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ORIGINAL PAPER

Characterization of a wheat–*Thinopyrum bessarabicum* (T2JS-2BS·2BL) translocation line

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Abstract *Thinopyrum bessarabicum* ($2n = 2x = 14$, JJ or E^bE^b) is an important genetic resource for wheat improvement due to its salinity tolerance and disease resistance. Development of wheat–*Th. bessarabicum* translocation lines will facilitate its practical utilization in wheat improvement. In this study, a novel wheat–*Th. bessarabicum* translocation line T2JS-2BS·2BL, which carries a segment of *Th. bessarabicum* chromosome arm 2JS was identified and further characterized using sequential chromosome C-banding, genomic in situ hybridization (GISH), dual-color fluorescent in situ hybridization (FISH) and DNA markers. The translocation breakpoint was mapped within bin C-2BS1-0.53 of chromosome 2B through marker analysis. Compared to the Chinese Spring (CS) parent and to CS-type lines, the translocation line has more fertile spikes per plant, longer spikes, more grains per spike and higher yield per plant, which suggests that the alien segment carries yield-related genes. However, plants with the translocation are also taller, head later and have lower 1,000-kernel weight than CS or CS-type lines. By using markers specific to the barley photoperiod response gene *Ppd-H1*, it was determined that the late heading date was conferred by a recessive allele located on the 2JS segment. In addition, four markers specific for the translocated segment were identified, which can be used for marker-aided screening.

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Introduction

Although genetic diversity in wheat was reduced during domestication, some of it can be restored through introgression from its progenitors or from the more distant wild relatives (Dubcovsky and Dvořák 2007). Introgression of alien genes, even from tertiary gene pool species, could increase genetic diversity for agronomic performance of cultivated wheat (Able et al. 2007), as illustrated by the application of the wheat–rye translocations, T1RS·1BL and T1RS·1AL, in wheat breeding (Jiang et al. 1994; Friebe et al. 1996).

Thinopyrum bessarabicum ($2n = 2x = 14$, JJ or E^bE^b) is a perennial maritime wheatgrass that possesses salinity tolerance and resistance to several diseases, and can be an important gene source for wheat improvement (Gorham et al. 1986; William and Mujeeb-Kazi 1993; King et al. 1997a). Both the octoploid amphiploid ($2n = 8x = 56$, AABBDDJJ) derived from CS and *Th. bessarabicum* (Forster and Miller 1985; Gorham et al. 1986; Forster et al. 1987), and the hexaploid amphiploid ($2n = 4x = 42$, AABBJJ) derived from tetraploid wheat and *Th. bessarabicum* (King et al. 1997a, b; Hassani et al. 2000) showed high levels of salt tolerance. The development of wheat–*Th. bessarabicum* alien chromosome addition lines provided useful tools for locating genes of interest in *Th. bessarabicum* and for its further utilization in wheat improvement (William and Mujeeb-Kazi 1993, 1995; Zhang et al. 2002). For example, a major gene for salinity tolerance was located on

Th. bessarabicum chromosome 5J by studying wheat–*Th. bessarabicum* disomic addition lines (Forster et al. 1988) and disomic substitution lines, where wheat chromosomes 5A and 5D were, respectively, substituted by chromosome 5J (King et al. 1993, 1996, 1997a; Forster et al. 1988). However, the low fertility and genetic instability of wheat–*Th. bessarabicum* amphiploids or alien chromosome addition/substitution lines make them unlikely to be directly useful in crop production (Hassani et al. 2000). Translocation lines, particularly involving small alien segments, would be genetically more stable and desirable. Development of wheat–*Th. bessarabicum* translocations have been reported in earlier studies. By using DNA markers, King et al. (1993) identified a T5AS·5JL wheat–*Th. bessarabicum* translocation line where the translocation involved wheat chromosome arm 5AS and *Th. bessarabicum* chromosome arm 5JL. Other 19 different wheat–*Th. bessarabicum* translocation lines involving chromosome 5J were also obtained through homoeologous pairing induction in the absence of *Ph1* (reviewed by King et al. 1997a). Translocations involving other *Th. bessarabicum* and/or wheat chromosomes were also observed and preliminarily characterized (Zhang et al. 2002; Zhuang et al. 2004; Chen et al. 2007; Qian 2007). A wheat–*Th. bessarabicum* alien telosomic addition line TJ04 ($2n = 6x = 44$), with one pair of chromosome arms 6JL added, was selected in the backcross F5 from CS × CS-*Th. bessarabicum* amphiploid (Zhuang et al. 2004). However, a translocation between wheat chromosome 2B and *Th. bessarabicum* 2J may also occur in this line (Zhuang et al. 2004; Chen et al. 2007; Qian 2007). In addition to its later heading date and increased plant height, line TJ04 has larger spikes and better yield than CS. Since TJ04 contains segments from both chromosomes 2J and 6J of *Th. bessarabicum*, it was not clear which segment accounted for the agronomic effects. In the current study, wheat translocation lines that were homozygous for the 2JS segment only were identified and characterized using cytogenetic and molecular techniques. The agronomic effect of the alien segment was also studied.

