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ORIGINAL ARTICLE

Characterization of a *Triticum aestivum–Dasypyrum villosum* T2VS·2DL translocation line expressing a longer spike and more kernels traits

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Abstract

Key message **By using 2V-specific EST–PCR markers and sequential GISH/FISH analysis, we identified four homozygous CS–2V translocation lines, including a novel compensating T2VS·2DL translocation line NAU422. This translocation line has longer spikes and produces more grains per spike than its recurrent parent CS and three other translocation lines, which could be a valuable resource in wheat yield improvement.**

Abstract *Dasypyrum villosum* ($2n = 14$, VV), the wild relative of wheat, possesses novel and superior alleles at many important loci and should be utilized to improve the genetic diversity of cultivated wheat and may be very helpful for the improvement of wheat yield. In this study, four homozygous Chinese Spring (CS)–*D. villosum* translocation lines containing different fragments of chromosome 2V were characterized from a pool, including 76 translocations that occur in chromosomes 1 V through 7 V of *D. villosum* by both molecular markers and cytogenetic analysis. A rough physical map of 2V was developed which included nine markers in three segments of the short arm and ten markers in the long arm. The photoperiod response gene of *D. villosum* (*Ppd-V1*) was physically mapped to the FL 0.33–0.53 region of 2VS, while the gene controlling bristles on the glume ridges (*Bgr-V1*) was mapped to 2VS FL 0.00–0.33. A novel compensating *Triticum aestivum–D. villosum* Robertsonian translocation line T2VS·2DL (NAU422) with good plant vigor and full fertility was further characterized by sequential genomic in situ hybridization and fluorescent in situ hybridization and the use of molecular markers. Compared to its recurrent parent CS and three other translocation lines, the T2VS·2DL translocation line has longer spikes, more spikelets and more grains per spike in two season years, which suggested that the alien segment may carry yield-related genes of *D. villosum*. The developed T2VS·2DL translocation line with its morphological and co-dominant molecular markers could be utilized as a novel germplasm for high-yield wheat breeding.

Introduction

Wheat, a major cereal crop produced worldwide, has been intensively bred over the past decades. Similar to other crops, the genetic diversity of cultivated wheat has been greatly eroded by the frequent use of the same parental genotypes for breeding cultivars. A fundamental method of increasing wheat yield would be to widen its narrow genetic background. The kernel number per spike is the most important factor of the many potential characteristics that determine wheat yield (Zhou et al. 2007; Zheng et al. 2011). Future yield increases may depend on the identification and exploration of novel genetic resources at specific loci. The wild relatives of wheat possess many novel and superior alleles at important loci and should be explored in depth and fully utilized (Zhong and Qualset 1993; Wu et al. 2006; Qi et al. 2010; Zhang et al. 2014).

In wheat, the introgression of alien genes from other *Triticeae* species to improve agronomic traits is possible, as

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in the case of the 1BL·1RS translocation line (Lukaszewski 2001). Although most of the major disease resistance genes on 1RS have now been overcome by virulent pathotypes, breeders in China still use this translocation line as a parent because of its positive effect on kernel numbers (Zhou et al. 2007; Zheng et al. 2011). A novel *Triticum aestivum–Thinopyrum bessarabicum* translocation line T2JS-2BS·2BL, which carries the segment of *Th. bessarabicum* chromosome arm 2JS, has been verified to positively affect grain yield (Qi et al. 2010). Wu et al. (2006) reported that gene(s) controlling the high number of florets and kernels per spike are located on chromosome 6P of the *T. aestivum–Agropyron cristatum* addition and substitution lines. In addition, Yang et al. (2010) produced the *T. aestivum–A. cristatum* 6P translocation and introgression lines, which had transferred the chromosome 6P fragment taking the multi-kernel gene(s) along into a wheat background and may improve kernel number, thus increasing crop yield. Du et al. (2013) found that gene(s) controlling the twin spikelets per spike of the *T. aestivum–Psathyrostachys huashanica* progeny line were located on the 6Ns chromosome and were amenable to the possibility of increasing the wheat yield based on this enlarged gene pool.

Dasypyrum villosum ($2n = 14$, genomes VV), a diploid wild relative of bread wheat possesses favorable genes for disease resistance, salt and drought tolerance and quality traits. It also has many high-yield characteristics, e.g., more tillers and more florets (reviewed by Gradzielewska 2006). The chromatins of several *D. villosum* accessions have been introgressed into wheat (the different V genome numbered by De Pace et al. 2011) and some alien genes have been transferred from *D. villosum* by Robertsonian translocation lines, which include powdery mildew resistance gene *Pm21* (Chen et al. 1995), wheat spindle streak mosaic virus resistance gene *Wss1* (Zhang et al. 2005), stem rust resistance gene *Sr52* (Qi et al. 2011), grain softness genes (Zhang et al. 2010) and seed storage protein genes (Zhang et al. 2014; Zhao et al. 2010; Vaccino et al. 2010). However, the yield-related genes transferred from *D. villosum* is not comprehensively researched. Liu et al. (2011) developed a set of compensating *T. aestivum–D. villosum* (V#3) Robertsonian translocation lines, with the exception of the 2VS and 5VL arms. Among the various transfers, the T6AL·6VS translocation line carrying *Pm21* has been exploited in production agriculture (Li et al. 2007). In the past decades, several wheat–*D. villosum* (V#4) disomic addition lines and substitution lines have been produced in our laboratory. A morphological marker, the long bristles on the glume ridges produced by the gene(s) located on chromosome 2V (Chen et al. 2008; Li et al. 2009), makes finding this chromosome easy among the genetic stocks. The agronomic evaluation of the genetic shocks showed that the 2V substitution line DS2V (2D) and addition line DA2V all have the longer spikes and higher kernel number per spike when compared to other lines, indicating that the production of *T. aestivum–D. villosum* 2V translocation lines may be beneficial for the improvement in kernel number, thus increasing wheat yield.

