Theor Appl Genet (2015) 128:1827–1837
DOI 10.1007/s00122-015-2550-9

ORIGINAL PAPER

Introgression of *Agropyron cristatum* 6P chromosome segment into common wheat for enhanced thousand-grain weight and spike length

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Received: 2 April 2015 / Accepted: 29 May 2015 / Published online: 21 June 2015

Abstract

Key message **This study explored the genetic constitutions of wheat-*Agropyron cristatum* 6P chromosomal translocation and determined the effects of 6P intercalary chromosome segment on thousand-grain weight and spike length in wheat.**

Abstract Crop wild relatives provide rich genetic resources for wheat improvement. Introduction of alien genes from *Agropyron cristatum* into common wheat can broaden its genetic diversity. In this study, radiation-induced wheat-*A. cristatum* translocation line Pubing3035 derived from the offspring of wheat-*A. cristatum* 6P chromosomes addition line was identified and analyzed using genomic in situ hybridization (GISH), dual-color fluorescence in situ hybridization (FISH), and molecular markers. GISH analysis revealed that Pubing3035 was a Ti1AS-6PL-1AS·1AL intercalary translocation. The breakpoint was pinpointed to locate near the centromeric region on the short arm of wheat chromosome 1A based on a constructed F_2 linkage map and it was flanked by markers *SSR12* and *SSR263*. The genotypic data, combined with the phenotypes, indicated that *A. cristatum* 6P chromosomal segment played an important role in regulating the thousand-grain weight and spike length. On average, the thousand-grain weight and spike length in translocation individuals were approximately 2.5 g higher and 0.7 cm longer than those in non-translocation individuals in F_2 and BC_1F_1 populations. The clusters of quantitative trait loci for thousand-grain weight, spike length, and spikelet density contributed by 6P chromosome segment were mapped between *A. cristatum* unique marker *Agc7155* and wheat marker *SSR263*, which, respectively, explained phenotypic variance of 24.96, 12.38 and 17.20 % with an LOD of 10.63, 4.89 and 5.59. Overall, the translocation Pubing3035 had a positive effect on the yield of wheat, which laid the foundation for the localization of *A. cristatum* excellent genes and made itself a promising and valuable germplasm for wheat improvement.

Communicated by S. S. Xu.

Jing Zhang and Jinpeng Zhang contributed equally to this work.

Electronic supplementary material The online version of this article (doi:10.1007/s00122-015-2550-9) contains supplementary material, which is available to authorized users.

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Introduction

Wheat (*Triticum aestivum* L., $2n = 6x = 42$, genomes AABBDD) is a major cereal crop produced worldwide. With the growth of population and climate change, expanding demand for agricultural products requires a substantial increase in the productivity of crop plants over future decades (Tilman et al. 2011). However, in long-term cultivation, elite wheat cultivars had resulted in the reduction of genetic diversity. Broadening the genetic base of wheat is an important means of improving its yield and quality, and enhancing the capacity of resistance to biotic and abiotic stresses for plant breeding (Tester and Langridge 2010; Trethowan and Mujeeb-Kazi 2008). The wild relatives of wheat possess many excellent genes and play an important role in wheat genetic improvement (Friebe et al. 1996; Jiang et al. 1993; Pingali and Rajaram 1999; Sharma and Gill 1983). Producing wheat-alien species translocation

lines and analyzing their genetic constitutions are the key steps for the effective transfer of useful genes into common wheat (Chen et al. 1995; Friebe et al. 1996; Gill et al. 2011; Klindworth et al. 2012; Larson et al. 2012). In recent years, many inter-generic heterologous translocation lines had been produced from crossing common wheat to *Aegilops speltoides*, *Thinopyrum intermedium*, *Leymus racemosus*, *Secale cereale*, *Haynaldia villosa*, and *Psathyrostachys huashanica*, which carried new disease resistance genes, including leaf rust, stem rust, stripe rust, Fusarium head blight, and powdery mildew resistance genes that can be used in plant breeding (Chen et al. 1995; Kang et al. 2011; Larson et al. 2012; Liu et al. 2011; Qi et al. 2011; Zhao et al. 2010; Li et al. 2007). Two particularly important examples of the introgression of genetic information from the crop wild relatives (CWR) are the use of the short arm of rye chromosome 1R and *H. villosa* 6V in wheat (Cao et al. 2011; Chen et al. 1995; Tester and Langridge 2010).

Agropyron cristatum (L.) Gaertn. ($2n = 4x = 28$, genomes PPPP), is an important CWR of wheat (Dewey 1984; Dong et al. 1992), which possess many desirable traits, such as high tiller number, high floret numbers, and resistance to wheat rusts, powdery mildew, and barley yellow dwarf virus (Li 1995). In the early 1990s, a series of inter-generic hybrids between common wheat cv. Fukuhokomugi (Fukuho) and *A. cristatum* accession Z559 were synthesized through wide cross and embryo rescue, and then an array of wheat-*A. cristatum* addition lines and several disomic substitution lines were produced (Li et al. 1998a, b; Wu et al. 2006). Compared with the recipient parent wheat variety Fukuho, the addition line 4844-12 has the characteristics of increased numbers of florets and kernels per spike (Li et al. 1998a). Wu et al. (2006) located agronomic trait of increased numbers of florets and kernels on the chromosome 6P of *A. cristatum*. A variety of wheat-*A. cristatum*6P translocation plants were gained by irradiation or *Aegilops*' gametocidal chromosomes (Luan et al. 2010). The wheat-*A. cristatum* translocation Pubing3035, produced from irradiated hybrid seeds (wheat-*A. cristatum* addition line/Gaocheng 8901), is a valuable wheat-*A. cristatum* 6P chromosome intercalary translocation line with 42 chromosomes (Huang et al. 2013).