Materials and methods

Plant materials

The CS-*Th. bessarabicum* alien telosomic addition line TJ04 (Zhuang et al. 2004) was crossed with CS to produce F1, F2 and F2:3 families for cytogenetic and molecular marker analysis, and for agronomic evaluation. The wheatgrass *Th. bessarabicum* (obtained from Sichuan Agricultural University, China) and the CS-*Th. bessarabicum* amphiploid (provided by Dr Mujeeb-Kazi, Centro Internacional de Mejoramiento de Maizy Trigo, Mexico) were used as controls. To identify and locate markers specific for wheat chromosome 2B and *Th. bessarabicum* chromosome 2J, the six CS nulli-tetrasomic (NT) lines N2AT2B, N2AT2D, N2BT2A, N2BT2D, N2DT2A and N2DT2B were used. Two CS deletion lines del2BS-1 and del2BS-3, which have deletion breakpoints at fracture length (FL) of 0.53 and FL 0.84, respectively, and divides chromosome arm 2BS into three deletion bins (C-2BS1-0.53, 2BS1-0.53-0.84 and 2BS3-0.84-1.00) (Conley et al. 2004), were used to determine the breakpoint of the translocated chromosome. The NT lines and deletion lines were kindly provided by Dr. Bikram S. Gill, Department of Plant Pathology, Kansas State University, Manhattan, KS, USA.

Cytogenetic analysis

Sequential C-banding and genomic in situ hybridization (GISH) were done according to Gill et al. (1991) and Jiang and Gill (1993). Total genomic DNA was isolated as described by Ma and Sorrells (1995). *Th. bessarabicum* genomic DNA (labeled with fluorescein-12-dUTP through nick translation) was used as a probe and genomic DNA of CS was used as a blocker with a ratio of probe:blocker of ca. 1:60. Chromosomes were stained with propidium iodide and analyzed with an Olympus BX60 fluorescence microscope.

To characterize the translocated chromosomes, dual-color FISH was employed using both a DNA clone *pSc119.2* and total genomic DNA of *Th. bessarabicum* as probes, but labeled with biotin-16-dUTP and tetramethyl-rhodamine-5-dUTP, respectively. The DNA clone *pSc119.2* contains a 120-bp tandem repeated DNA sequence from rye (*Secale cereale* L.) (Bedbrook et al. 1980; McIntyre et al. 1990) and can bind specifically with wheat chromatin. Dual-color FISH can therefore not only differentiate wheat and *Th. bessarabicum* chromatin, but also show identity of particular wheat chromosomes. After hybridization with the probes and the blocker, chromosomes were treated with FITC-avidin antibody, stained with DAPI and analyzed with an Olympus BX60 fluorescence microscope. Images were captured using a CCD camera and the computer programs Spot 32 and Photoshop (v7.0). The staining patterns of wheat chromosomes as revealed by the clone *pSc119.2* and by C-banding and described in Kubaláková et al. (2005) and Gill et al. (1991) were used as reference patterns.

Molecular marker analysis

To identify F2 plants that carried *Th. bessarabicum* chromatin from chromosome arm 2JS but not 6JL, three markers that were specific for 2JS (*Xscm144-2JS*), 6JL (*Xbtd8-6JL*) and 2BS (*Xgwm257-2BS*) (Zhuang et al. 2004;

Table 1 Primers that produced specific markers for detecting the T2JS-2BS·2BL translocated chromosome

Primer	Origin	Gene/EST	Sequence (5′–3′)[a]	Tm	Location[b]
HVF11F	Barley	*Ppd-H1*	ATCGAATCACCCGTTTCAATC	47	2H
HVF11R			GACACCATCAGAGATAGTAAC		
HVF12F	Barley	*Ppd-H1*	CAAATGTTCATCTGCTCCACC	52	2H
HVF13R			CGCACACATATTGTACCTTGC		
CINAU696F	Wheat	BF483211	TTCTTCACGCCGCTTGTT	50	2B, 2D
CINAU696R			TCATTGCTGGAGGATTGC		
CINAU730F	Wheat	BE471132	CCAAAGATTGCCACGAAA	50	2D
CINAU730R			CCAAACCAGCAGCACATT		
CINAU737F	Wheat	BE606324	CGTATGGACCGTCTTCACAG	50	2B
CINAU737R			ATGCCGCAGATGGAGTTG		
CINAU807F	Wheat	BE500206	GACACGGTCTTCCCTGAT	50	2B
CINAU807R			AAGGGCTGAAAGTGTTGC		

[a] Sequences of primers HVF11F, HVF11R, HVF12F and HVF13R were obtained from Turner et al. (2005). The other primers were designed based on EST sequences (accession ID included) that are available in Genbank (http://www.ncbi.nlm.nih.gov/Genbank/)

[b] The location of markers for gene *Ppd-H1* was obtained from Turner et al. (2005), and the locations of the remaining markers were determined in this study