The purposes of this work were to produce *T. aestivum–D. villosum* 2V translocation lines with the characteristics of multi-kernel and to stabilize their inheritance in a wheat background. The gene(s) controlling the bristles on the glume ridges (*Bgr-V1*) and the photoperiod response gene (*Ppd-V1*) of *D. villosum* were physically mapped to a small segment of 2VS. The evaluation of agronomic traits showed that the newly created translocation line containing morphological markers could provide a novel germplasm for high-yield wheat breeding.

Materials and methods

Plant materials

The plant materials used in this study included *T. aestivum* cv. Chinese Spring (CS), *D. villosum* accession GP005, introduced from the Cambridge Botanical Garden, UK (which is the donor of the V chromatins in the present study; its chromosomes were numbered #4 by De Pace et al. 2011), *T. durum* cv. 1286 (AABB), *T. durum* cv. 1286–*D. villosum* GP005 amphiploid (AABBVV), a set of *T. aestivum–D. villosum* disomic addition lines, a disomic substitution line DS2V (2D), and two telosomic addition lines 2VL and 2VS. These cytogenetic materials were all developed and maintained at the Cytogenetic Institute, Nanjing Agricultural University (CINAU), China. To identify and locate markers specific for wheat group 2 chromosomes and *D. villosum* chromosome 2V, the CS nulli-tetrasomic (NT) lines N2AT2D, N2BT2D and N2DT2B, which were kindly provided by Dr. BS Gill, were also used in this study.

To produce wheat–*D. villosum* translocations, the *T. durum–D. villosum* amphiploid (AABBV) pollen was irradiated by ^{60}CO-γ ray and pollinated to the CS genetic background (Bie et al. 2007). Seventy-six translocation lines involving 1 V–7 V chromosomes were identified in the progeny by genomic in situ hybridization (GISH) and backcrossed three to four times, using CS as a recurrent parent (Cao et al. 2009a).

Cytogenetic analysis

The procedure of chromosome in situ hybridization described by Zhang et al. (2014) was followed. Sequential GISH and fluorescence in situ hybridization (FISH) was employed using total genomic DNA of *D. villosum* and

the repetitive DNA clone *pAs1* as probes, respectively. The genomic DNA of *D. villosum* was labeled with fluorescein-12-dUTP. The clone *pAs1* from *Aegilops tauschii* Coss. was used to detect most of the D-genome chromosomes (Mukai et al. 1993) and was labeled with digox-igenin-11-dUTP (Roche Diagnostics GmbH, Germany). After hybridization with the probes, the chromosomes were visualized under an Olympus BX60 fluorescence microscope and photographed with a SPOT Cooled Color Digital Camera.

EST selection and STS primer design

A sample of 211 EST-based primers from different regions of each arm of the wheat group 2 chromosomes (http://wheat.pw.usda.gov/cgi-bin/westsql/map_locus.cgi) was used to select the 2V-specific markers. The primers were designed using the software Primer 3 (http://frodo.wi.mit.edu). Five previously reported 2V-specific EST–PCR markers (CINAU33, CINAU34, CINAU35, CINAU36 and CINAU37) were also used to screen 2V chromosome segments (Cao et al. 2009b) (Table 1). The *XHvF11* marker (Turner et al. 2005) specific for the barley *Ppd-H1* gene was used to evaluate the photoperiod response allele of *D. villosum*.

Genomic DNA was isolated from young leaves with the DNAsecure Plant Kit (TIANGEN BIOTECH CO., LTD, Beijing, China), according to manufacturer's instructions. PCR reactions were conducted in a 10-μL system containing 25 ng of template DNA, 10 mM of each primer, 2.5 mM of each dNTP, 2.5 mM $MgCl_2$ and 0.08 U of *Taq* polymerase (TaKaRa). The samples were denaturated at 94 °C for 3 min and subjected to 32 cycles of the following: 30 s of denaturation at 94 °C, 45 s at 50–58 °C, according to the different primers (Table 1), and a 1.2-min elongation at 72 °C. A final cycle with an extension of 10 min at 72 °C was applied to complete the reactions. The PCR products were separated on 8 % polyacrylamide gels and visualized by silver staining.

Evaluation of agronomic traits

Four CS–2V translocation lines with their recurrent parent CS were planted in greenhouses at the experimental farm of Nanjing Agricultural University during the 2013–2014 and 2014–2015 sowing seasons. The five entries were planted in four rows and repeated three times with spacing in 25 cm rows, 1.0 m row lengths and 10 plants per row. Plant height (PH, cm), spike length (SL, cm), spikelet per spike (SS), grains per spike (GPS) and 1,000-kernel weight (TKW, g) were evaluated for each entry and plot. Analysis of variance and multiple comparisons among entries were performed using the SAS 8.2 system.

