNA introgression from 6VS into NIL, we cloned and sequenced 21 AFLP fragments of the Type VIII, including groups D5, D6, F2, F4, F8, F13 (Supplementary Table 2), which were found in *H. villosa*, T6VS.6AL, and the NIL, but were not amplified in Jing411. The results revealed that the majority of sequences of these AFLP fragments exhibited weak homology among the NIL, *H. villosa*, and T6VS.6AL. However, in the groups D6 and F8, strong sequence homology was observed. For instance, a 344bp-long amplified sequence from group F8 exhibited a very small difference (2bp) between NIL and T6VS.6AL. In the group D6, all of the fragments (D6H, D66, D6N) were 301bp-long (designated as EACG/M14-301, Genbank accession number: KJ427807), and displayed 100% sequence homology among the NIL, *H. villosa* and T6VS.6AL (Fig 6). Thus, it was logical to conclude that the 301bp-long sequence had introgressed into NIL from 6VS.

Discussion

Specific molecular markers located on chromosome 6VS of H. villosa in NIL

The NILs were generated mainly by crossing, backcrossing for 6-7 generations, and finally self-pollination. Owing to the

Table 1. The response of plants to powdery mildew

	Infection type					
Materials	0	0;	1	2	3	4
H. villosa	√					
Jing411						√
T6VS.6AL		√				
NIL(Jing411/T6VS.6AL//Jing411^{7})			√			

Note：0, represents no visible flecks, immunity; 0;, represents necrotic flecks; 1, represents highly resistant; 2, represents resistant; 3, represents susceptible; 4, represents highly susceptible

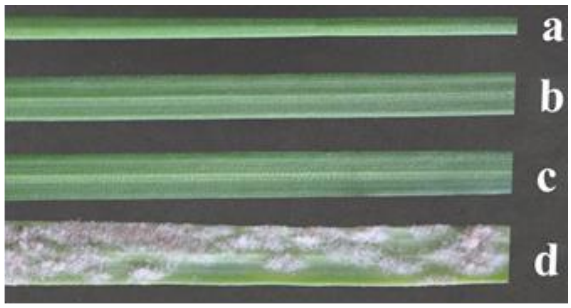

Fig 1. Characterization of the response of *H. villosa*, T6VS.6AL, NIL, and Jing411 to *Bgt* infection. *H. villosa* (a) and T6VS.6AL (b) were highly resistant to *Bgt* and no colonies nor necrotic symptoms were observed when they were inoculated with *Bgt*. The NIL (c) appeared to be highly resistant to *Bgt*, although the morphological features were nearly identical to the recurrent parent Jing411 (d). These results indicated that NIL inherited the T6VS.6AL resistance trait and maintained the resistance against powdery mildew after back-crossing with Jing411 for 7 generations.

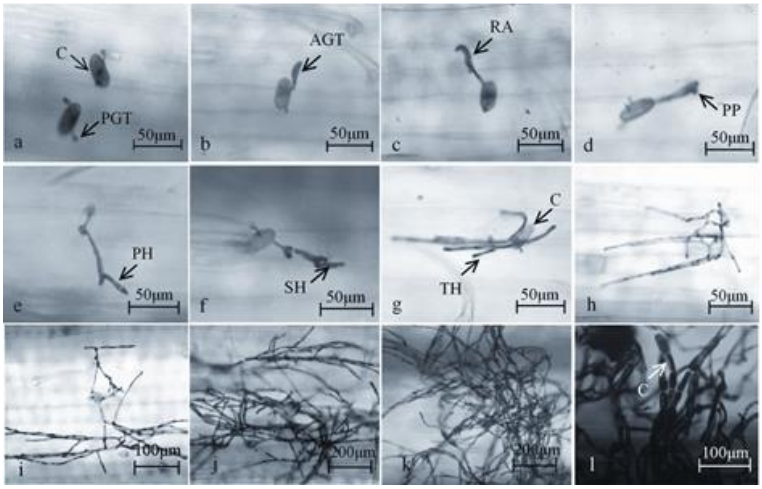

Fig 2. Infection process of powdery mildew on susceptible wheat Jing411 leaf surface. Primary germ tube were observed at 0-4h (a); appresorium germ tube was formed at 8h (b); rostriform appresorium at 12h (c); penetration peg at 16h (d); primary hypha at 20h (e); secondary hypha at 24h (f); triple hypha at 36h (g); and the hyphae were formed at 48h (h). Subsequently, the host cells were covered with hyphae and new conidiospores were formed (i-l). C: conidiospore; PGT: primary germ tube; RA: rostriform appresorium; AGT: appresorium germ tube; PP: penetration peg; PH: primary hyphae; SH: secondary hypha; TH: triple hypha.