To understand detailed genetic constitution of Pubing3035 and further exploit these desirable genes on the *A. cristatum* 6P chromosome, strict backcrossing and self-pollination were carried out. A segregating F_2 population and a segregating BC_1F_1 population were obtained from crossing between Pubing3035 and Fukuho. Surprisingly, the translocation lines contained 6P translocation chromosome segment exhibited more superior agronomic traits (thousand-grain weight and spike length) in two populations. In this study, the genomic constitutions of Pubing3035 using GISH, FISH and molecular markers were examined, but also localized superior agronomic characters to *A. cristatum* 6P intercalary chromosomal segments.

Materials and methods

Plants materials

Wheat-*A. cristatum* 6P disomic addition line 4844-12 ($2n = 44$) and disomic substitution line 4844-8 ($2n = 42$) were obtained by hybridization between *A. cristatum* accession Z559 ($2n = 4x = 28$, PPPP, from Xinjiang, China) and *T. aestivum* cv. Fukuho (Li et al. 1998a). Wheat-*A. cristatum* 6P translocation lines WATP6-32 (M_2 generation) were produced from irradiated hybrids between Gaocheng 8901 and 4844-12 (Luan et al. 2010). The recipient parent Fukuho and a high-quality strong gluten *T. aestivum* cv. Gaocheng 8901 were common wheat varieties. The translocation line Pubing3035 was isolated from offspring following seven generations of consecutive self-pollination of WATP6-32, being assisted by cytogenetic analysis.

An F_2 population was developed from the hybrid between Pubing3035 and Fukuho. The BC_1F_1 backcross population was gained through backcrossing Pubing3035 with the recurrent parent Fukuho. The sizes of F_2 and BC_1F_1 populations are 310 and 88, respectively. These two populations were used to examine the association of excellent agronomic traits with the alien chromosome segment. All materials were collected or developed by our laboratory.

GISH and FISH analysis

To detect P-genome chromosome, genomic in situ hybridization (GISH) was carried out in root tip cells using *A. cristatum* genomic DNA labeled with the DIG-Nick Translation Mix (Roche, Mannheim, Germany) as a probe. Fukuho genomic DNA was used for blocking. Root tips were prepared as described in Cuadrado et al. (2000). Genomic DNA was isolated from *A. cristatum* and wheat cv. Fukuho separately using a modified CTAB method (Dellaporta et al. 1983). The hybridization procedure was carried out as described by Han et al. (2004).

The probe pAs1, containing a 1 kb insert from *Ae. tauschii* Coss.($2n = 2x = 14$, DD) repeated sequences (Rayburn and Gill 1986), enables us to identify all the D-genome chromosomes of wheat (Pedersen and Langridge 1997). The clone pHvG39 can particularly hybridize strongly with B genomes while it hybridizes weakly with the A and D genomes (Pedersen and Langridge 1997). Dual-color fluorescence in situ hybridization (FISH) was performed using pAs1 and pHvG39 as probe to identify translocated wheat chromosomes. The FISH procedure

Table 1 Sequences of *A. cristatum* EST-STS, SLAF markers and wheat SSR markers

Marker	Type	Annealing temperature (°C)	Left primer (5′–3′)	Right primer (5′–3′)
barc148	SSR	52	GCGCAACCACAATGTATGCT	GGGGTGTTTTCCTATTTCTT
CAAS118	SSR	60	GTTCTGCTTTCAACGTTGCTC	ACGTTTCCAATTCAAGGGTTC
SSR283	SSR	56	GGGTTGCAATAGTTGCTTCAT	CACGCACACTCTCTCCCTTAC
SSR12	SSR	56	ATTTATGATCTTGGAGCAGT	CTTTGTTTTTCACGAAAATGGTC
gwm357	SSR	55	TATGGTCAAAGTTGGACCTGG	AGGCTGCAGCTCTTCTTCAG
Agc46261	STS	59	TTCCATACTCGGGGTGAATC	CCGTGCTATCTGAAGATGTCG
Agc21572	STS	59	GGCAAGTCTTGGTAACGATCTC	CGCAAGCTCACAACAAACATC
Agc64456	STS	59	ATATCTTGCTGCCAAGTGGG	AAACATCAAGGCAAGATGGC
Agc51639	STS	59	GATAGATCGCCGGTTTACCA	TGCATGTGGATACCTTGGAG
Agc52189	STS	59	CCAAGGGTCAGATCCACCTA	TCACTTCTGAACGGCAACAC
Agc24350	STS	59	TGATTGCGTACAAAAGCTTGC	CATTTGCACTATCTTGAAATACACTGA
Agc38380	STS	59	CTTGTGGCACCCCGATAG	GTGTACCCAGCCAGTCCTGT
Agc7155	STS	59	CCTCTTTATATTTTCGGTGGTGA	CTGTTCCTGTGCCCCCG
SL12433	SLAF	59	GGCTCTAAAATCGCCAGAAG	CCCAAACTAACGCCCAGTG
SL4746	SLAF	58	GTTGGGCGGTTGACAGGT	AACAACCATCGATGAAGCAA
SL4100	SLAF	58	GCAGATTTCCAACCCTACTCC	GTTGCATCCATTGTCAAAGC
SL10594	SLAF	59	TAGGGCAGGGGCATCTTACT	CGGCAACAGAGTGATGGAAT
SL26496	SLAF	58	GCCATAAGAGGCAGTGATG	GCCAAGGGATTGACAACCT
SL17120	SLAF	56	ATCCGTCACCTGCCACCTAT	GCAAAAGCTCCGCATCAT
SL12396	SLAF	58	TCCTGTCCTTGGACTATCAT	TCGGAGGTCGTCCTAGTGAT
SL45115	SLAF	57	GCTCGAGCAATCATAGCTTCC	AAGTCAAGCTGATTTGCGACA
SL11313	SLAF	60	TCCTCTGGTTGATCTAGCTTGT	GGGTACGCTCCTCCTGATG