Results

Development of chromosome 2V arm-specific STS markers

Fourteen out of the 211 STS primer pairs (6.6 %) displayed polymorphism among CS, *T. durum* cv. 1286 (AABB), *T. durum* cv. 1286–*D. villosum* amphiploid (AABBV) and 2V chromosome disomic addition lines. When the primer pairs were used to amplify DNA from the telosomic addition lines DT2VL and DT2VS, it was possible to assign nine of them (CINAU182, CINAU183, CINAU184, CINAU185, CINAU186, CINAU187, CINAU188, CINAU189 and CINAU190) to 2VS and five of them (CINAU191, CINAU192, CINAU193, CINAU194 and CINAU195) to 2VL (Table 1). The five previously reported 2V-specific markers were all located on 2VL. These 19 EST–STS loci covering the short and long arms of 2V chromosome were all used to detect the 2V alien segments present in the translocations pool.

Identification of 2V introgression lines

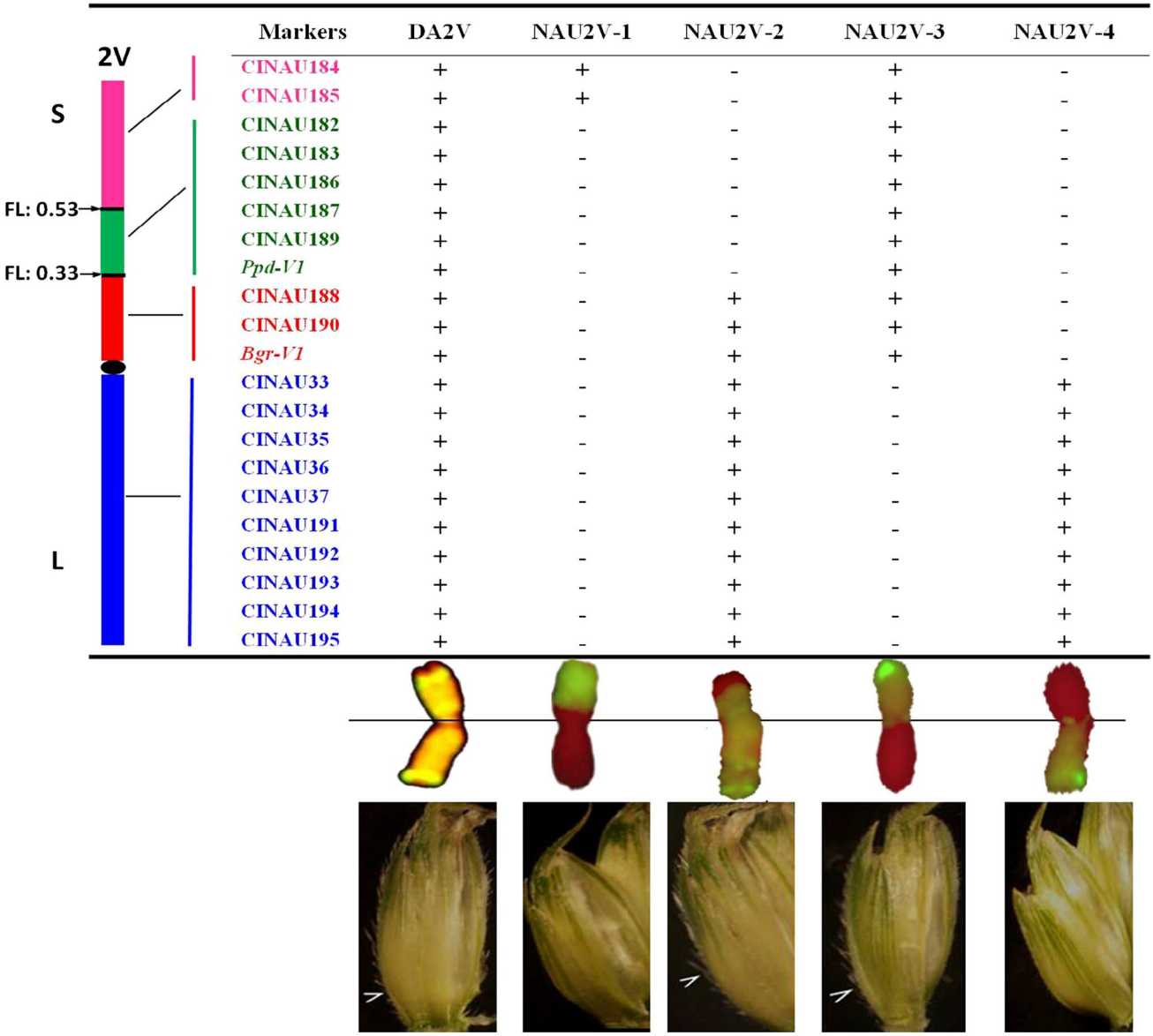
The 19 EST–STS molecular markers and mitotic and meiotic GISH analysis allowed for the identification of 4 CS–2V introgression lines with different alien-fragment sizes from 76 introgression lines of the translocation pool (Table 2; Figs. 1, 2). The results showed that NAU2V-1 was a small alien-fragment terminal translocation with the breakpoint at FL 0.53. The alien segment was the terminal part of 2VS and the diagnostic bands of two 2VS-specific molecular markers (CINAU184 and CINAU185) were present, while NAU2V-2 was a large-fragment translocation with the breakpoint at FL 0.33, involving all of 2VL and a part of 2VS. Ten 2VL- and two 2VS-specific molecular markers were present in NAU2V-2. The diagnostic bands of nine 2VS-specific molecular markers were present in NAU2V-3 while the diagnostic bands for ten 2VL-specific molecular markers were present in NAU2V-4; these results indicated that NAU2V-3 and NAU2V-4 were all homozygous whole-arm translocations, having a pair of 2VS·W and 2VL·W, respectively. The sequential GISH/FISH analysis showed that the *pAs1* patterns were present in the translocated chromosome of the NAU2V-3 line (Fig. 2i, j). The standard FISH pattern for *pAs1* established for the wheat variety CS (Mukai et al. 1993) was compared with the *pAs1* pattern of NAU2V-3. It was found that the translocation involved wheat chromosome arm 2DL. Hence, NAU2V-3 was a compensation whole-arm translocation and was designated T2VS·2DL (Permanent No. NAU422).