fact that it takes a relatively long time to establish NILs, many researchers focused on determining whether the target genes of NILs are even still present or absent as a result of genetic variations, such as, deletions, insertions, substitutions, or selections. In previous reports, Weissmann et al. (2005) used molecular markers to demonstrate the sporadic intergeneric gene introgression from cultivated wheat into a wild *Aegilops* species in nature, and found that a 120bp-long DNA fragment of *T. aestivum* has introgressed into a wheat-wild relative, *Anadenanthera peregrina*. There were no sequence differences between the 120bp-long fragments at the same loci found in the samples collected in different years. Stam and Zeven (1981) estimated the theoretical proportion of the donor genome in NILs by backcrossing, and found that about 1.56%, and 0.78% of donor genomic DNA were introgressed into NILs (BC_5, BC_6). Zhou et al. (2005) established 11 NILs for the powdery mildew resistance genes, and the results from the AFLP analysis indicated that the genetic similarity between the NILs and their recurrent parents varied from 0.96 to 0.98, and these were all found to carry the targeted genes. NILs have been developed in many crops and applied widely in plant breeding, as well as in classical and molecular genetic studies (Eichten et al., 2011; Martin et al., 1991; Obando et al., 2008; Stam and Zeven 1981; Wang et al., 2008; Zhang et al., 2010; Zhang et al., 2012; Zhou et al., 2005; Zhu et al., 2008). In this study, we developed a pair of NILs by using 6VS as the donor parent. A total of 21 AFLP fragments located on chromosome 6VS of

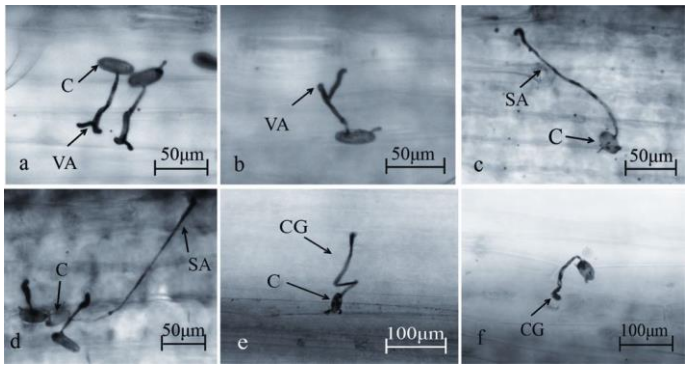

Fig 3. The abnormal development types of *Bgt* on resistant wheat leaf. After *Bgt* infection, some abnormal development types of *Bgt* were observed on resistant wheat leaf, such as valving appresorium on NIL leaves (a and b), slender appresorium on *H. villosa* leaves (c and d), contorted germ tube on T6VS.6AL leaves (e and f). The presence of these abnormal development types suggested that the wheat resistance is causing abnormal appressoria and that these abnormal appressoria would lose infection ability.
C: conidiospore; VA: valving appresorium; SA: slender appresorium; CG: contorted germ tube

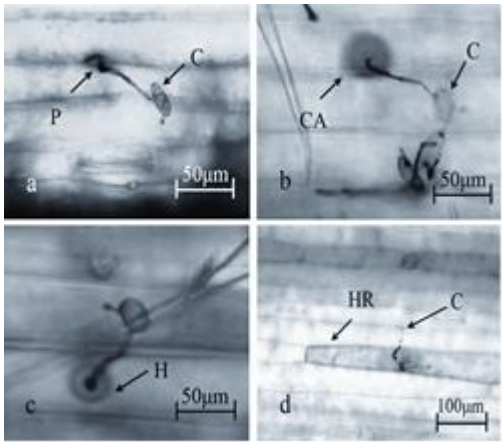

Fig 4. Defense response of resistant wheat to powdery mildew infection. After *Bgt* infection, the host cell's resistance structures were detected in the resistant wheat leaves, such as papillae (a), cytoplasm agglomeration (b), halo (c) and hypersensitive reaction (d). C: conidiospore; P: papillae; CA: cytoplasm agglomeration; H: halo; HR: hypersensitive reaction