followed that described by Liu et al. (2010). All cytological images were observed under an OLYMPUS AX80 (Olympus Corporation, Tokyo, Japan) fluorescence microscope and captured with a CCD camera (Diagnostic Instruments, Inc., Sterling Heights, MI, USA).

Molecular marker analysis

To determine the translocated 6P chromosome segment and its location, a total of 461 SSR primer pairs and 13 STS markers, which were located on wheat chromosome 1A, were screened from the GrainGenes 2.0 website (http://www.wheat.pw.usda.gov/GG2/index.shtml) (Table S1; Table S2). Moreover, 680 EST-STS markers specific to chromosome 6P were used in the study according to the published *A. cristatum* transcriptome sequences (Zhang et al. 2015). The primer sequences and the annealing temperature are listed in Table 1. The PCR amplification procedure and electrophoresis were performed according to published method (Wu et al. 2006).

Evaluation of agronomic trait

Evaluation of traits is primarily aimed at parents and populations. For parents, evaluation was conducted in a field trial in two locations (Beijing and Xinxiang of Henan in China) with three replications in the 2013–2014 growing season. For each replication, 20 grains of each line were evenly planted in 2.0-m rows, spaced 0.3 m apart. All parents were evaluated for several key agronomic traits (i.e., plant height, fertile tillers, spike length, kernels per spike, thousand-grain weights, and spikelet density).

Similarly, an F_2 population including 310 individuals and a BC_1F_1 population including 88 individuals from one cross involving Pubing3035 and the wheat cultivar Fukuho were planted in 2013–2014 growing season to assess all of the key agronomic traits. Each plant was identified with the P-genome specific STS markers, and then two populations were divided into two groups: 6P translocation plants and non-translocation plants according to the positive and negative results of *A. cristatum* 6P-specific markers. After harvesting, traits were measured on each plant from an F_2 population and a BC_1F_1 population and on 20 plants randomly selected from the parents Pubing3035, 4844-12, Gaocheng 8901, and Fukuho. Statistical analyses were conducted using the Statistical Analysis System version 9.2 (SAS Institute Inc., Cary, NC, USA), and a *t* test was used to test the difference of the agronomic traits between 6P translocation plants and non-translocation plants and among the parents.

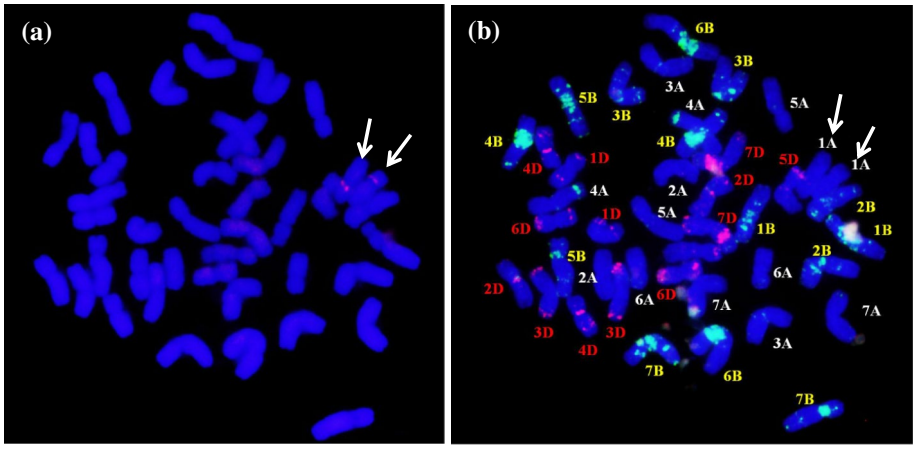

Fig. 1 GISH/FISH identification of the homoeologous group of translocated wheat chromosomes. **a** GISH detection of the 6P segment in Pubing3035 using P-genomic DNA (*red*) as a probe. **b** Dual-color FISH identification of Pubing3035 using pHvG39 (*green*) and pAs1 (*red*) as probes (color figure online)

Linkage map construction and QTL mapping

To further determine the translocation breakpoint and the association between *A. cristatum* chromosomes and agronomic trait, a genetic map was constructed using the F_2 population. QTL mapping and correlation analysis were conducted using IciMapping3.3 software (http://www.isbreeding.net/), with an LOD threshold of 2.5.

Results

GISH and FISH analysis

GISH was used to obtain a clear picture of the genomic constitution of translocation line Pubing3035 based on its visible signals, which was conducted during the mitotic metaphases using the whole genomic DNA of *A. cristatum* as the probe and Fukuho as the blocker. The root tip somatic cells of the translocation Pubing3035 had very small segments of *A. cristatum* chromosomes with red hybridization signals (Fig. 1a). The fluorescent signal patterns of Pubing3035 showed that an inserted exogenous chromosome segment was close to a centromeric position. The GISH results confirmed that Pubing3035 contained 42 chromosomes from wheat, so it was designated as a wheat-*A. cristatum* intercalary translocation line. Dual-color FISH analysis combined with GISH revealed that the chromosome 6P segment was translocated to the short arm of wheat chromosome 1AS (Fig. 1b). Hence, Pubing3035 was suggested to be a 1AS-6P intercalary translocation.