Table 1 Sequence of 2V-specific EST–STS markers

Marker	EST location	Arm location on 2V	T_m (°C)	Primer sequence 5′–3′
HVF11	2HS	2VS	50	Forward: ATCGAATCACCCGTTTCAATC
				Reverse: GACACCATCAGAGATAGTAAC
CINAU182	2BS 0.00–0.53	2VS	55	Forward: GAGCTCGACCAGTACAAGAC
				Reverse: AGATCGTTCCTCTTCCTCTC
CINAU183	2BS 0.00–0.53	2VS	55	Forward: CGGGAGGAGTAGCTCTTAAT
				Reverse: ACTTCAGGTCAGCCTCTGTA
CINAU184	2AS 0.00–0.78	2VS	58	Forward: AGAAACTGGTGCTCAACCTA
				Reverse: AACTTTTGCTTCTCATCTCG
CINAU185	2AS 0.00–0.78	2VS	55	Forward: GTCATCCTGTTCGTCATGTT
				Reverse: GTAGAGGAGTGGCACACAAT
CINAU186	2AS 0.00–0.78	2VS	54	Forward: CATGGAGCTGTTCCCGCAGAG
				Reverse: CCAGCACCTTCCCGCCATAGA
CINAU187	2AS 0.00–0.78	2VS	55	Forward: GTGTCGAAGTACGGGTTCCA
				Reverse: TCCTTGAGGAACTGCTCGAT
CINAU188	2DS 0.00–0.33	2VS	56	Forward: GTTCCGCAAGTTCAGCTACC
				Reverse: CAGCAGGAGCATCCTTTTTC
CINAU189	2AS 0.00–0.78	2VS	55	Forward: AGATCGAGCGCCTCATGC
				Reverse: CCTTCTCGCACAGTGCTCTA
CINAU190	2DS 0.00–0.33	2VS	55	Forward: CCGAGCGTGAGAGAGGTATC
				Reverse: CAGTCAAGGTTGGTGGACCT
CINAU33	2DL 0.49–0.76	2VL	55	Forward: GACCCAAGAGGCGTTGATTA
				Reverse: CATGTGTGCCAAATTCAAGC
CINAU34	2DL 0.76–1.00	2VL	55	Forward: CATGATGCTGTAGGGCTGAA
				Reverse: TCGCTGACAAGGCATTACAC
CINAU35	2DL 0.76–1.00	2VL	55	Forward: AAGTGGAGCACCTGGATTTG
				Reverse: ATGTGACCAGCTCCTTCGAT
CINAU36	2DL 0.76–1.00	2VL	55	Forward: ATTTGGATGAGGCAAAGGTG
				Reverse: TCTGCTGGTCCTCTGATGTG
CINAU37	2DL 0.76–1.00	2VL	55	Forward: AGATTGGAAGCATGGTTTGC
				Reverse: ACAGGGTCGTAGCCATTCAC
CINAU191	2DL 0.76–1.00	2VL	55	Forward: GACAAGAAGATCGAGCAGAT
				Reverse: ACATGACGTAGAGGAACCAC
CINAU192	–	2VL	55	Forward: AAGCACAAGGCCGTCGTC
				Reverse: AGACGCCGCGGGAGTAGT
CINAU193	–	2VL	55	Forward: CTGACCTGCGTCTGGATTTT
				Reverse: CACACCTGCCAGTCGTACAG
CINAU194	–	2VL	57	Forward: TGGAACCACACATCTGGAAA
				Reverse: GGCAGTCCATTCACCATCTT
CINAU195	–	2VL	58	Forward: CCGCCAAGACCTTCCACGAC
				Reverse: CCGCAGAGAACAGGCAACGA

Because genes controlling photoperiod response in wheat (*Ppd-D1*, *Ppd-B1*, *Ppd-A1*) and barley (*Ppd-H1*) were located on chromosomes 2D, 2B, 2A and 2H, respectively, the *XHvF11* marker specific for the barley *Ppd-H1* gene was used to evaluate the translocation lines. The results showed that a diagnostic *XHvF11* marker band was present in lines DA2V4, DT2VS and NAU2V-3 (Fig. 1c), but absent in NAU2V-1, NAU2V-2 and NAU2V-4, indicating that a photoperiod response allele of *D. villosum* (designed as *Ppd-V1*) was located on 2VS bin FL 0.33–0.53 (Table 1; Fig. 1c). However, the photoperiod response allele *Ppd-D1* was absent in NAU2V-3 (Fig. 1c) and 2DS-specific bands for other molecular markers, such as CINAU184 (Fig. 1b), were also absent, which further