Chen et al. 2007; Qian 2007) were used to screen a total of 106 F2 plants derived from the cross CS/TJ04. The DNA sequences of the primer pair SCM144 (SCM *Secale cereale* microsatellite) (Hackauf and Wehling, 2003) were provided by Dr. Peter Wehling, Institute of Agricultural Crops, Germany. The primer pair BTD8 was designed by Dr. Bie Tongde (Cytogenetic institute, Nanjing Agricultural University, China) based on an EST (expressed sequence tag) sequence (GenBank ID: BE490082). Primer pair GWM257 was synthesized based on the sequence information provided by Somers et al. (2004). To determine the breakpoint of the chromosome 2B translocation, a total of 22 SSR markers that were physically (Sourdille et al. 2004) or genetically (Somers et al. 2004) mapped on 2B were synthesized and used to analyze parental lines CS and TJ04, two wheat deletion lines that had different deletions on chromosome 2B, and F2 plants that carried *Th. bessarabicum* 2JS chromatin only. In addition, a total of 140 CINAU primer pairs (designed based on sequences of ESTs that have been physically mapped on wheat homoelogous group 2 chromosomes; Conley et al. 2004) were also used to develop markers specific for the 2JS segment (Table 1). All primers used in this study were synthesized by Invitrogen Life Technologies Ltd (Shanghai, China). PCR reactions were conducted in a 10-μL system and the PCR mixture contained 25 ng template DNA, 10 μM of each primer, 2.5 mM of each dNTP, 2.5 mM $MgCl_2$ and 0.5 units *Taq* polymerase. Amplification was done for 3 min at 94°C, followed by 32 cycles of 30 s at 94°C, 45 s at 50–60°C according to the different primers, 1 min 20 s at 72°C and a final step of 10 min at 72°C. PCR products were separated in an 8% polyacrylamide gel and visualized by silver staining.

Evaluation of agronomic traits

Measurements of heading date, plant height and spike length in the parents (CS and TJ04) and F1 involved multiple plants, whereas the F2 measurements were based on single plants. To evaluate other yield-related traits such as the number of fertile spikes, number of spikelets per spike, number of grains per spike, yield per plant and 1,000-kernel weight, a total of 19 F2 plants that were homozygous for the 2JS translocation and contained no other *Th. bessarabicum* chromatin (Type I plants, Table 2) and 19 F2 plants without any *Th. bessarabicum* chromatin (CS-type plants, Table 2) were selected to produce F2:3 families. Trait evaluation in these families was conducted in a field trial with two replications in Nanjing, China. For each replication, 20 grains of each family were evenly planted in a 0.50-m wide and 1.50-m long row. Except for heading date, F2:3 traits were measured on approximately five randomly selected plants in each family. Student's *t* test was used to test the difference of family means for each trait.

Results

Marker-assisted characterization of plants segregating for the 2JS and 6JL introgressed regions

Since line TJ04 had both alien chromosome arms present (Zhuang et al. 2004; Chen et al. 2007; Qian 2007), a total of 106 F2 plants derived from cross CS/TJ04 were first analyzed using three markers that were specific for 2JS (*Xscm144*), 6JL (*Xbtd8*) and 2BS (*Xgwm257*),

Table 2 Presence of *Th. bessarabicum* chromatin and averaged measurements of plant height, spike length and heading date in Chinese Spring (CS), line TJ04 and F_2 plants derived from CS/TJ04

Line/type	No. of plants	Presence of *Th. bessarabicum* chromatin		Plant height (cm)	Spike length (cm)	Heading date (days)
Parent						
CS	5	–	–	105.50 ± 3.84	6.76 ± 0.58	173
TJ04	5	2JS seg. (homo)	6JL	126.40 ± 4.34	10.68 ± 0.75	182
CS/TJ04 F_1	5	2JS seg. (heter)	6JL	126.20 ± 2.49	10.32 ± 0.88	173
CS/TJ04 F_2	106					
Type I	24	2JS seg. (homo)	–	124.08 ± 6.13	10.29 ± 0.84	182.75 ± 2.44
Type II	36	2JS seg. (heter)	–	116.41 ± 6.68	9.09 ± 0.96	177.39 ± 2.27
Type III	8	–	6JL	106.06 ± 11.15	8.45 ± 0.82	177.36 ± 2.92
Type IV	2	2JS seg. (homo)	6JL	115.5 ± 7.78	9.7 ± 0.14	182.5 ± 2.12
Type V	11	2JS seg. (heter)	6JL	118.41 ± 5.71	9.89 ± 0.88	177.82 ± 1.4
CS-type	25	–	–	105.58 ± 11.4	7.68 ± 0.63	176.16 ± 3.62