H. villosa were found using the AFLP technique. Among these AFLP fragments, EACG/M14-301 in NIL exhibited 100% sequence homology to the fragments from *H. villosa* and T6VS.6AL. However, in Jing411 that lacks chromosome 6VS, this fragment was not detected. These results indicated that the EACG/M14-301 fragment is a specific sequence evidence for intergeneric DNA introgression from *H. villosa* into NIL. To verify the reliability and robustness of using the EACG/M14-301 fragment as sequence evidence, we further investigated this AFLP fragment using individuals of *H. villosa*, T6VS.6AL, NIL, and RP Jing411. A PCR amplification step was run using DNAs from *H. villosa*, T6VS.6AL, NIL, and Jing411 as templates with primers from EACG/M14. We found that the EACG/M14-301 fragment was present in *H. villosa*, T6VS.6AL, and all of the NIL individuals, but was not detected in Jing411 (Fig 7). This suggested that the resistance gene located on 6VS was transferred into the NILs, and provided a new sequence evidence for intergeneric DNA introgression from *H. villosa* 6VS chromosome into wheat powdery mildew resistance NIL. The identification of a sequence-specific marker linked with the donor gene was crucial for the evaluation of genetic background and the development of NILs. Our work also advances the understanding and examination into the molecular mechanism of the donor gene in NILs.

The application of EACG/M14-301 in developing Bgt resistance in wheat

Powdery mildew is a severe disease encountered in wheat breeding. To date, 72 alleles at the loci for powdery mildew resistance have been identified and assigned to a specific chromosome (Fu et al., 2013); however, most of the correlated genes have already been overcome by new virulent *B. graminis* strains owing to a single source of the resistance gene and its repeated use (Xie et al., 2001). It is therefore necessary to broaden the investigation to search for new genetic sources of resistance to powdery mildew in cultivated wheat. Chen et al. identified a potential source of powdery mildew resistance in *H. villosa* and established a wheat-*H. villosa* translocation line (T6VS.6AL). The 6VS chromosome has been transferred into cultivated wheat in order to enrich the vulnerable gene pool for resistance to powdery mildew (Chen et al., 1995). Cytogenetical studies and resistance breeding revealed that no pairing was found between 6VS from *H. villosa* and 6AS from cultivated wheat. Crossover was inhibited in chromosomes of pollen mother cells that are in mitotic metaphase. A single gene on the short arm of chromosome 6VS of *H. villosa* was demonstrated to be responsible for this resistance. This resistance gene was originally designated as *Pm21* (Qi et al., 1995). So far, it appears that *Pm21* can be inherited. In China and many regions of the world, this gene conferred broad-immunity to all of the assessed *Bgt* strains. Therefore, T6VS.6AL has been widely used as a parent in breeding programs. Over the last decade, more than ten novel resistant varieties have been developed, and in fact the most developed varieties have already been cultivated on more than 3.4 million hectares of farm land in China since 2002 (Cao et al., 2011). Molecular markers of closely linked target genes prove to be useful in breeding programs. These markers can help in detecting resistance genes of interest without the need to perform disease tests, and may shorten the breeding time in marker-assisted breeding (Huang and Röder, 2004). Therefore, researchers have identified molecular markers specific to *Pm21*, and considerable progress has been made in the last twenty years. In this study, we identified a sequence-specific molecular marker, EACG/M14-301. This marker is located on the chromosome 6VS of *H. villosa*, and was co-presented in T6VS.6AL and NILs; it may represent a sequence evidence for intergeneric DNA introgression into common wheat. Our work on the EACG/M14-301 molecular marker should benefit future investigative efforts in wheat resistance breeding.

Materials and Methods

Plant materials and breeding of wheat near-isogenic line

Common wheat cultivar Jing411 (*Triticum aestivum*, 2N=6X=42, AABBDD), wheat – *H. villosa* chromosome translocation line (TVS.6AL), resistant wheat near-isogenic

