Targeted sequencing of the short arm of chromosome 6V of a wheat relative *Haynaldia villosa* for marker development and gene mining

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Abstract

Background: Short arm of chromosome 6V (6VS) of *Haynaldia villosa* has been used in wheat breeding programs to introduce Pm21 resistance gene against powdery mildew and some other genes. **Results:** In this work, 6VS was isolated from a wheat (*Triticum aestivum*) - 6VS telosome addition line by flow cytometric sorting and sequenced by illumina technology. The assembly length was 230.39 Mb with contig N50 of 9,788 bp. The sequence annotation identified 3,276 high confidence genes supported by RNA sequencing data, representing about 2.3% of the chromosome arm sequence; repetitive elements accounted for 74.91% of the arm sequence. Sequences homologous to 6VS genes were identified on short arms of chromosomes 6A of *T. urartu*, 6D of *Aegilops tauschii*, 6A and 6B of *T. dicoccoides*, 6A, 6B and 6D of *T. aestivum* and 6H of *Hordeum vulgare*, revealing synteny relationships among these chromosome arms. Based on differences in intron size between the homologous genes on 6VS and 6AS/6BS/6DS of *T. aestivum*, 222 primer pairs were designed. Out of them, 120 amplified 6VS-specific products and are suitable as intron-target (IT) markers to trace the 6VS chromatin introduced into wheat. **Conclusions:** The results obtained and markers developed in this work will facilitate introduction of important genes to common wheat from its wild relative, while reducing the presence of unfavorable genes due to linkage drag.

Background

Haynaldia villosa L. (2n=14, genome VV) is a wild relative of common wheat (*Triticum aestivum* L.) carrying resistance genes to numerous wheat diseases, including powdery mildew, wheat yellow mosaic virus, eyespot, take-all and rusts [1]. It has also been credited for improving tillering [2, 3], high grain protein content [4-6], and tolerances to frost and drought of wheat [7, 8]. These characters make *H. villosa* a highly attractive source of important genes and alleles for wheat improvement [1]. In the previous study, several useful genes were mapped on short arm of chromosome 6V, such as the *Pm21* locus, which provides immunity or high resistance to all powdery mildew isolates, and *NAM-V1*, which contributes to increased grain protein content (GPC) in the wheat-*H. villosa* 6AL/6VS translocation lines [9, 10]. However, the lack of the genome sequence hampered the efforts to mine other important genes from *H. villosa* and the use of molecular tools to introduce them to wheat, while avoiding unfavorable alien chromatin.

The progress in DNA sequencing technology now makes the production of whole genome sequence assemblies feasible by whole genome shotgun approaches and the number of sequenced genomes of wheat relatives keeps on increasing [11-13]. However, if chromosomal location of the loci of interest is known, the option is to sequence only the chromosome, or chromosome arm of interest. This approach significantly reduces the project costs and thus enables sequencing chromosomes from multiple lines of a species, if needed. It also simplifies bioinformatic analyses due to reduced volume of sequence data.

Targeted sequencing of a particular chromosome is possible after isolating a required number of chromosomes by flow cytometric sorting [14-21]. Next generation sequencing of flow-sorted chromosomes has been used to develop molecular markers in *Aegilops geniculata* and *H. villosa* [22-24].

Importantly, sequencing DNA from flow sorted chromosomes facilitated the production of draft genome assemblies of barley [25], rye [26] and common wheat [27] and to isolate genes in wheat and barley either by the MutChromSeq strategy [28] or the TACCA approach [29].

Purification of a particular chromosome by flow sorting may be hampered by the inability to discriminate the chromosome from other chromosomes in a karyotype if its size or relative DNA content is not different. Various strategies have been developed to overcome this difficulty and one of them is to sort translocation or deletion chromosomes with altered size [15-19]. Larger deletions are not viable in diploids, but they may be developed from wild type chromosomes after they are introduced to a polyploid species, such as wheat, which tolerate aneuploidy. Thus, Tiwari et al. sorted chromosome 5Mg from a wheat/*Ae. geniculata* disomic substitution line [20]. In a similar way, Xiao et al. used a wheat-alien ditelosomic addition line "NAU1201" to isolate chromosome arm 4VS of *H. villosa*. Thus, the line needs not to be prepared exclusively for flow cytometric sorting and may be already available [21].