2JS seg. translocated segment from *Th. bessarabicum* chromosome arm 2JS, *6JL* the complete 6JL chromosome arm, *homo* homozygous, *heter* heterozygous, respectively

respectively. Twenty-five F2 plants had *Xgwm257-2B* but not *Xscm144-2JS* and *Xbtd8-6JL*, which suggested that no *Th. bessarabicum* chromatin was present (subsequently referred to as CS-type plants) (Table 2). Sixty F2 plants had only the *Xscm144-2JS* locus and therefore possessed the 2JS translocation, but not 6JL chromatin (Table 2, Fig. 1a–c). Among these 60 plants, 24 showed the simultaneous absence of marker *Xgwm257-2B*, which indicated that they were translocation homozygotes (subsequently referred to as Type I plants) (Table 2). The remaining 36 plants also had the *Xgwm257-2B* locus, which indicated that they were translocation heterozygotes (Type II plants, Table 2). Among the remaining 21 F2 plants with the marker locus *Xbtd8-6JL*, eight plants carried *Xgwm257-2B* but not *Xscm144-2JS*, indicating that they possessed 6JL telosomes only (Type III plants, Table 2); two plants had *Xscm144-2JS* but not *Xgwm257-2B* and thus were 2JS translocation homozygotes with the 6JL telosome (Type IV plants, Table 2); and 11 plants had both *Xscm144-2JS* and *Xgwm257-2B*, which indicated that they possessed 6JL and were heterozygous for the 2JS translocation (Type V plants, Table 2).

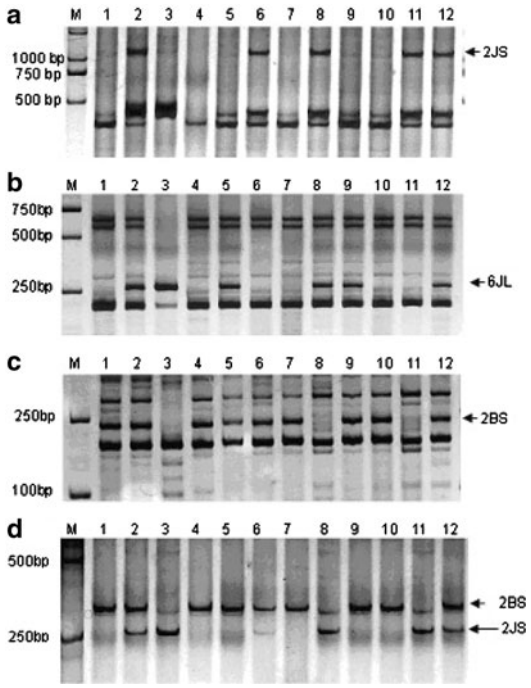

Fig. 1 PCR amplification patterns of markers specific for chromosome arms **a** 2JS (*Xscm144*), **b** 6JL (*Xbtd8*), **c** 2BS (*Xgwm257*) and **d** 2JS/2BS (*Xcinau696*). The *arrows* indicate the chromosome arm specific loci. *Lanes*: *M* size standard, *1* Chinese Spring, *2* CS-*Th. bessarabicum* amphiploid, *3 Th. bessarabicum*, and *4–12* F2 individuals from cross CS/TJ04

Cytogenetic characterization of the translocated chromosome

Sequential C-banding and GISH of root tip chromosomes confirmed that the Type I F2 plants derived from cross CS/TJ04 were homozygous for a terminal translocation (Fig. 2a, b). Regular chromosome pairing occurred in pollen mother cells during meiosis I(MI), which suggested that the plants were cytologically stable. Dual-color FISH using both the DNA clone *pSc119.2* and genomic DNA of *Th. bessarabicum* as probes further confirmed that the translocated chromosome contained a segment of *Th. bessarabicum* chromatin and a large segment of wheat chromatin (Fig. 2c) and has the constitution: T2JS-2BS·2BL (Fig. 2d).