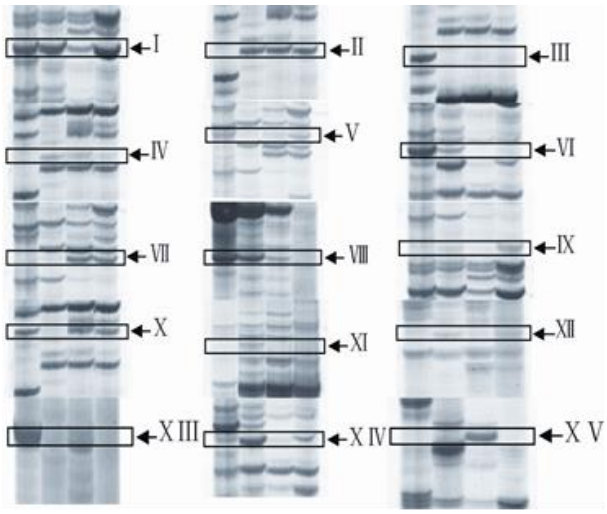

Fig 5. AFLP fragment types of the NIL, the donors *H. villosa*, T6VS.6AL, and recurrent parent Jing411
The lanes from left to right were *H. villosa*, T6VS.6AL, NIL, and Jing411, respectively. The boxes framed the same bands and every box was marked by Roman numerals. The Roman numerals represented AFLP fragment types from I to XV, respectively (for additional explanation see supplementary Table 1).

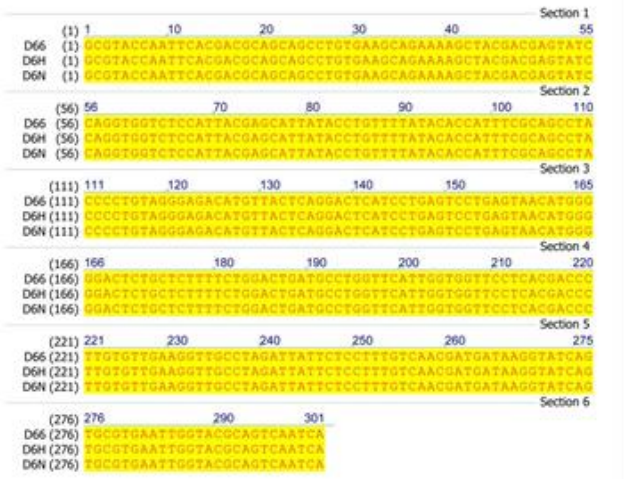

Fig 6. DNA sequence alignment of D66 (T6VS.6AL), D6H (*H. villosa*) and D6N (NIL)
A specific fragment co-presented in *H. villosa*, T6VS.6AL and NIL, which carried the chromosome short-arm 6VS, was cloned and sequenced. The length of this specific fragment was 301bp, and was named EACG/M14-301. EACG/M14-301 in NIL (D6N, cloning number) was 100% homologous to the fragments in *H. villosa* (D6H) and T6VS.6AL (D66).

Line (Jing411/T6VS.6AL//Jing411[7]), and *H. villosa* (2N=2X=14, VV) were used in the experiments. Common cultivated wheat Jing411 is a fine wheat variety and has been used in agricultural production, but is susceptible to powdery mildew. T6VS.6AL is a donor of the resistance gene. NIL was obtained by crossing T6VS.6AL with Jing411, and then backcrossed with Jing411 for 7 generations, and finally self-crossed and selected under *Bgt* inoculation.

Plant culture and powdery mildew resistance evaluation

The seeds of Jing411, T6VS.6AL, NIL, and *H. villosa* were germinated at 20℃ for 2 days. The seedlings were kept in the artificial climate chamber (Clima cell CLC707, MMM, Germany) at 25℃ with 16 h light / 8 h darkness and 60% humidity. The seedlings were inoculated with race 15 of *Bgt* at the time when the first leaves were fully expanded (*Bgt* race 15 is a virulent wheat powdery mildew species in northern China). The reactions were scored 7-8 days after inoculation when susceptible Jing411 was heavily infected. Plant reactions to powdery mildew inoculation was recorded on a scale of 0 to 4, with 0 representing no visible symptoms, 0; for necrotic flecks (hypersensitive response, HR), and values of 1, 2, 3 and 4 for highly resistant, resistant, susceptible and highly susceptible reactions, respectively (Mains and Diktz, 1930).

Cytological observation of Bgt inoculation on resistant and susceptible wheat leaves

Wheat leaves of lengths 2cm were excised from seedlings that were inoculated with *Bgt* for 2h, 4h, 8h, 12h, 24, 36h, 48h, and 72h. Then the excised leaves were fixed in the fixative solution containing methanol, glacial acetic acid (3:1), and acetocaustin (0.15%), for 72h, and then the leaves were stained with 0.6% Coomassie Brilliant Blue R250 (0.6g of Coomassie Brilliant Blue R250 diluted in methanol, 15% acetocaustin, 3:1) for 24h. Resistant reactions of NIL to *Bgt* and *Bgt* development on the leaves of the NIL were observed under the light microscope (Nikon 80i) in bright field. Statistical analysis of the total number of conidia, germ tubes, appressoria, and irregular appressoria were performed based on a scoring of at least 200 cells per treatment.