In this work, 6VS of *H. villosa* was flow-sorted from a *T. aestivum*-*H. villosa* ditelosomic addition line containing a pair of short arms of chromosome 6V. The isolated 6VS was sequenced and assembled. The draft sequence obtained made it possible to characterize molecular composition of 6VS including DNA repeat content, identify genes and characterize syntenic relationships with the genomes of tribe *Triticeae* and other sequenced grasses. The 6VS sequences would also be used to develop 6VS-specific markers to support alien introgression breeding of wheat and the cloning of favorable genes from 6VS.

Results

Flow sorting and sequencing of chromosome arm 6VS of *H. villosa*

Flow cytometric analysis of chromosomes isolated from *T. aestivum*-*H. villosa* 6VS ditelosomic addition line resulted in bivariate flow karyotypes FITC (log scale) vs. DAPI (linear scale) fluorescence on which a number of populations could be resolved (Figure 1). The population representing 6VS telosome was identified after screening all populations with lower DAPI fluorescence, which were expected to correspond to smaller chromosomes. Microscopic analysis of flow-sorted particles after FISH with probes for

pSc119.2 and Afa family repeats enabled unambiguous identification of the population representing 6VS telosomes (Figure 1). A detailed microscopic analysis showed that 6VS telosome could be sorted at an average purity of 89.41%. The sorted DNA was amplified by multiple displacement amplification (MDA) reactions before Illumina sequencing. Sequencing of DNA amplified from flow-sorted chromosome 6VS in Illumina MiSeq system generated 47.7 Gb high-quality paired-end reads from two libraries, with insert sizes of 500 bp and 1,000 bp, respectively.

After assembly using Hecate software, a total of 230.39 Mb draft sequences was obtained. The sequences consist of 153,177 scaffolds, with the maximum and minimum lengths of the scaffold of

138,620 bp and 100 bp, respectively, and contig N50 length of and mean length of 9,788 bp and 1,464 bp, respectively.

Identification of repetitive DNA elements

Using RepeatMasker software, a total of 181.29 Mb out of 230.39Mb 6VS assembly was identified as repetitive sequences which accounted for 74.91% (Table. 2). Among the repeat elements, the most abundant were LTR retrotransposons comprising about 88%, out of which *Gypsy* superfamily repeats accounted for about 69.3%, followed by *Copia* superfamily, which comprised 14.43%. DNA transposons were mainly represented by *TIR* family, which made up about 8.72% of all repeats. After masking all repetitive DNA elements, the remaining non-repetitive sequence reads from 6VS equaled 49.1 Mb, which was used for the following gene prediction and sequence comparisons.

Gene content of chromosome 6VS

Ab initio gene prediction using AUGUSTUS software preliminarily identified 5,973 predicted coding genes from repeat-masked scaffold of 6VS. After using transcriptome data of *H. villosa* (data not show) as the evidence of coding loci, 3,276 genes on 2,871 scaffolds of 6VS were retained and deemed as high-confidence. The gene length distribution is shown on Fig. S1A. The genic sequences represented a total length of 5,278,412 bp, which accounted for 2.3% of the 6VS assembly. Totally, 1,672 genes were classified to one or more Gene Ontology (GO) terms (Fig. S1B). To summarize, the number of genes which was annotated into biological process, molecular function and cellular component were 1,432, 1,150 and 1,441, respectively.

In order to test the annotation quality of the 6VS, we used genes *NLR-V* [32], *STPK-V* [33] and *NAM-V1* [9] cloned from 6VS to perform BLASTn search. We found sequences homologous at 99.93%, 100.00% and 99.93%, respectively, implying a high quality of *H. villosa* 6VS annotation. Thus, the 6VS draft sequence obtained in this work will facilitate extensive mining of 6VS genes in wheat breeding.