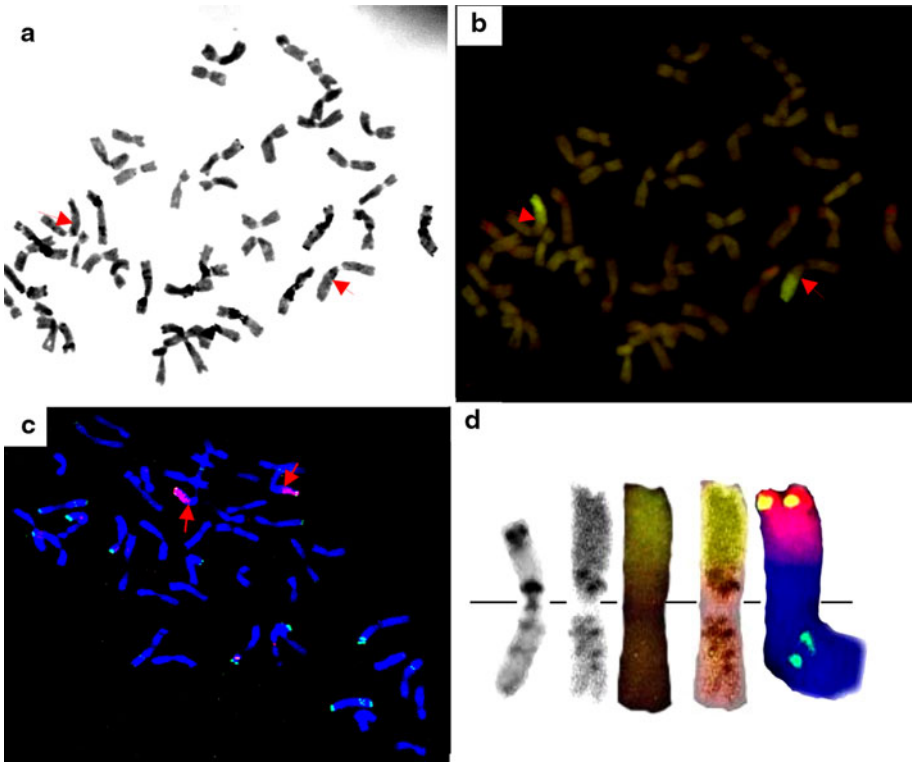

Fig. 2 Sequential C-banding, GISH and dual-color FISH of mitotic chromosomes in a root tip cell of T2JS-2BS·2BL translocation homozygote ($2n = 42$). **a** C-banding, **b** GISH using *Th. bessarabicum* total genomic DNA labeled with fluorescein-12-dUTP as probe and Chinese Spring genomic DNA as block. The *green signal* shows the *Th. bessarabicum* chromatin. *Arrows* indicate the breakpoint in the translocated chromosomes; **c** Dual-color FISH using DNA clone *pSc119.2* labeled with fluorescein-12-dUTP (*green*) and total genomic DNA of *Th. bessarabicum* labeled with tetramethyl-rhodamine-5-dUTP (*red*) as probes and Chinese Spring genomic DNA as block. *Arrows* indicate the breakpoint in the translocated chromosomes; and **d** magnified picture of the translocated chromosome, from left to right: C-banded wheat chromosome 2B; sequential C-banding and GISH; composite C-banding and GISH image, and dual-color FISH of the translocated chromosome (color figure online)

Molecular mapping of the breakpoint in the translocated chromosome

To determine the breakpoint in the translocated chromosome, 15 and 7 SSR markers that were, respectively, physically (Sourdille et al. 2004) or genetically (Somers et al. 2004) mapped on wheat chromosome 2B were used to analyze the T2JS-2BS·2BL translocation and the two chromosome 2B deletion lines. Use of the two deletion lines allowed for physical mapping of the seven genetically mapped markers (*Xwmc257*, *Xbarc35*, *Xwmc154*, *Xwmc344*, *Xwmc477*, *Xgdm86 and Xwmc27*; Somers et al. 2004) (Fig. 3). After analyzing the translocation line using these 22 markers, five loci (*Xbarc13*, *Xgwm410*, *Xbarc55*, *Xwmc154* and *Xgwm148*) in bin 2BS1-0.53-0.84 and four loci (*Xgwm257*, *Xbarc200*, *Xwmc257* and *Xbarc35*) in bin 2BS3-0.84-1.00 were not amplified. Of the 11 markers that mapped in bin C-2BS1-0.53 on chromosome 2B, three (*Xgwm630*, *Xgwm319* and *Xbarc114*) were present, but eight (*Xbarc18*, *Xbarc230*, *Xgwm374*, *Xgwm429*, *Xwmc344*, *Xwmc477*, *Xgdm86* and *Xwmc27*) were absent in the translocation line. In addition, markers *Xgwm47.2*-2BL and *Xbarc167*-2BL that are located on chromosome arm 2BL were also present in the translocation line. Thus, the marker results confirmed that the translocation occurred in the chromosome arm 2BS and showed that the breakpoint was located within bin C-2BS1-0.53 (Fig. 3).

Development of markers that are specific for the translocated 2JS segment

A total of 140 primer pairs were designed based on the sequences of ESTs that have been physically mapped on wheat homoeologous group 2 chromosomes (Conley et al. 2004) and these were subsequently used to analyze the translocation and CS-type lines. Four primer pairs amplified translocation-specific markers, of which primer pair CINAU696 generated a 260-bp fragment in translocation homozygotes, but a 350-bp fragment in plants with a normal 2BS (Fig. 4a); primer pair CINAU737 amplified a 440-bp fragment from the translocation line T2JS-2BS·2BL and a 470-bp fragment from 2BS (image not shown); primer