EST-STS analysis

Pubing3035 was characterized using 680 pairs of the P-genome-specific STS primers developed from *A.*

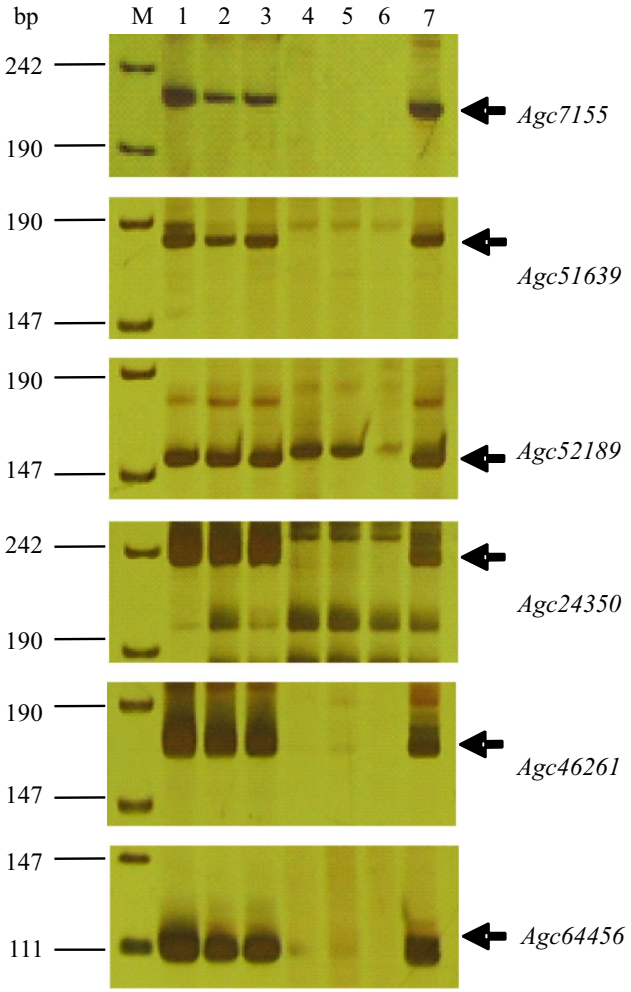

Fig. 2 Amplification patterns of EST-STS markers for translocation line Pubing3035 and the negative controls. *M*, pUC19 DNA/MspI (HpaII); *1 A. cristatum* accession Z559, *2* 4844-12, *3* 4844-8, *4* Gaocheng8901, *5* Fukuho, *6* Chinese Spring, *7* Pubing3035. *Arrows* the diagnostic amplification products of *A. cristatum*

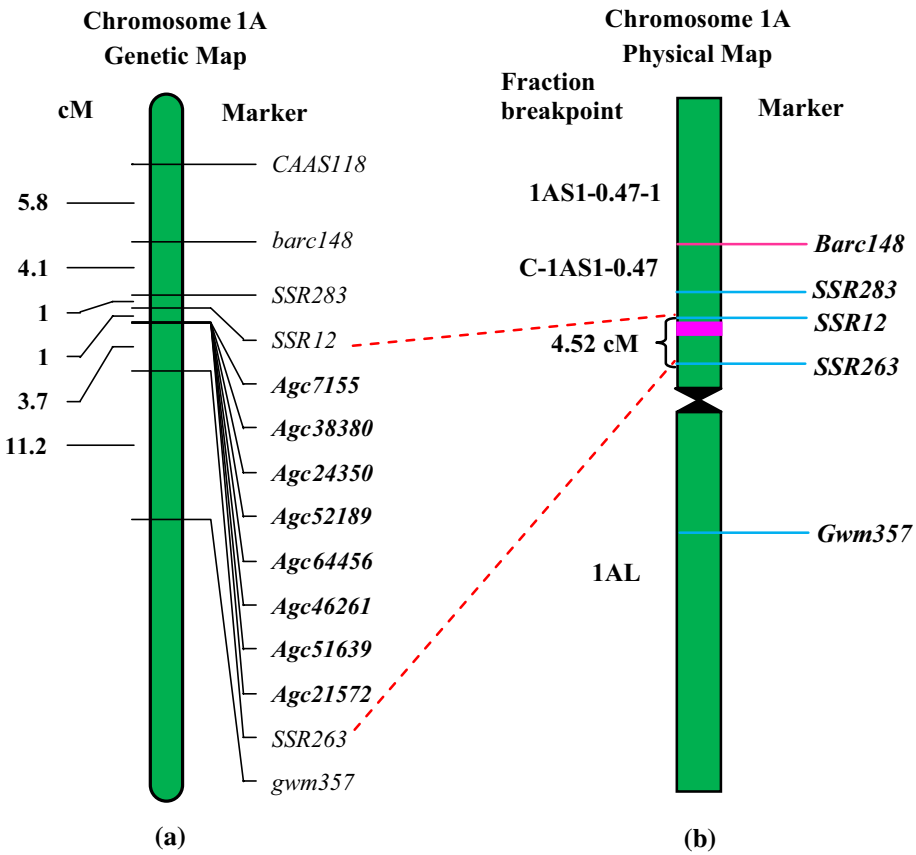

Fig. 3 Wheat chromosome 1A genetic linkage (*left*) and physical (*right*) maps. Centimorgan (cM) distances are shown in the left side of genetic map and markers in the *right side*. *A. cristatum*-specific markers are shown in *bold*. The map on the *right* is the physical map of wheat chromosome 1A, including C-1AS1-0.47, 1AS1-0.47-1 and 1AL. *Deletion line* designations and fraction breakpoints are indicated to the left and bin-mapped markers to the *right*. Region in *orange* indicate the presence of *A. cristatum* chromosome 6P. The physical map was derived from the GrainGenes website (http://avena.pw.usda.gov/GG2/index.shtml) (color figure online)