Comparative analysis of 6VS sequence composition

The genomic reference of common wheat cv. Chinese Spring released by IWGSC [34] was used to identify the syntenic regions of 6VS on wheat chromosomes 6A, 6B and 6D, using the high-confidence genes. We have also identified 6VS syntenic regions on chromosomes 6A and 6B of tetraploid *T. dicoccoides*, 6D of *Ae. tauschii*, 6A of *T. urartu* and 6H of *H. vulgare*. After filtering, 2,867 6VS genes had 1,499, 1,577 and 1,430 blastn hits with homologous genes in wheat chromosomes 6A, 6B and 6D, respectively; the number of hits with homologous genes in *T. dicoccoides* chromosomes 6A and 6B was 1,323 and 1,374, respectively; in *Ae. tauschii* chromosome 6D it was 1,301, in *T. urartu* 6A it was 1,424 and in *H. vulgare* 6H it was 1,307. Moreover, 634 out of 2,867 genes were shared among all eight genomes. The syntenic genes on *T. aestivum* chromosomes 6A, 6B and 6D, *T. dicoccoides* chromosomes 6A and 6B, *Ae. tauschii* chromosome 6D, *T. urartu* chromosome 6A and *H. vulgare* chromosome 6H were plotted on chromosomes to highlight the syntenic regions, according to their physical position (Fig. 2). As expected,

the syntenic regions with high gene density were observed on chromosomes 6AS, 6BS and 6DS of *T. aestivum*, 6AS and 6BS of *T. dicoccoides*, 6DS of *Ae. tauschii*, 6AS of *T. urartu* and 6HS of *H. vulgare*.

NB-ARC domain proteins are commonly known as disease resistance genes. In the 6VS assembly, a total of 45 genes were predicted to encode NB-ARC domain proteins using HMMER model [35]. In a separate project, we analyzed transcriptome of the wheat-*H. villosa* T6VS/6AL translocation line after the treatment with two *Blumeria graminum f.sp tritici* (*Bgt*) isolates E26 and E31 (data not shown). We found that 28 genes were expressed after inoculation of both isolates within 24 hours, with 15 genes up-regulated two-fold or more when compared to the control (Figure 3). As 6VS chromatin introduced to wheat showed the main contributor of the resistance to various *Bgt* isolates, 6VS genes might be involved in the innate immunity of *H. villosa* to powdery mildew.

Wheat cultivars with 6VS/6AL translocation have been used extensively in wheat production with more than four million hectares in China [32], not only due to broad-spectrum resistance to powdery mildew, but also due to their contributions to higher 1000-grain weight (TGW) [36]. In a previous study, *TaGW2-6A* was described as a negative regulator of grain-width and grain-weight [37-39]. Four SNPs that occurred in the promotor region of *TaGW2-6A* were reported to be associated with TGW at positions -998bp, -739bp, -593bp and -494bp, in which SNP at -494 bp showing significant association with TGW and was located in the 'CGCG' motif [37]. SNP-494 has most effect on *TaGW2-6A* expression level and TGW, with haplotypes of A allele having significantly lower *TaGW2-6A* expression and higher TGW compared with those with G allele. To figure out if the increased TGW of 6VS/6AL translocations was due to the substitution of 6AS with 6VS, the *TaGW2-6A* gene homologue *HvGW2-6V* was identified in the 6VS assembly. The *HvGW2-6V* in *H. villosa* belongs to G allele at SNP-494, which was associated with low TGW (Figure 4). We speculate that higher TGW of 6VS/6AL translocations might be affected by other genes rather than *GW2-6V*, or that the expression of alien gene is suppressed due to genomic shock in wheat background although the genotype at position -494 was the same with low TGW.

Development of intron targeted (IT) markers

Zhang et al. and Wang et al. developed IT markers for all chromosomes except for 6VS of *H. villosa* [22, 23]. With the shotgun sequences of 6VS, IT markers for this short arm could now be developed. All 2,063 annotated genes from *Ae. tauschii* 6DS were aligned against the wheat genome reference and 6VS assembly sequences to determine exon-exon junction lengths on chromosomes 6AS, 6BS, 6DS of *T. aestivum* as well as on 6VS. A total of 222 genes had the intron length in *H. villosa* differing by at least 10% as compared to those in wheat subgenomes A, B and D. Then, we designed PCR primers in the conserved exon regions which flanking the targeted introns using Primer 3 software (<http://frodo.wi.mit.edu/primer3/>).