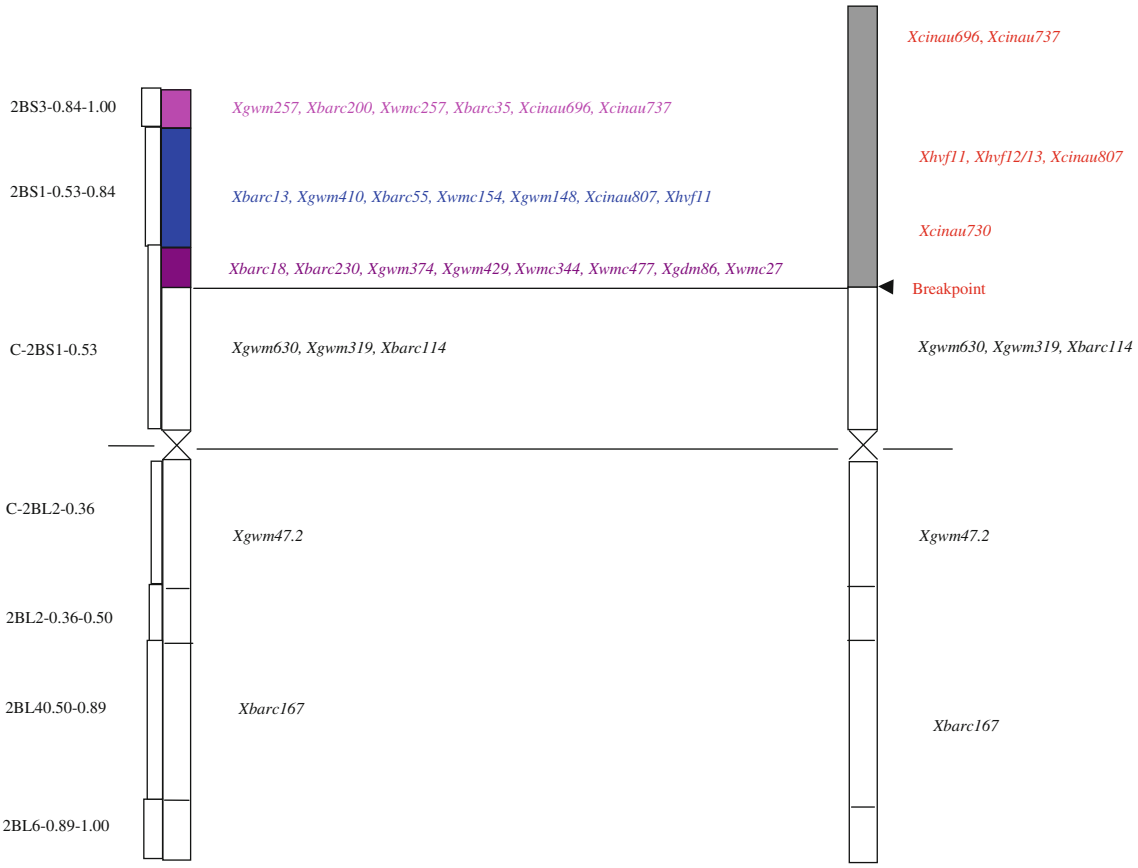

Fig. 3 Physical map of the T2JS-2BS·2BL translocation based on wheat chromosome 2B SSR and STS marker loci. The map on the left is of a normal wheat chromosome 2B and includes 11 markers identified in this study and 15 markers reported by Sourdille et al. (2004). Markers indicated in *pink*, *blue* and *brown* were allocated to the corresponding deletion bins by using two wheat 2B deletion lines. The map on the right is of the T2JS-2BS·2BL translocated chromosome. Six markers in *red* were specific for *Th. bessarabicum* chromatin (*gray*) (color figure online)

pair CINAU807 amplified a 510-bp fragment from the 2JS segment, but a 470-bp fragment from 2BS (Fig. 4b); and primer pair CINAU730 amplified a 200-bp fragment in 2JS, but a 210-bp fragment in chromosome 2D (based on the data of N2AT2D and N2BT2D) (image not shown). Therefore, amplification products of the four primer pairs can be used to detect *Th. bessarabicum* chromosome arm 2JS.

Effect of the introgressed genes on grain yield

Wheat cultivar Chinese Spring had an average plant height of 105 cm, spike length of 6.76 cm and heading date of about 173 days, whereas line TJ04 was 126.40 cm tall with spike length of 10.68 cm and heading date of about 182 days (Table 2). Compared with CS, TJ04 produced more grains per spike and gave a higher yield per plant (data not shown), which indicated that TJ04 probably carries genes that affect yield. Since TJ04 differs from CS with respect to the presence of *Th. bessarabicum* chromosome arm 6JL and a 2JS translocation, the alien chromatin must be responsible for the observed agronomic effects. Five F1 plants derived from cross CS × TJ04 had an average plant height (126.2 cm) and spike length (10.32 cm) similar to that of line TJ04, but its heading date (173 days) was close to that of CS (Table 2). Therefore, alleles for taller plant height and longer spikes in TJ04 may be dominant, but those for later heading date in TJ04 may be recessive. To determine whether the genes were located on the translocated 2JS segment or on the added chromosome 6JL, a total of 106 F2 plants from cross CS/TJ04 were characterized for heading date, plant height and spike length. The F2 population included 25 CS-type (without any *Th. bessarabicum* chromatin), 24 type I (homozygous for the 2JS translocation), 36 Type II (heterozygous for the 2JS translocation), eight Type III (*Th. bessarabicum* chromatin from 6JL only), two Type IV (homozygous for the 2JS translocation and with the 6JL addition) and 11 Type V (heterozygous for the 2JS translocation and with the 6JL addition) plants (Table 2). The results showed that the CS-type and Type III plants had average plant heights similar to CS, while the Type I plants were similar to TJ04 (Table 2). Therefore, it is the 2JS translocated segment rather than the chromosome