cristatum transcripts. From 680 chromosome 6P specific primers, we screened eight EST-STS primers pairs that could amplify unique DNA bands from *A.cristatum*, disomic addition line 4844-12, disomic substitution line 4844-8 and Pubing3035, but amplified no bands from the common wheat parents Fukuho and Gaocheng8901 (Fig. 2). The amplification length of these primers was mostly from 110 to 250 bp. The results indicated that these EST-STS primers could be used as molecular markers to track the alien chromatin of 6P from *A.cristatum*.

Translocation breakpoint of wheat-*A. cristatum* 6P translocation lines

To confirm the translocated wheat chromosomes and intercalary position in wheat, a total of 461 pairs of wheat SSR primers and 13 STS markers mapped on wheat chromosome 1A were used to detect whether the translocation is an insertion of a 6P chromosome segment without any loss of wheat 1A chromatin (Table S1; Table S2). The marker analysis displayed that no markers for 1A were lost in Pubing3035. The method of linkage analysis was then attempted to pinpoint the translocation breakpoint. These markers were tested and compared for polymorphisms in Pubing3035 and Fukuho. Of these, 34 (7 %) pairs of SSR primer pairs could amplify polymorphic bands between the two parents. The polymorphic wheat SSR markers and *A. cristatum* EST-STS markers were chosen for the translocation breakpoint determination. In the F_2 segregation population, Chi-squared test analysis showed that the proportion between translocation and non-translocation plants complied with the theoretical ratio 3:1 ($\chi^2_{3:1} = 0.21 \ll \chi^2_{0.05,\,1} = 3.84$). According to the linkage relationship between the wheat markers and *A. cristatum*-specific markers, the translocated wheat chromosomes and translocation breakpoints were determined. Linkage map construction based on the F_2 population showed that the *A. cristatum* 6P-specific markers were linked with wheat markers located on 1AS. The 6P chromosome segments were localized at a distance of 1 cM from *SSR12*, and 3.52 cM from *SSR263* (Fig. 3a). The two markers corresponding to the wheat draft genome sequences were located near the centromeric section (FL0~0.47) on wheat chromosome 1AS (Fig. 3b).

The source analysis of *A. cristatum* 6P intercalary chromosome segments

To pinpoint the origin of *A. cristatum* 6P intercalary segments, a high-density genetic map for P-genome of *Agropyron* Gaertn. based on specific locus-amplified fragment

sequencing (SLAF-seq) was used (submitted). Nine SLAF markers mapped on *A. cristatum* chromosome 6P were developed to analyze the origin of alien segment in Pubing3035. As shown in Fig. 4, four markers were screened (*SL4746*, *SL4100*, *SL10594* and *SL12433*) from *A. cristatum* chromosome arm 6PS and five markers (*SL11313*, *SL26496*, *SL17120*, *SL12396* and *SL45115*) from *A. cristatum* chromosome arm 6PL. Only one SLAF marker (*SL11313*) could amplify specific bands in Pubing3035. Combined with position information of the genetic map of P-genome, we found that marker *SL11313* was located at the same position as two markers (*SL12396* and *SL45115*). The results indicated that *A. cristatum* 6P intercalary chromosome segments in Pubing3035 originated in *A. cristatum* chromosome 6PL.

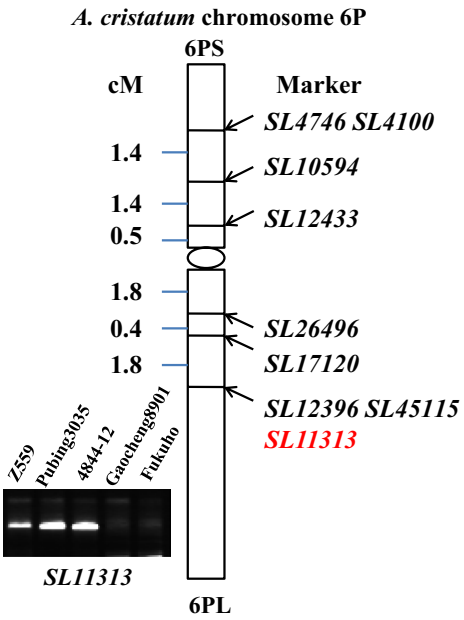

Fig. 4 Linkage map of *A. cristatum* chromosome 6P. On the model, *A. cristatum* chromosome 6P is divided into eight sections according to genetic distance. Eight markers are expanded to the corresponding regions. PCR amplification patterns of *A. cristatum*-specific marker *SL11313* is shown in the *lower left corner*. Genetic distances (cM) are shown on the *left end side*

Evaluation of agronomic traits in Pubing3035

In growing stages, Pubing3035 exhibited compact plant type and was taller than common wheat Gaocheng 8901 in the field. After harvesting, each of these parents was evaluated for eight agronomic traits, including plant height, fertile tillers, spike length, spikelet number per spike, kernels per spikelet, kernels per spike, thousand-grain weight, and spikelet density. Trait observations over two locations showed the similar significance level, indicating that the phenotype of Pubing3035 was genetically stable, independent of environment. Among three parents, spikelet number per spike, kernels per spikelet, and grain number per spike in Pubing3035 were lower than those of 4844-12 and Gaocheng 8901. Plant height in Pubing3035 was higher than that of Gaocheng 8901, but lower than that of 4844-12. For fertile tillers, no significant difference was observed between Pubing3035 and Gaocheng 8901.