In order to test the 222 IT primers on 6VS of *H. villosa*, we perform PCR analysis for the DNA from *T. aestivum* cv. Chinese Spring (AABBDD), *H. villosa* (VV) and *T. aestivum*-*H. villosa* T6AL·6VS translocation line. If the primer pair amplify a distinct PCR product visualized only in *H. villosa*, and *T. aestivum*-*H. villosa* T6AL·6VS translocation line while not in common wheat, it was considered 6VS-specific marker. In

total, 120 6VS-specical markers were obtained with a success rate of 54.05% (Table S1). All IT markers were tested on three different translocation lines, NAU418, NAU419 and NAU1203, involving 6VS with different introgressed segments. The chromosome arm could be dissected into four bins: bin1 to bin4 (Fig 5), which contained 34, 11, 46 and 29 markers, respectively. Given that all three translocation lines are resistant, the resistant gene *Pm21* was mapped within bin3. The 40 markers within this physical bin are suitable for marker-assisted breeding.

Discussion

Aneuploidy germplasm facilitate flow-sorting target chromosomes or its arms

In order to characterize short arm of *H. villosa* chromosome 6V (6VS) at DNA level, we combined flow cytometric chromosome sorting and next generation DNA sequencing. When compared to whole genome sequencing, this approach provided a massive and lossless reduction of DNA sample complexity and facilitated DNA sequence analysis. A chromosome can be purified by flow sorting if it differs in relative DNA content from other chromosomes in a karyotype, which is not the case of chromosome 6V in *H. villosa*. Thus, we have sorted 6VS chromosome arm from *T. aestivum*–*H. villosa* 6VS ditelosomic addition line, where the telocentric chromosome 6VS is smaller than other chromosomes. With the aim to achieve high resolution of 6VS, we employed bivariate analysis of DNA content (DAPI fluorescence) and the amount of GAA microsatellites labelled by FITC following the FISHS protocol [40]. This approach permitted sorting 6VS arm at almost 90% purity.

The 6VS sequences would accelerate breeding program

H. villosa has been an important donor of disease resistance in wheat breeding, and *Pm21* transferred from *H. villosa* to wheat remains the most effective powdery mildew resistance gene [10]. Although *Pm21* has been cloned, its introduction by genetic transformation may not be acceptable by the market [41]. On the other hand, *Pm21* transferred from wheat-*H. villosa* translocation line T6AL·6VS, has been successfully utilized in wheat breeding, and more than 20 wheat varieties have been released in China [42]. Thus, the introgression of alien chromatin harboring traits of interest by chromosome engineering remains a priority. However, due to linkage drag, this strategy often introduces favorable traits together with deleterious loci, compromising yield and quality [43]. Thus, advanced chromosome engineering is needed to minimize alien chromatin during alien introgression breeding. The main procedures for reducing alien chromatin in wheat is to induce chromatin break-rejoining by ionizing radiation, or induce meiotic recombination between the alien chromatin and its homoeologous common wheat counterpart. In order to preserve beneficial genes and remove deleterious loci, it is important to know the location of beneficial and deleterious genes and define the size of introgressed chromatin.

Development specific molecular markers using chromosome sorting strategy

Development of molecular markers is now much easier than before due to falling costs of next-generation sequencing. As shown in this work, this is true also in species without genome sequence,

especially if a chromosome of interest can be purified by flow sorting. The sequences from alien chromosome could then be combined with available wheat genome sequence to develop molecular markers suitable for detecting alien chromatin. Tiwari et al. developed 2,178 5MgS-specific SNPs for *Ae. geniculata* by combining chromosome flow sorting and sequencing and highlighted the power of this approach for mining markers specific for alien chromatin [24]. Zhang et al. developed 1,624 intron targeting markers for all *H. villosa* chromosomes, except 4VS and 6VS arms, and out of them 841 (51.79%) markers were specific for tracing *H. villosa* chromatin in wheat background [22]. Wang et al. developed 359 intron targeting primers by combining chromosome sorting and sequencing, among which 232 (64.62%) can be used to trace the 4VS chromatin in the wheat background [23]. In this study, with the availability of the 6VS sequence, we designed 222 IT primer pairs and 120 (54.05%) were proved to be 6VS specific. Apart from improving the knowledge of genome structure of an important donor of genes in wheat improvement and development of markers to support its use in alien introgression breeding of wheat, the results of this work confirm that chromosome sorting combined with next-generation sequencing is an efficient strategy for IT marker development.

Conclusions

Here, we report a draft DNA sequence of *H. villosa* chromosome arm 6VS and annotation of high-confidence of 3,276 genes. The coding genes showed a fine synteny with Triticeae group 6 chromosomes. A total of 120 IT markers specific to 6VS were developed and used to identify 6VS chromatin in three alien introgression lines. The results and resources developed will support further analysis of molecular organization of 6VS and accelerate its utilization in improvement of bread wheat.