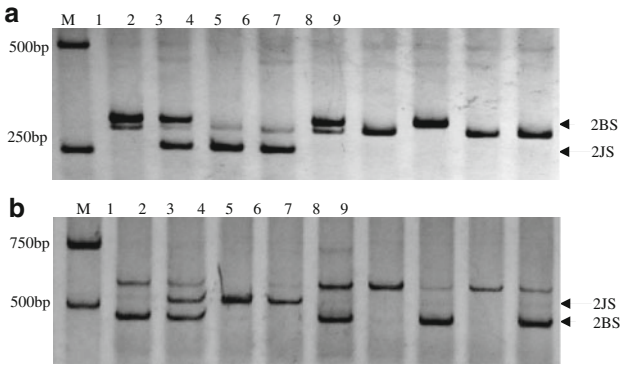

Fig. 4 PCR amplification patterns that were produced using wheat EST-derived primers **a** CINAU696 and **b** CINAU807. The long arrow indicates the marker mapped on the introgressed 2JS segment and the short arrow indicates the 2BS band. *Lanes*: *M* size standard, *1* Chinese Spring (CS), *2* CS-*Th.bessarabicum* amphiploid, *3 Th. bessarabicum*, *4* the translocation line T2JS-2BS·2BL, *5* NT2A/2B, *6* NT2B/2D, *7* NT2D/2B, *8* del2BS-1 (with breakpoint at FL0.53) and *9* del2BS-3 (with breakpoint at FL0.84)

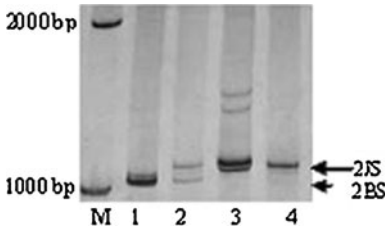

Fig. 5 PCR amplification pattern obtained following screening for *Xhvf11,* a marker specific for the barley *Ppd-H1* gene. The *long arrow* indicates the product associated with chromosome arm 2JS and the *short arrow* indicates the product from 2BS. *Lanes*: *M* size standard, *1* Chinese Spring, *2* CS-*Th. bessarabicum* amphiploid, *3 Th. bessarabicum* and 4 = the translocation line T2JS-2BS·2BL

arm 6JL that affects plant height. The Type II and Type V plants that were heterozygous for the 2JS translocation had average plant heights (116.4 and 118.4 cm) and spike lengths (9.09 and 9.89 cm) that were intermediate (with slight bias toward those of line TJ04). Furthermore, the heading dates (177.4 and 177.8 days) of Type II and Type V plants were close to that of CS (173 days) (Table 2), which suggested that the 2JS translocation carried partially dominant alleles for taller plant height and longer spikes, but a recessive allele for later heading date. Since genes controlling photoperiod response in wheat and barley (*Ppd-D1*, *Ppd-B1*, *Ppd-A1*, and *Ppd-H1*) were located on chromosomes 2D, 2B, 2A and 2H (reviewed by Snape et al. 2001), markers specific for the barley *Ppd-H1* gene were used to evaluate the translocation lines as well as line TJ04 and CS. Markers *XHvF11* (1,100 bp, Fig. 5) and *XHvF12/13* (210 bp, data not shown) that are specific for *Ppd-H1* (Turner et al. 2005) were present in the translocation lines and in TJ04, which confirmed that the 2JS translocation carried a photoperiod response allele different from that of CS. Also, *XHvF11* and *XHvF12/13* could be diagnostic markers for identifying this allele in the CS background (Fig. 5).

Validating the effects of the 2JS translocation with F2:3 families

Nineteen Type I F2 plants were used to produce F2:3 families homozygous for the 2JS translocation and another 19 CS-type F2 plants were used to produce F2:3 families without any *Th. bessarabicum* chromatin. The F2:3 families were evaluated for plant height, spike length, heading date and other yield-related traits such as the number of fertile spikes per plant, spikelets per spike, grains per spike, grain weight per spike, yield per plant and 1,000-kernel weight. With the exception of heading date, the data of the other traits for each type were collected from 87 harvested plants (approximately five random plants per family) within each of the two categories (2JS chromatin present versus 2JS chromatin absent). Comparisons (*t* test) of mean values indicated that grain weight per spike was not significantly different between the two types of F2:3 families (2.07 and 2.03 g, respectively), but that the average yield per plant in translocation lines was significantly higher (2.74 g more, $P < 0.05$) than that of CS-type derivatives (Table 3). Highly significant differences ($P < 0.001$) were also observed for other traits (Table 3). Translocation lines were taller (11 cm), had longer (3 cm) spikes, headed 10 days later, had three more fertile spikes per plant, three more spikelets per spike and produced eight more grains per spike; however, their 1,000-kernel weight was 2.78 g lower (Table 3).