DNA preparation for amplified fragment length polymorphism (AFLP) analysis

About 0.2g of fresh leaves from each of Jing411, TVS.6AL, NIL, and *H. villosa* were used as the starting materials for DNA isolation. Total wheat genomic DNA was extracted using the CTAB method (Zhang et al., 2010). DNA concentration was measured by Nanodrop 2000 (Nanodrop, Thermo). DNA integrity was determined by running 5μl of the samples on 0.7% agarose gel.

AFLP amplification and product test

The AFLP reaction was performed as described by Vos et al. (1995) and in accordance to the manufacturer's instructions (Takara). Adapters and primers of AFLP were listed in Supplementary Tables 3 and 4. Genomic DNA digestion, adapter ligation and pre-amplification, selective amplification, amplification, and product test were performed as described by Zhang et al. (2010). Target fragments were excised from agarose gel under UV light and 50μl ddH_2O was added. Sample was incubated at 95℃ for 30min and centrifuged at 3000 ×*g* for 2min. A total of 2μl of the supernatant was used as template for selective amplification. Purification of the amplification products was performed using 1.2% low melting temperature agarose gel. pGEM®-T Easy vector was used as the cloning vector. Sequence analysis was carried out at Sangon Biotech Co. Ltd. (Shanghai). The result was analyzed with the software Bioedit 5.0. Sequence homology searches were conducted using BLAST against the NCBI database.

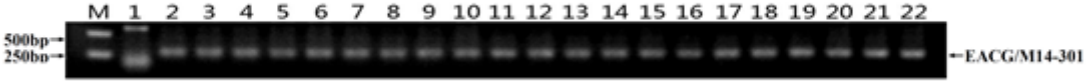

Fig 7. Amplification products with primer EACG/M14 in Jing411, *H. villosa*, T6VS/6AL and NIL individuals. We screened *H. villosa*, T6VS.6AL, NIL individuals and RP Jing411 with the AFLP primer pair EACG/M14. The fragment EACG/M14-301 was detected in *H. villosa* (lane 2), T6VS.6AL (lane 3) and all the NIL individuals (lane 4 to 22), but not in Jing411 (lane 1). This result further suggested that EACG/M14-301 was a sequence evidence for intergeneric DNA introgression from *H. villosa* into common wheat. DL2000 Marker (lane M).

Statistical analysis of AFLP patterns

Statistical analysis of AFLP patterns was carried out according to Zhang et al. (2010). The experiments were repeated three times with three replicates for each sample for all analyses. The significance of the differences between the control and *Bgt*-treated samples of susceptible and resistance wheat was determined using the *t*-test, and $p < 0.05$ represents a significant difference. A total of 15 types of AFLP patterns were summarized in Supplementary Table 1. However, in this study, only type VIII fragments – which were co-presented in *H. villosa*, T6VS.6AL, and NIL, but did not appear in Jing411 – were cloned and sequenced.

Conclusion

In this study, we generated a pair of wheat near-isogenic line (Jing411/T6VS.6AL//Jing411^{7}) that was resistant to *Blumeria graminis* f. sp. *tritici*. The AFLP fragment EACG/M14-301 shared 100% identity in NIL, T6VS.6AL and *H. villosa*, which carried the 6VS chromosome. This is very likely to be a sequence evidence for intergeneric DNA introgression from *H. villosa* into the common wheat.

Acknowledgments

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References

Brevis JC, Chicaiza O, Khan IA, Jackson L, Morris CF, Dubcovsky J (2008) Agronomic and quality evaluation of common wheat near-isogenic lines carrying the leaf rust resistance gene. Crop Sci. 48: 1441-1451.

Cao AZ, Xing LP, Wang XY, Yang XM, Wang W, Sun YL, Qian C, Ni JL, Chen YP, Liu DJ (2011) Serine/threonine kinase gene *Stpk-V*, a key member of powdery mildew resistance gene *Pm21*, confers powdery mildew resistance in wheat. Proc Natl Acad Sci. 108: 7727-7732.

Chen PD, Qi LL, Zhou B, Zhang SZ, Liu DJ (1995) Development and molecular cytogenetic analysis of wheat-Haynaldia villosa 6VS/6AL translocation lines specifying resistance to powdery mildew. Theor Appl Genet 91: 1125-1128.