However, thousand-grain weight, spike length and spikelet density showed significant variations among three materials. Pubing3035 and 4844-12 produced higher thousand-grain weight, longer spike length, and lower spikelet density than those of wheat parent Gaocheng 8901 (Table 2). These results indicated that there were positive regulators of thousand-grain weight and spike length on *A. cristatum* 6P intercalary segments, which provide a possibility of improving spike traits and increasing yield without prolonging the growth period or maturity.

Genetic effect of *A. cristatum* 6P translocation segment

A *t* test was performed on an F_2 population and a BC_1F_1 population to further dissect genetic effect of 6P

Table 2 Agronomic traits of Pubing3035 and its contrast parents, common wheat cv. Gaocheng 8901, and disomic addition line 4844-12

Materials	Locations	Plant height (cm)	Fertile tillers	Spike length (cm)	Spikelet number per spike	Kernels per spikelet	Grains per spike	Thousand-grain weight (g)	Spikelet density
Pubing3035	Beijing	104.9b	13.8a	10.8a	19.4b	3.0b	32.0c	41.5a	17.0b
	Xinxiang	116.2b	16.4a	9.5a	19.0b	3.6b	42.2c	47.8a	19.0c
4844-12	Beijing	113.9a	8.0b	9.9ab	21.4ab	4.8a	88.4a	36.4b	20.6a
	Xinxiang	141a	13.8a	9.3a	21.0a	5.6a	79.2b	43.8b	21.5b
Gaocheng 8901	Beijing	63.6c	9.0ab	9.7b	22.2a	5.0a	69.4b	30.9c	22.0a
	Xinxiang	77.2c	14.2a	7.6b	21.6a	4.0b	56.8b	40.9c	27.0a

Data in the column indicate mean

Significant differences in the mean are indicated at the $P < 0.05$ (lowercase letters), based on Duncan's multiple range tests

Table 3 Comparison of yield traits between translocation and non-translocation individuals in segregating populations

Population	Type	Plant height (cm)	Fertile tillers	Spike length (cm)	Kernels per spikelet	Thousand-grain weight (g)	Spikelet density
F_2	6P(+)	111.0a	18.0a	10.2a	3.8a	45.3a	18.9b
		101.8-120.2	12.2-23.7	9–11.3	3.3–4.3	39.3–51.3	16.9–20.9
	6P(−)	108.7b	19.3a	9.5b	3.7a	42.8b	19.6a
		98.3–119.2	12.3–26.3	8.4–10.5	3.2–4.2	34.7–51	17.5–21.7
BC_1F_1	6P(+)	103.8a	23.1a	10.4a	4.0a	44.7a	18.8b
		94.4–113.2	15.3–31	9.3–11.5	3.4–4.6	40.6–48.8	17.2–20.4
	6P(−)	102.9a	19.6b	9.7b	3.9a	42.1b	19.7a
		95–110.8	11.8–27.4	8.6–10.7	3.4–4.4	37.9–46.2	18–21.3

Data in the column indicate mean (variance range), respectively

Significant differences in the mean are indicated at the $P < 0.05$ (lowercase letters), based on Duncan's multiple range tests

chromosome fragment. The results showed that spike length, thousand-grain weight, and spikelet density differed significantly; whereas fertile tillers and kernels per spikelet from the F_2 population were not significantly different between translocation and non-translocation plants. On average, the thousand-grain weight of translocation individuals (45.3 g) was approximately 2.5 g higher than that of non-translocation individuals (42.8 g). The spike length of translocation individuals (10.2 cm) was about 0.7 cm longer than that of non-translocation individuals (9.5 cm). Similar results were found in translocation and non-translocation individuals from the BC_1F_1 population (Table 3). In the BC_1F_1 population, the thousand-grain weight and spike length of translocation individuals was also about 2.5 g higher and 0.7 cm longer than those of non-translocation individuals. These results showed that the wheat-*A. cristatum* 6P translocation individuals contributed to increasing thousand-grain weight and spike length phenotypes, suggesting that they may be useful for improvement of yield and spike traits in wheat.

Quantitative trait locus (QTL) mapping was also conducted to detect QTLs of wheat-*A. cristatum* 6P translocation individuals for different traits including plant height, tiller, spike length, kernels per spike, thousand-grain weight, and spikelet density. A linkage group of chromosome 1AS was constructed with six wheat SSR markers and eight *A. cristatum*-specific EST-STS markers. A total of three QTL clusters were detected and localized around SSR loci *Agc7155* to *SSR263* (Fig. 5). A QTL cluster was detected for increasing spike length, explaining 12.38 % phenotypic variance with an LOD of 4.89. The second QTL cluster explained 24.96 % phenotypic variance of thousand-grain weight with an LOD of 10.63. The third QTL was related to spikelet density, with an effect from alien gene(s) on decreasing spikelet density, and explained 17.2 % phenotypic variance with an LOD of 5.59 (Table 4). Altogether, QTL mapping results further suggested that the 6P alien chromosome segment contains desirable genes for improving grain weight and spike length of common wheat.