Table 2 Molecular analysis of wheat–2V introgression lines with their different alien chromatin and the physical location of *Ppd-V1* and *Bgr-V1* loci



Markers	DA2V	NAU2V-1	NAU2V-2	NAU2V-3	NAU2V-4
CINAU184	+	+	-	+	-
CINAU185	+	+	-	+	-
CINAU182	+	-	-	+	-
CINAU183	+	-	-	+	-
CINAU186	+	-	-	+	-
CINAU187	+	-	-	+	-
CINAU189	+	-	-	+	-
Ppd-V1	+	-	-	+	-
CINAU188	+	-	+	+	-
CINAU190	+	-	+	+	-
Bgr-V1	+	-	+	+	-
CINAU33	+	-	+	-	+
CINAU34	+	-	+	-	+
CINAU35	+	-	+	-	+
CINAU36	+	-	+	-	+
CINAU37	+	-	+	-	+
CINAU191	+	-	+	-	+
CINAU192	+	-	+	-	+
CINAU193	+	-	+	-	+
CINAU194	+	-	+	-	+
CINAU195	+	-	+	-	+

'+' indicates the presence of the marker loci, and '−' the absence
Yellowish-green, the chromatin of 2V; red, the chromatin of wheat
Arrowhead shows bristles on the glume ridges

confirmed that the translocated chromosome in NAU2V-3 was T2VS·2DL.

Agronomic traits evaluation

The presence of long bristles on glume ridges is a typical characteristic of *D. villosum* (De Pace et al. 2011). The gene(s) determining this phenotype were located on chromosome 2V and are dominantly expressed in wheat genetic backgrounds (Chen et al. 2008, Li et al. 2009). The long bristles was present on glume ridges in the NAU2V-2 and NAU2V-3 lines and absent in NAU2V-1 and NAU2V-4, confirming that the gene locus (designed *Bgr-V1*) controlling the bristle-length trait was located on 2VS bin FL 0.00–0.33 (Table 2). The results of other agronomic traits controlled by putative genes in the chromosome 2V translocation lines are shown in Table 3. In the 2 years, there were no obvious difference among the four translocation lines and their recipient parent CS in plant height ($P > 0.05$) and TKW ($P > 0.05$). The heading dates of the four lines were in the range of 179–185 days in two growing