Methods

Plant materials

H. villosa (VV, 2n=14, Accession No. 91C43) was obtained from Cambridge Botanical Garden, UK. The *T. aestivum* - *H. villosa* ditelosomic addition line Dt6VS [2n=42(AABBDD) + 2t(6VS)] (Accession No. NAU1202), three *T. aestivum* - *H. villosa* small fragment translocation lines (Accession No. NAU418, NAU419 and NAU1203), and *T. aestivum* - *H. villosa* T6VS·6AL translocation line 92R137 (Accession No. NAU405) were developed at the Cytogenetics Institute, Nanjing Agricultural University (CINAU, hereafter). Common wheat (*T. aestivum*, AABBDD) cv. Chinese Spring maintained at CINAU was used as a control in this work.

Chromosome sorting and DNA sequencing

Suspensions of mitotic metaphase chromosomes were prepared from synchronized meristem root tips of young seedlings according to Vrána et al. and Kubaláková et al. [44, 45]. GAA microsatellite repeats on isolated chromosomes were fluorescently labelled by FISHIS [40] using GAA-fluorescein isothiocyanate (FITC) conjugate (Sigma, Saint Louis, USA) and counterstained by DAPI (4',6-diamidino 2-phenylindole)

at 2 µg/ml. The samples were analyzed by FACSaria II SORP flow cytometer and sorter (Becton Dickinson Immunocytometry Systems, San José, USA) at rates of 2000-3000 particles per second and sort windows were set on bivariate flow karyotypes FITC vs. DAPI fluorescence. The identity of sorted particles and contamination of sorted fractions by other chromosomes were determined following Kubaláková et al. [46]. Briefly, one thousand particles were sorted from each sample into a 7 µl drop of P5 buffer on a microscope slide. After air-drying, the slides were used for FISH with probes for pSc119.2 and Afa family repetitive DNA sequences and evaluated by fluorescence microscopy.

Chromosomes were sorted at rates of 15 - 20 / sec into 40 µl sterile deionized water in 0.5 ml PCR tubes and two different 6VS DNA samples were prepared and sequenced. The first was produced by multiple displacement amplification (MDA) of DNA prepared from two batches of 100,000 copies of 6VS telosomes. The amplification was done using Illustra GenomiPhi V2 DNA Amplification Kit (GE Healthcare, Piscataway, USA) as described by Šimková et al. [47] and the two MDA products were pooled into one sample to reduce amplification bias. Two micrograms of amplified DNA were used to prepare sequencing library using TruSeq DNA PCR-Free Library Prep Kit (Illumina, San Diego, USA). The library was sequenced in one run on Illumina MiSeq System (1000 bp insert, 2 x 300 bp) yielding 14 Gb sequence data (~44x coverage of 6VS). The second type of 6VS DNA sample was not amplified and DNA from 100,000 copies of 6VS telosome was purified and directly used to prepare sequencing library using Nextera DNA Library Prep Kit (Illumina). The library was sequenced in one run on Illumina MiSeq System (500 bp insert, 2 x 300 bp) yielding 10.2 Gb sequence data (~32x coverage of 6VS). The sequenced reads data of this research were available in NCBI (PRJNA590539). Four k-mer sizes (41, 45, 49, and 63) were used to de novo assemble the raw data using the software of Hecate (<http://bgi-international.com/us/>, unpublished). The k-mer sizes which generated the assembly with the best sequence coverage and N50 size were finally selected.

Identification of DNA repeats

The repetitive DNA regions of 6VS assembled sequence was identified and masked using the software of RepeatMasker (<http://www.repeatmasker.org/>). Two repeat libraries, TREP database and Repbase Update, were used to search the repetitive sequences of the 6VS with the default settings.

Prediction of coding gene across 6VS sequence

The gene prediction of repeat-masked 6VS sequences was performed through AUGUSTUS program. The transcriptome data of *H. villosa*, which containing 204,258 unigenes, was used to provide the evidence of the loci with coding genes. The predicted genes were blastn against the transcriptome data to define the evidenced gene, with more than 95% identity and at least 300bp coverage on a unigene of transcriptome. For GO analysis of predicted genes, Blast2GO and WEGO software were performed to get GO annotation and GO functional classification, respectively.