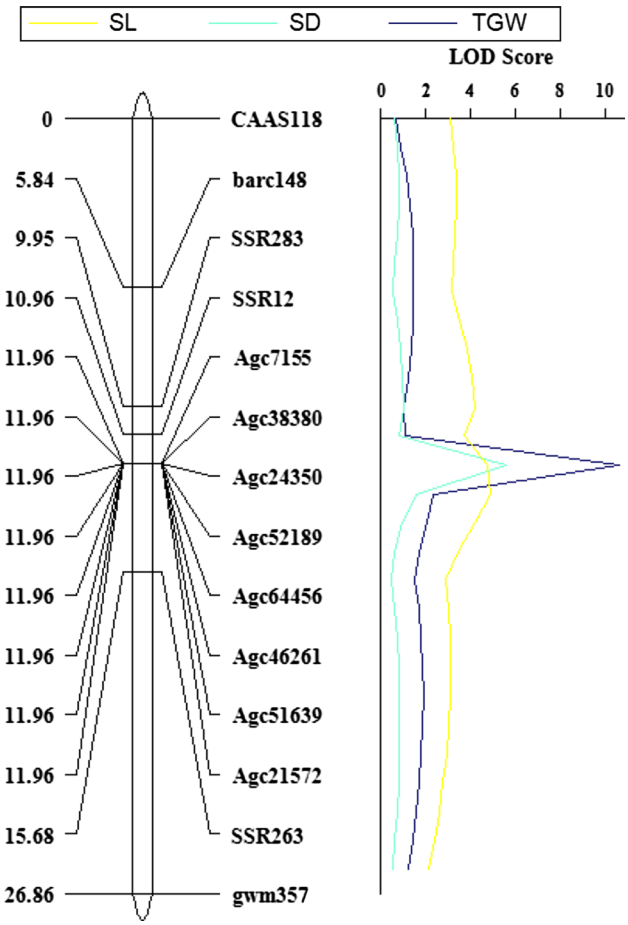

Fig. 5 QTL mapping to detect the genetic effect of alien *A. cristatum* 6P translocation segment for different traits. Linkage map of microsatellite markers and *A. cristatum* EST-STS markers were used for F_2 QTL analysis. Putative QTLs are shown on the *right* side. *TGW* thousand-grain weight, *SL* spike length, *SD* spikelet density

Table 4 QTLs for thousand-grain weight, spike length, and spikelet density based on interval mapping analysis using F_2 population

Trait	Position (cM)	Marker interval	LOD score	Additive effect	Variation (%)
Spike length	13	Agc7155-SSR263	4.89	0.40	12.38
Thousand-grain weight	12	Agc7155-SSR263	10.63	0.86	24.96
Spikelet density	12	Agc7155-SSR263	5.59	−0.33	17.20

Discussion

Wheat-*A. cristatum* 6P intercalary translocation line Pubing3035 is a valuable small segmental translocation line

Crop wild relatives provide a rich genetic resource for wheat improvement (Baum et al. 1992). Wide hybridization is a practical way to introduce excellent alien genes from the wheat wild relative species into the common wheat to enhance the diversity of the wheat genetic pool (Jauhar et al. 2009; Jiang et al. 1993; Niu et al. 2011). Success of chromosome engineering for targeted introgression of alien genes is dependent on elimination of the deleterious effects of the introgressed alien chromatin in the crop plant. However, it has been a challenge to transfer a small amount of alien chromatin containing the gene of interest from one genome to another non-homologous genome (Niu et al. 2011). The acquirement of breeding-usable materials through alien gene introgression is very difficult. For example, only two intercalary translocation lines were detected from 57 spontaneous or induced wheat-alien chromosome translocation lines (Jiang et al. 1993). Generally, translocations with smaller alien segments are genetically more stable and less likely to have deleterious effects (Faris et al. 2008). Many researchers extensively explored strategies to develop small segmental translocation lines with less negative effects using irradiation and *Ph1* system, such as an improved scheme of chromosome engineering for efficient elimination of a large amount of goatgrass (*Aegilops speltoides*) chromatin surrounding *Sr39*, a reduced *H. Villosa* containing chromatin the *Pm21* locus for powdery mildew resistance in wheat, and the creation of wheat-rye terminal and intercalary chromosomal translocations (Bie et al. 2007; Chen et al. 2013; Lukaszewski 2000; Mukai et al. 1993; Niu et al. 2011).

In our study, Pubing3035 was identified as an intercalary translocation line ($2n = 42$) and conferred increasing thousand-grain weight and spike length without negative effects observed in the field environment. Although the translocation Pubing3035 occurred between wheat chromosome arm 1AS and *A. cristatum* chromosome arm 6PL, it was probably genetically stable. Pubing3035 harbored the small *A. cristatum* 6P segment without the possible loss of wheat 1A chromatin. Stable genetic characteristic and phenotypic data suggest that Pubing3035 has no obvious deleterious traits (Table 2). Moreover, previous study shows that *A. cristatum* 6P chromosome generates genetic rearrangements during evolution (Han et al. 2014). Similar spontaneous chromosomal rearrangements have also been reported in wheat-*H. villosa* and wheat-*L. racemosus* translocation lines (Cao et al. 2009; Wang et al. 2010). Hence, it is speculated that the insertion of the 6P chromosome segment in Pubing3035 may have a partial compensation effect for wheat chromosome 1AS. New technology will reveal its sequence collinearity relationship of 6P segment with wheat chromosome 1AS in future works.