Table 3 Comparison of T2JS-2BS·2BL translocation lines with Chinese Spring (CS)-type lines for certain agronomic traits

Traits	T2JS-2BS·2BL	CS-type line	*T* test
Plant height (cm)	131.88 ± 8.89	120.74 ± 7.63	8.12***
Number of fertile spikes per plant	11.54 ± 6.15	8.64 ± 4.85	3.92***
Spike length (cm)	11.15 ± 0.72	8.11 ± 0.84	22.61***
Spikelets number per spike	27.51 ± 2.10	24.22 ± 2.11	9.54***
Number of grains per spike	77.53 ± 11.63	69.90 ± 13.55	3.99***
Grain weight per spike (g)	2.07 ± 0.43	2.05 ± 0.48	n.s.
Heading date (days)	189.05 ± 1.39	179.21 ± 1.08	24.3***
Yield per plant (g)	15.57 ± 9.72	12.83 ± 7.82	2.04*
Thousand-kernel weight (g)	26.48 ± 3.31	29.26 ± 4.38	−4.72***

*, **, *** Significant differences at the probability levels of $P < 0.05$, 0.01 and 0.001, respectively; whereas *n.s.* non-significant difference

Discussion

The transfer of useful genes from the tertiary gene pool could be an effective way of increasing genetic diversity and the improvement of cultivated wheat. The production of translocation lines is desirable as it involves smaller amounts of alien chromatin as compared to addition and substitution lines. In this study, we identified and characterized a segmental wheat T2JS-2BS·2BL translocation line that carries *Th. bessarabicum* chromatin from chromosome arm 2JS. Additionally, our results showed that the alien segment may carry genes affecting yield.

Analysis of the translocation line and two wheat deletion lines, using chromosome arm 2BS markers from previous studies (Sourdille et al. 2004; Somers et al. 2004) or developed during this study, determined that the breakpoint in the translocation chromosome occurs in bin C-2BS1-0.53 on chromosome arm 2BS. By combining the chromosome patterns revealed by both C-banding and FISH, the breakpoint was confirmed in the distal part of the bin. Markers specific for the *Th. bessarabicum* segment have been developed, which will facilitate its selection in wheat improvement.

Line TJ04 was derived from the F5 of the cross CS × CS-*Th. bessarabicum* amphiploid (Zhuang et al. 2004) and resulted from a spontaneous translocation in the presence of *Ph1*. The translocated chromosome T2JS-2BS·2BL could therefore be the result of either homoeologous recombination or spontaneous breakage and reunion between wheat chromosome 2B and *Th. bessarabicum* chromosome 2J. Primers that produced locus-specific markers for the small 2JS segment also detected homoeo-loci in chromosome arm 2BS (for example HVF11, CINAU696, CINAU737, CINAU807, etc.), which confirms a high level of genetic similarity (or synteny). Therefore, homoeologous recombination might have resulted in the translocation and, in future studies, either homoeologous pairing induction or irradiation treatment may be used to further shorten the alien segment.

From the cytogenetic analysis, it was apparent that the translocated chromosome 2B had a relatively longer short arm resulting in an arm ratio different from that of the normal wheat chromosome 2B (Fig. 2). Translocation homozygotes 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 longer short arm of the translocated chromosome could result from the presence of comparatively more heterochromatin in the alien segment, which may not necessarily affect plant growth and fertility. However, given the size of the alien segment, it is also possible that areas of the introgressed region may not have homoeology to 2BS. Currently, we are using DNA markers or DNA clones derived from the repetitive sequences to investigate the translocation and to compare the heterochromatin content of the 2JS segment with that of the corresponding wheat 2B segment.

The F1 CS/TJ04 data showed that alleles for increased plant height and longer spikes may be dominant, while later heading date appears to be recessive. However, data obtained from Type II and Type V (translocation heterozygotes) F2 plants suggested that translocation heterozygotes had intermediate plant height and spike length. The average plant height of these translocation heterozygotes was significantly taller (11.18 cm, $P < 0.0001$) than that of CS-type or Type III plants (non-translocation homozygous), but was significantly shorter (6.54 cm, $P < 0.001$) than that of Type II or Type IV plants (translocation homozygotes). The average spike length of translocation heterozygotes was significantly longer (1.40 cm, $P < 0.001$) than that of CS-type or Type III plants, but were significantly shorter (0.97 cm, $P < 0.001$) than that of translocation homozygotes (Table 2). Since only five F1 plants, but more than 40 F2 plants were evaluated, the F2 results would be more accurate, i.e., the translocation most probably has partially dominant alleles for increased plant height and longer spikes. Chromosome 2B of CS contains a dominant photoperiod response gene (*Ppd-B1*) that confers earlier heading date (reviewed by Snape et al. 2001). In translocation line T2JS-2BS·2BL, the dominant allele *Ppd-B1* was substituted by the recessive alien allele causing the translocation line to head about 10 days later than CS or CS-type lines. Besides the late heading date, the translocation line also showed increased plant height and spike length. It is likely that genes other than *ppd* could be responsible for the effects on plant height and spike length since alleles for these traits are dominant, or possible gene interactions may also exist and affect these traits.

In conclusion, by using sequential chromosome C-banding, GISH, dual-color FISH and DNA markers, we identified and characterized a new wheat–*Th. bessarabicum* T2JS-2BS·2BL translocation line. The translocation line has longer spikes, produce more grains per spike and yields more per plant than the recurrent parent (CS) or CS-type lines, which suggest that the 2JS segment carries yield-related genes. Markers specific for the translocated segment were developed for marker-aided screening in wheat improvement.

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