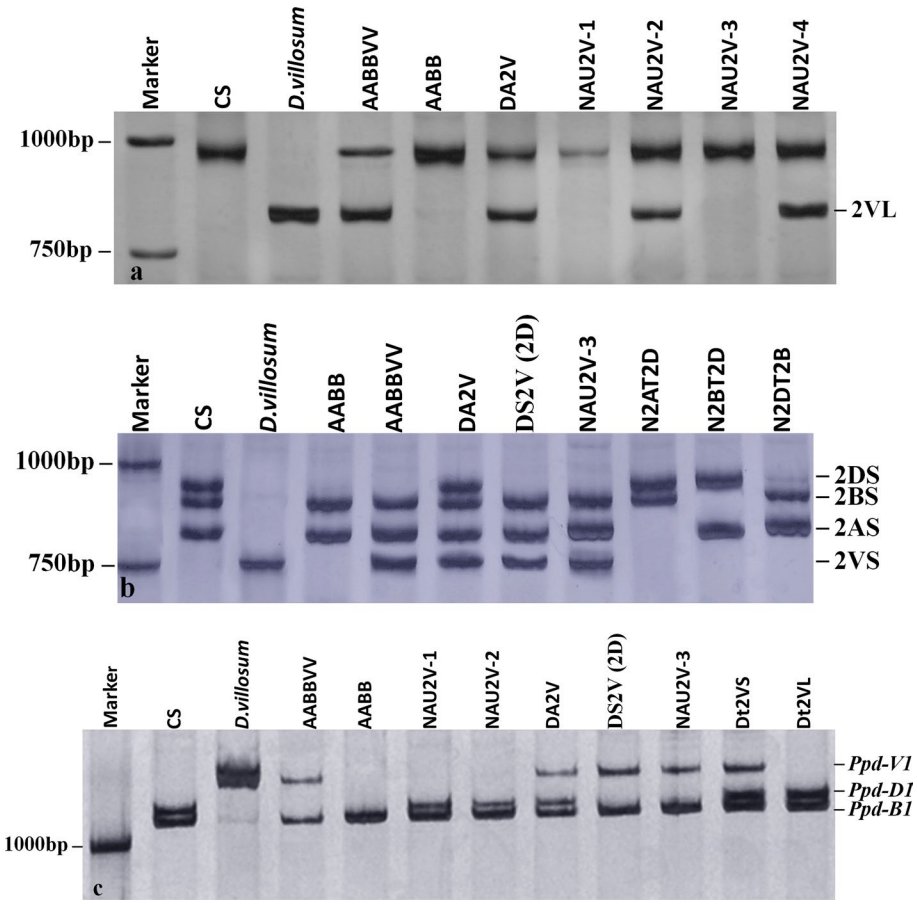


Fig. 1 Electrophoresis patterns of 2V-specific EST–PCR markers. The *straight line* indicates the fragment which can be assigned to a certain chromosome. **a** 2VL-specific fragment amplified by the CINAU194 primer was present in NAU2V-2 and NAU2V-4. **b** 2VS-specific fragment amplified by CINAU184 primer was present in NAU2V-3, while the 2DS-specific fragment was absent in substitution line DS2V (2D), N2DT2B and NAU2V-3. **c** The *Ppd-V1* specific fragment amplified by the Xhvf11 primer was present in NAU2V-3 and telosomic addition line DT2VS, while the *Ppd-D1* fragment was absent in substitution line DS2V (2D) and NAU2V-3

seasons. NAU2V-2 was present 4–5 days later and others were essentially in line with that of CS. However, there were significant differences in spike length, spikelets per spike and GPS. The average values of spike length, spikelets per spike and GPS of NAU2V-3 were all significantly higher than those of CS, NAU2V-1, NAU2V-2 and NAU2V-4. However, the mean values of spike length, spikelets per spike and GPS of NAU2V-1 were all significantly lower than those of CS, except for spikelets per spike in 2013–2014. By comparison, the mean values for spike length, spikelets per spike and GPS of NAU2V-2 were not obviously different from the mean values for the same traits measured in CS except for spikelets per spike in 2014–2015. There were no obvious differences between NAU2V-4 and CS for spike length, spikelets per spike or GPS measured in 2013–2014, but these traits were significantly lower in 2014–2015 except for GPS. These results implied that the alien segment in 2VS FL 0.33–0.53 may control the characteristics of longer spikes and multi-kernel. Therefore, the T2VS·2DL (NAU2V-3) translocation line could be a novel germplasm with high yield potential.

Discussion

Introducing alien genes from the tertiary gene pool has been proven to be an effective way of increasing genetic diversity and improving cultivated wheat (Friebe et al. 1996). As the important tertiary gene pool of common wheat, the V chromatins originating from seven different *D. villosum* accessions have been introgressed into wheat (De Pace et al. 2011). In addition, the beneficial alien genes located on chromosome 1 V, 4 V, 5 V and 6 V were transferred into common wheat (Chen et al. 1995; Zhang et al. 2005, 2010, 2014; Zhao et al. 2010; Qi et al. 2011). In this study, we identified and characterized four translocation lines that contained *D. villosum* chromatin from chromosome 2V in CS genetic backgrounds. The substitution line DS2V (2D) obtained from crossing (*T. turgidum* cv. γ-80 × *D. villosum* GP005) F1 to *T. aestivum* Yangmai5 (Liu et al. 1988) has a different genetic background than T2VS·2DL translocation line NAU422. However, agronomical evaluation showed that the two lines all presented with longer spikes and more kernels (Fig. 3). Accordingly, the chromosome arm 2VS of *D. villosum* most likely has alleles for increasing longer spikes and higher kernel