Eichten SR, Foerster JM, de Leon N, Kai Y, Yeh CT, Liu SZ, Jeddeloh JA, Schnable PS, Kaeppler SM, Springer NM (2011) B73-Mo17 near-isogenic lines demonstrate dispersed structural variation in maize. Plant Physiol 156: 1679-1690.

Fu BS, Chen Y, Li N, Ma HQ, Kong ZX, Zhang LX, Jia HY, Ma ZQ (2013) *pmX*: a recessive powdery mildew resistance gene at the *Pm4* locus identified in wheat landrace Xiaohongpi. Theor Appl Genet. 126: 913 – 921.

Huang XQ, Röder MS (2004) Molecular mapping of powdery mildew resistance genes in wheat: a review. Euphytica. 137: 203-223.

Kaczorowski KA, Kim KS, Diers BW, Hudson ME (2008) Microarray-based genetic mapping using soybean near-isogenic lines and generation of SNP markers in the Rag1 aphid-resistance interval. Plant Genome. 1: 89-98.

Mains EB, Diktz SM (1930) Physiologic forms of Barley mildew, Erysiphe graminis hordei. Phytopathology. 20: 229-239.

Martin GB, Williams J, Tanksley SD (1991) Rapid identification of markers linked to a Pseudomonas resistance gene in tomato by using random primers and near-isogenic lines. Proc Natl Acad Sci. 88(6): 2336-40.

Obando J, Fernández-Trujillo JP, Martínez JA, Alarcón AL, Eduardo I, Arús P, Monforte AJ (2008) Identification of melon fruit quality quantitative trait loci using near-isogenic lines. J Am Soc Hortic Sci. 133: 139-151.

Qi LL, Chen PD, Liu DJ, Zhou B, Zhang SZ, Sheng BQ, Xiang QJ, Duan XY, Zhou YL (1995) The gene *Pm21* - a new source for resistance to wheat powdery mildew. Acta Agron Sin. 21: 257-262.

Stam P, Zeven AC (1981) The theoretical proportion of the donor genome in near-isogenic lines of self-fertilizers bred by backcrossing. Euphytica. 30: 227-238.

Vos P, Hogers R, Bleeker M, Reijans M, van De Lee T, Hornes M, Friters A, Pot J, Paleman J, Kuiper M (1995) AFLP: a new technique for DNA fingerprinting. Nucleic Acids Res. 23: 4407-4414.

Wang Y, Zhu J, Zhao HM, Lei DH, Wang ZY, Peng YK, Xie CJ, Sun Q, Liu ZY, Yang ZM (2008) Screening and identification of the AFLP markers linked to a new powdery mildew resistance gene in wheat cultivar Brock. J Mol Cell Biol. 41: 294.

Weissmann S, Feldman M, Gressel J (2005) Sequence evidence for sporadic intergeneric DNA introgression from wheat into a wild Aegilops species. Mol Biol Evol. 22: 2055-2062.

Xie CJ, Ni ZF, Sun QX, Yang ZM, Liu BS, Wei YL (2001) Molecular tagging of a major powdery mildew resistance gene MlG in wheat derived from wild Emmer by using microsatellite marker. Acta Genet Sin. 28: 1034.

Young ND, Zamir D, Ganal MW, Tanksley SD (1988) Use of isogenic lines and simultaneous probing to identify DNA markers tightly linked to the tm-2a gene in tomato. Genetics. 120: 579-585.

Zhang R, Wang Y, Liu XY, Wang ZY, Peng YY, Xie CJ, Yang ZM (2010) Molecular evaluation on the background of wheat Brock near isogenic lines for powdery mildew resistance. Sci Agric Sin. 43: 3059-3066.

Zhang YL, Wang MJ, Zhanf Y, Chu CP, Lin ZS, Xu QF, Ye XG, Zhang XS (2012) Development and application of functional markers specific to powdery mildew resistance on chromosome arm 6VS from different origins of *Haynaldia villosa*. Acta Genet Sin. 38: 1827-1832.

Zhou RH, Zhu Zo, Kong X, Huo NX, Tian QZ, Li P, Jin CY, Dong YC, Jia JZ (2005) Development of wheat near-isogenic lines for powdery mildew resistance. Theor Appl Genet. 110: 640-648.

Zhu S, Walker DR, Boerma HR, All JN, Parrott WA (2008) Effects of defoliating insect resistance QTLs and a Cry1Ac transgene in soybean near-isogenic lines. Theor Appl Genet. 116: 455-463.