Transcriptome data

6VS/6AL translocation line grown in a growth chamber with 20°C/16°C (day/night), 16 h/8 h (light/dark). The translocation line was inoculated with *Bgt* isolates E26 and E31 at two-leaf stage, respectively, and inoculated water as control. RNAs were isolated at 1 h, 8 h, 18 h and 24 h after *Bgt* and water inoculation, respectively, followed by freezing in liquid nitrogen for subsequent RNA extraction. The samples were submitted to the BGI for sequencing using the Illumina HiSeq 4000 platform. After sequencing, Trinity was used to de novo assembly of clean reads. In the transcript three-level classification of Trinity results, taking the longest transcript of gene level as unigenes and then were Blast to NT, NR, COG, KEGG and SwissProt for annotation. Bowtie2 to compare clean reads to unigenes. Then, using the RSEM to calculate the expression level of each sample. *Bgt* isolates E26 and E31 were collected from Institute of Plant Protection, Chinese Academy of Agricultural Sciences.

Development of Intron Target markers

Firstly, we extracted the annotated coding sequence (CDS) of the unigenes from the two gene databases of *Ae. tauschii* chromosome 6DS and *T. aestivum* chromosome 6DS. Then, all genes were compared with the genomic sequences of Chinese Spring short arm chromosomes of group 6 and *H. villosa* 6VS through BLASTn program. The genes which has homologous copy and predicted at least one intron among 6DS, 6BS, 6AS and 6VS chromosomes were selected. Thirdly, we determined and compared the intron sizes of selected genes, and chose the target introns to design the primer pairs with predicted amplification sizes in 6VS differed from 6DS, 6BS and 6AS simultaneously at least 10%. Primer 3 (<http://frodo.wi.mit.edu/primer3/>) was used to design primers in the exons which flanking the target introns.

Abbreviations

DAPI: 4', 6-diamidino-2-phenylindole; FITC: fluorescein isothiocyanate; MDA: multiple displacement amplification; GO: gene ontology; NR: non-redundant protein sequence database; NT: nucleotide sequence database; COG: clusters of orthologous groups; KEGG: kyoto encyclopedia of genes and genomes; FISH: fluorescence in situ hybridization; TREP: triticeae repeat sequence database; LTR: long terminal repeat; TGW: thousand grain weight; IT: intron targeting; SNP: single nucleotide polymorphism; SINE: short interspersed nuclear elements; TIR: terminal inverted repeat. CDS: coding sequence

Declarations

Authors' Contributions

WXE and JD conceived, designed and coordinated the work; JV and JD flow-sorted telosome 6VS, determined the purity in flow-sorted fractions and amplified chromosomal DNA; KH sequenced amplified chromosomal DNA; XJ, WWT, JD, and WHY wrote the manuscript; WWT, LML, YZY and LJ performed experiments; XJ, WWT, ZX, and WYF analyzed the data. and all authors have read and approved the final manuscript.

Availability of data and materials

The sequence read data of 6VS chromosome was deposited in the (NCBI) Sequence Read Archive (SRA) and is available under accession number PRJNA590539.

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Ethics approval and consent to participate

Not applicable.

Consent for publication

Not applicable.

Competing interests

The authors declare that they have no competing interests.

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Tables

Table 1 The statistics of assembly of flow-sorted short arm of *H. villosa* 6V chromosome

Total bases (Gbp)	47.7
Number of assembly scaffolds	153,177
Total assembly bases (bp)	230,388,792
Max. length of assembly scaffolds (bp)	138,620
Min. length of assembly scaffolds (bp)	100
N50 (bp)	9,788
Mean length (bp)	1,464
GC-content (%)	45.68

Table 2 Identification of repetitive DNA elements in short arm of *H. villosa* 6V chromosome

Type	Sub-type	Total length (bp)	% genome
DNA transposon	TIR	11,269,751	6.53
	Helitron	189,843	0.11
retrotransposon	LTR_Copia	18,656,357	10.81
	LTR_Gypsy	89,588,481	51.91
	LTR_Unknown	3,831,370	2.22
	SINE	1,691,326	0.98
	Unknown	4,694,291	2.72
tandem repeat		535,011	0.31
unknown		4,694,291	2.72

Additional File Legends

Additional file 1: Fig. S1 (A) The length distribution of predicted genes of 6VS; (B) Percent distribution of the GO entries for *H. villosa* 6VS genes. The most represented entries within the three ontologies (Molecular function, Biological process and Cellular component) are indicated.