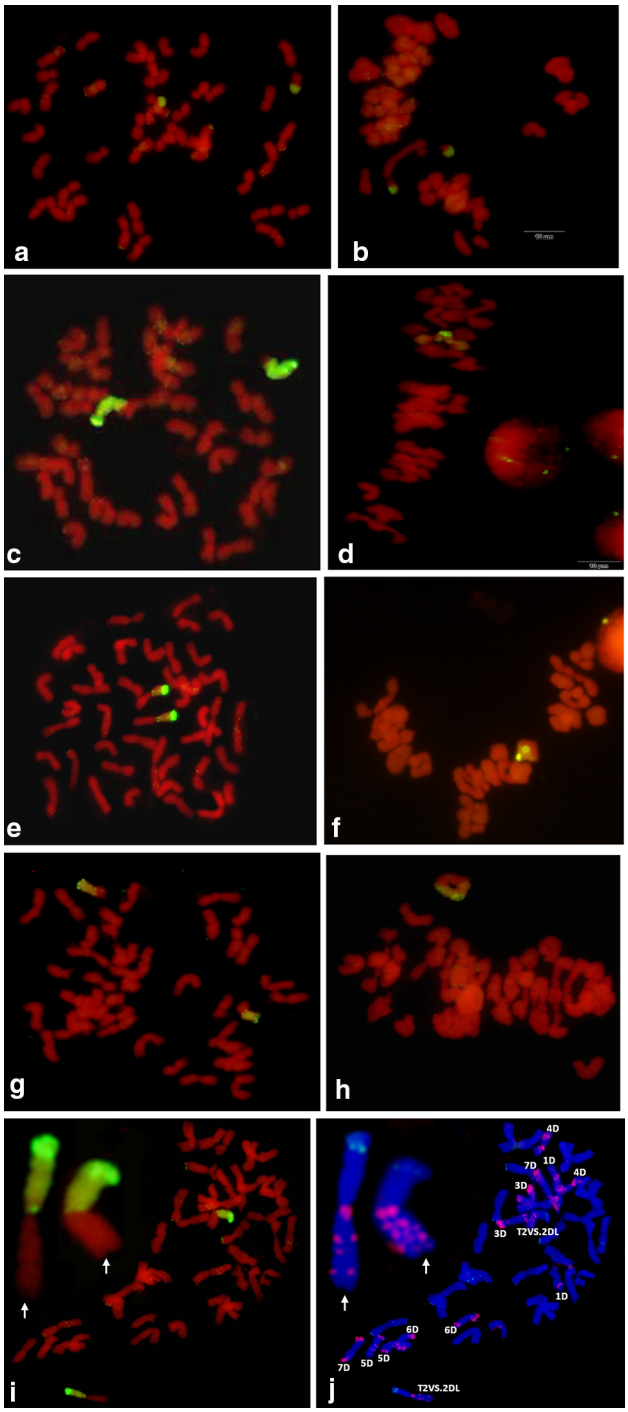


Fig. 2 Mitotic and meiotic analysis of the wheat–2V introgression lines. GISH using total genomic *D. villosum* DNA labeled with fluorescein-12-dUTP and detected by yellow-green fluorescence and wheat chromosomes were counterstained with propidium iodide and fluoresce red. FISH was employed using repetitive DNA clone *pAs1* as probes labeled with fluorescein-11-dUTP and detected by red fluorescence and wheat chromosomes were counterstained with DAPI and fluoresce blue color. **a** and **b** Mitotic and meiotic GISH patterns of NAU2V-1, which contained a pair of wheat–2V small-fragment terminally translocated chromosomes; **c** and **d** GISH patterns of NAU2V-2 with a pair of wheat–2V large-fragment translocation chromosomes; **e** and **f** mitotic and meiotic GISH patterns of NAU2V-3 ($2n = 42$) with 21II, which included 40 wheat chromosomes and one pair of CS–2VS whole arm translocated chromosomes; **g** and **h** mitotic and meiotic GISH patterns of NAU2V-4 with a pair of CS–2VL whole arm translocated chromosomes; sequential GISH (**i**) and FISH (**j**) patterns of T2VS·2DL translation stock. The enlarged chromosomes indicated by *arrows* are the T2VS·2DL chromosomes and all the D-genome chromosomes painted by the *pAs1* probe are marked in **j**

numbers per spike than that of the 2DS chromosome arm of CS. The gene loci located on wheat group 2 chromosomes were already shown to be involved in the control of number of the spikelets and kernel number per spike in wheat (Peng et al. 1998; Dobrovolskaya et al. 2009; Li et al. 2011; Zhang et al. 2013). Muramatsu (2009) presumed that the homoeologous group 2 chromosomes were involved in a genetic system that determines the number of spikelets in the tribe *Triticeae*. However, the disomic addition line CS-DA2V#2 produced by E.R. Sears using the so-called "Greek" *D. villosum* accession did not show longer spikes than the other disomic addition lines (De Pace et al. 2011). This difference in outcomes may be due to a polymorphism of the 2VS locus controlling this phenotype in different accessions of *D. villosum*. Other *T. aestivum–D. villosum* 2V translocations including one small segmental translocation T6BS·6BL-2VS and two whole arm translocations (designated T3DS·2VL and T7DL·2VS) have been developed and characterized by Chen et al. (2008). The gene controlling the tufted bristles located on 2VS was verified, but the spike phenotype of these lines was not clarified.

EST–PCR markers are based on gene sequences that are typically conserved but vary in some degree among species in *Triticeae* (Lee et al. 2009; Wang et al. 2010). Because of the rare EST information in *D. villosum*, 211 EST sequences of common wheat group 2 were used to develop the specific markers for chromosome 2V. Fourteen markers on different physical loci were effectively found and exploited in the current study to track different regions of alien chromosomes in the pool of translocation lines. Four CS–2V introgression lines with different alien-fragment sizes were successfully identified using the electrophoretic patterns of diagnostic bands for five reported EST–STS molecular markers. Using the EST–STS molecular markers, the detection procedure of the introgressed alien chromosome fragments can be completed in a shorter time than conventional cytological examinations. Moreover, very small alien segments cannot be detected by traditional cytological methods. After quick marker assisted selection in the pool of translocation lines, we had a general idea of the introgression status of each translocation line. Combined with the GISH/FISH analysis, the identification of the translocated chromosome was quickly and accurately detected, as in the case of T2VS·2DL found in the current

Table 3 Comparison of four translocation lines with Chinese Spring (CS) for agronomic traits

Lines	2013–2014						2014–2015					
	PH (cm)	SL (cm)	SS	GS	TKW (g)	Heading date (days)	PH (cm)	SL (cm)	SS	GS	TKW (g)	Heading date (days)
CS	155.3a	8.3b	23.4b	50.0b	34.1a	179b	152.7a	7.9b	24.5b	49.0b	38.5a	180b
NAU2V-1	161.7a	7.0c	22.1b	38.0c	31.8a	180b	153.7a	6.6d	20.2c	38.3c	34.4a	181b
NAU2V-2	152.3a	8.3b	20.7b	44.8bc	33.5a	183a	156.0a	7.4bc	21.4c	46.7bc	36.8a	185a
NAU2V-3	152.7a	15.8a	27.1a	62.0a	36.1a	180b	152.7a	16.7a	28.7a	68.7a	37.9a	181b
NAU2V-4	152.9a	8.2b	22.4b	47.3bc	34.3a	180b	152.3a	7.3c	21.3c	42.3bc	36.4a	181b