Additional file 2: Table S1 Markers specific for short arm of *H. villosa* 6V chromosome

Figures

Figure 1

Flow sorting of *H. villosa* chromosome arm 6VS Bivariate flow karyotype obtained after the analysis chromosomes isolated from bread wheat-Haynaldia villosa 6VS ditelosomic addition line. Prior to analysis, GAA microsatellite repeats on chromosomes were labelled by fluorescein isothiocyanate (FITC) and counterstained by 4',6-diamidino 2-phenylindole (DAPI). The population representing 6VS telosome

is marked by red rectangle. The inset shows examples of flow-sorted 6VS telosomes after fluorescence in situ hybridization (FISH) with probes for pSc119.2 (yellow-green) and Afa family repeats (red).

Figure 2

Comparative analysis of 6VS DNA sequence The distinguish of 6VS homologous genes in group 6 of Triticeae genome. The blue lines represent the homologous genes of 6VS located on the chromosomes of

Triticeae genome. Ta, Td, At, Tu and Hv represent *T. aestivum*, *T. dicoccoides*, *Ae. tauschii*, *T. urartu* and *H. vulgare*, respectively. The blue arrows represent the position of centromere of common wheat.

Figure 3

Gene expression patterns in response to powdery mildew infection and gene structures of the resistance gene analogs (RGAs) from 6VS of *H. villosa*. Left: The heatmap for the expression of predicted RGAs that treated with two Bgt isolates, E26 and E31, respectively at 3 and 24 hours after inoculation. Right: The structure of resistance gene analogs which predicted from 6VS of *H. villosa*. Different colors represent different domains. The scale represents the length of amino acids of genes.

HvGW2-6V-promotor	AAG ⁻⁹⁸⁸ GCG.....CCCGTGCAG ⁻⁷³⁹ TAGCT.....TCG ⁻⁵⁹³ GAC.....CC ⁻⁴⁹⁴ GCGG	High TGW
TaGW2-6A-promotor Hap_1	AAG ⁻⁹⁸⁸ GCG.....CCCGTACAG ⁻⁷³⁹ CAGCT.....TCG ⁻⁵⁹³ GAC.....CC ⁻⁴⁹⁴ GCGG	Low TGW
TaGW2-6A-promotor Hap_2	AAG ⁻⁹⁸⁸ GCG.....CCCGTACAG ⁻⁷³⁹ CAGCT.....TCG ⁻⁵⁹³ AAC.....CC ⁻⁴⁹⁴ GCGG	Low TGW
TaGW2-6A-promotor Hap_3	AAG ⁻⁹⁸⁸ GCG.....CCCGTACA ⁻⁷³⁹ ACAGCT.....TCG ⁻⁵⁹³ GAC.....CC ⁻⁴⁹⁴ GCGG	Low TGW
TaGW2-6A-promotor Hap_4	AAG ⁻⁹⁸⁸ ACG.....CCCGTACAG ⁻⁷³⁹ CAGCT.....TCG ⁻⁵⁹³ AAC.....CC ⁻⁴⁹⁴ GCGG	Low TGW
TaGW2-6A-promotor Hap_5	AAG ⁻⁹⁸⁸ GCG.....CCCGTACA ⁻⁷³⁹ ACAGCT.....TCG ⁻⁵⁹³ GAC.....CC ⁻⁴⁹⁴ ACGG	High TGW

Figure 4

The SNPs at the promotor region of TaGW2-6A and HvGW2-6V Characters that highlighted with red color were the SNPs that reported association with thousands of grain weight (TGW) within the promotor region of TaGW2-6A and HvGW2-6V. 'CGCG' motif was underlined.

Figure 5

Development of IT markers and construction of cytogenetic physical map of *H. villosa* 6VS chromosome arm Left: Molecular markers in each bin Right: The amplification results of representative marker in each bin which was underlined in the left 1: *T. aestivum* (AABBDD); 2: *H. villosa* (VV); 3: *T. aestivum*-*H. villosa* translocation line (T6VS·6AL); 4-6: Three *T. aestivum*-*H. villosa* small fragment translocation lines (NAU418, NAU419 and NAU1203); 7: DNA Ladder

Supplementary Files

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