Means followed by the same letter within a column are not significantly different at 5 % as determined by the least significant difference

PH plant height, *SL* spike length, *SS* spikelets per spike, *GS* grains per spike, *TKW* 1000-kernel weight

study. Therefore, these EST–STS markers have proven to be very helpful in detecting the alien segment in the pool of the translocation lines. Additionally, the 19 primers that produced locus-specific markers for the 2VS also detected homoeo-loci in chromosome 2AS, 2BS or 2DS arms, which confirmed that *D. villosum* chromatin 2VS showed homoeologous relationships to the short arms of wheat chromosome 2. Translocation homozygotes of NAU2V-3 also showed regular MI chromosome pairing and had normal seed set, which suggested that the translocated chromosome was genetically stable and without obvious negative effects on plant growth and fertility.

The photoperiod response is a very important physiological character in wheat, mainly controlled by three major genes, *Ppd-D1*, *Ppd-B1* and *Ppd-A1*, which are located in the short arm of homoeologous group 2 chromosomes. The *Ppd* alleles also have been verified to be effective in progress in young spike development, heading date, plant height and spike length (Qi et al. 2010). In the T2VS·2DL translocation line, the allele *Ppd-D1* of CS was substituted by the alien allele *Ppd-V1*, but its heading dates were not obviously delayed (Table 3). This result suggested that this exchange event might accelerate progress in immature spike development and, therefore, longer spike phenotypes (Fig. 3). As with the longer spike loci, the *Ppd-V1* was also physically located on 2VS FL 0.33–0.53. Therefore, we presume that the photoperiod response gene of *D. villosum* could be a valuable allele in wheat yield development.

The spike number per acre, kernel number per spike and TKW are the main parameters that influence wheat yield. To coordinate the relationship between the "three parameters" is the basic challenge faced by wheat breeders. Theoretically, increasing any one parameter and keeping the other two parameters invariant could lead to significant improvements in wheat yield. Long-term breeding practices have shown that the kernel number per spike is the factor that can most easily be improved to increase crop yield (Zhou et al. 2007; Zheng et al. 2011). However, kernel number per spike and TKW usually conflict with spike number per acre. Sometimes large-spike cultivars exhibit low ratio of spikes to tillers and insufficient spike number in the field resulting in low yield compared with small-spike cultivars. The results of agronomic traits evaluation showed that the T2VS·2DL translocation line has longer spikes, more spikelets and GPS, but no obvious differences were found between the average plant height and TKW of this line and their recurrent parent CS (Table 3). Compared to CS the number of spikes per plant of the T2VS·2DL translocation lines showed no obvious differences (Fig. 4), which may contribute to improving the wheat yield. However, spike number per area of the T2VS·2DL translocation line in different current dwarf varieties backgrounds should be explored in depth.

In the process of wheat breeding, it is very important to select the desired individuals from the large population at each generation. With the aid of the EST–PCR markers linked to useful genes, the selection for the desired traits in the early generation of the breeding program could greatly increase accuracy, breeding efficiency and breeding speed in the identification of desired wheat lines. Morphological markers are also used in breeding program, but only a few morphological markers are available and some of them are limited by many conditions such as the seasons and the environment (Arzani et al. 2004). In this study, we suggest using co-dominant 2VS-specific EST–PCR markers (for example HVF11 and CINAU184) to distinguish those organisms homozygous and heterozygous for the T2VS·2DL chromosome in early filial generation. The presence of long bristles on glume ridges is a typical morphological marker aroused by alien chromosome arm 2VS (Fig. 3c). Comparing to molecular marker techniques, this dominant morphological marker *Bgr-V1* used as a way to select the individual involving T2VS·2DL chromosome could be more efficient, safety and non-destructive.

In conclusion, using 2V-specific EST–PCR markers and sequential GISH/FISH, four homozygous translocation lines, including a novel T2VS·2DL translocation line,

Fig. 3 The young spike and mature spike morphologies of the 2V genetic germplasm and its CS parent. **a** The young spike of T2VS·2DL (*left*) and CS (*right*) were taken at 150 days after sowing the seed. **b** The young spike of T2VS·2DL (*left*) and CS (*right*) were taken in 160 days. The results showed that the T2VS·2DL has faster young spike development than its recurrent parent CS. **c** Comparing the spike length of Yangmai15, DS2V (2D), T2VS·2DL (NAU422) and CS (from *left to right*). The morphological marker, bristles on the glume ridges were present in DS2V (2D) and NAU422

Fig. 4 The morphology of spikes per plant of the T2VS·2DL translocation line NAU422 and its recurrent parent Chinese Spring (CS)

were identified. This translocation line has longer spikes and produces more GPS than its recurrent parent CS, and the three other translocation lines and could be a valuable resource for both basic and applied research for high-yield wheat breeding. The 2VS-specific markers and a morphological marker could be used in marker-aided screening in wheat breeding programs.

Author contribution statement Conceived and designed the experiments: Ruiqi Zhang; Performed the experiments: Fu Hou, Yigao Feng and Wei Zhang; Analyzed the data: Mingyi Zhang; Wrote the paper: Ruiqi Zhang and Peidu Chen.

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Compliance with ethical standards

Conflict of interest The authors declare no conflicts of interest.

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