Moreover, a number of valuable genes from the wild relative of wheat have been shown to be related to the sixth homoeologous chromosome groups. A 6VS/6AL translocation line was created and it carried powdery mildew resistance gene *Pm21* (Chen et al. 1995; Cao et al. 2011). Du et al. (2013) identified that a wheat-*Ps. Huashanica* Keng 6Ns disomic addition line had twin spikelets and multiple florets and kernels. Powdery mildew resistance gene *PmG3 M* was located on chromosome 6BL of wild emmer wheat by Xie et al. (2012). Here, the results suggest that the 6P chromosome segment also has promising agronomic traits, i.e., higher thousand-grain weight and longer spike length gene(s), and Pubing3035 is a valuable small segmental translocation line. The results from this study have laid the foundation for further research on gene mining.

The introgression of 6P chromosome segment can enhance the thousand-grain weight of common wheat

Thousand-grain weight is an important component of the grain yield in cereals (Campbell et al. 1999; Ketata et al. 1976). However, a range of adverse weather events has brought negative effects on wheat thousand-grain weight, and significantly affected wheat yield in recent years (Lizana and Calderini 2013; Trnka et al. 2014). Thus, the search for new genetic pools that can enhance grain production is urgent. In rice, many genes including *GS3*, *GW2* and *GIF1* have been identified related to grain weight (Ashikari et al. 2005; Fan et al. 2006; Li et al. 2010; Song et al. 2007). In wheat, *TaGW2*, an orthologue of the rice *OsGW2* gene, is considered as a candidate gene related to grain development. The haplotype Hap-6A-A is formed in the promoter region of *TaGW2*-6A and largely associated with wider grains and higher thousand-grain weight (Su et al.

2011). Hou et al. (2014) show that the endosperm starch synthesis pathway related to thousand-grain weight is a major target of indirect selection in global wheat breeding for higher yield. In wheat relatives, 1RS carrying QTL for thousand-grain weight can significantly improve thousand-grain weight, grain length, and width (Xiao et al. 2011). Moreover, 6VS/6AL translocation line was identified to have positive effects on increasing thousand-grain weight and spike length (Li et al. 2011). In this study, intercalary translocation line Pubing3035 with the *A. cristatum* 6P segment had higher thousand-grain weight than common wheat (Table 1). Furthermore, genetic effect analysis from F_2 and BC_1F_1 populations revealed that Pubing3035 had a significantly positive effect on improving grain weight. The increased thousand-grain weight means Pubing3035 could be an important potential donor stock for enhancing wheat yield during breeding.

The intercalary translocation lines showed potential applications for enhancement of wheat spike

Like other crops, spikes are the places where wheat produces grains, and thus they are related to the yield components. Moreover, spikes participate in photosynthesis after heading and they significantly contribute to grain filling, especially under drought conditions (Araus et al. 1993; Maydup et al. 2010; Sánchez-Díaz et al. 2002; Tambussi et al. 2007). Much research attention has, therefore, been paid to spike morphology, which is primarily determined by length, spikelet density, and fertile floret number. A number of studies showed that spike length is positively correlated with shoot biomass, straw biomass per plant, harvest index, and grain yield (Donmez et al. 2001; Moghaddam et al. 1997). Spike length also showed a strong correlation with spikelet density (Schuler et al. 1994). This study showed that the translocation line Pubing3035 possessed the characters of elongation of spike and lowering spike density. Meanwhile, longer spike is frequently associated with less spike compactness (Jantasuriyarat et al. 2004; Ma et al. 2007); it is advantageous for reducing the severity of Fusarium head blight (Buerstmayr et al. 2009, 2011; Mesterhazy 1995), and subsequently alleviating the yield loss and quality deterioration caused by the disease.

Like most agronomic traits, spike length is controlled by multiple genes and affected by environmental conditions (Sharma et al. 2003; Wu et al. 2014). Currently, in field trials conducted in different locations, some differences in spike length existed in the same genotypes (Table 1). The QTL peak for spike length was not obvious, suggesting that expression of the QTL may be involved in genetic and environmental interactions. It was reported that wheat spike length is dependent on genetic and environmental factors; however, genetic factors had a higher influence on expression of spike length in wheat than environmental factors (Šekularac 2013; Zečević et al. 2008). Likewise, Pubing3035 consistently had longer spikes than the recurrent parent Gaocheng8901 and genetic effects of *A. cristatum* 6P on spike length could account for spike length observation in the F_2 population and BC_1F_1 population (Tables 2, 3). Similar results were found in wheat-*A. cristatum* 6P small segmental translocation line (Dai 2012). The same is true for *A. cristatum* with lax spikelet density (Li et al. 1998b). Therefore, it is possible that the desirable genes conferring spike length from *A. cristatum* chromosome 6P could be transferred into wheat for improvement of spike traits.

In summary, the chromosomal segments of *A. cristatum* 6P positively regulating thousand-grain weight and spike length in wheat were pinpointed by dissecting the chromosomal constitution, behavior, and agronomic traits of wheat-*A. cristatum* intercalary translocation line. This work not only lays the foundation for further research on wheat spike traits, but also provides the bridge material for high-yield wheat breeding.

Author contribution statement Li LH conceived the research. Zhang J, Zhang JP performed the research. Zhang J wrote the paper. Liu WH produced the translocation lines. Han HM, Lu YQ, Yang XM and Li XQ participated in the preparation of the reagents and materials in this study.

Acknowledgments This work was supported by the Grants from the National Basic Research Program of China (973 Grant No. 2011CB100104), the National High Technology Research and Development Program of China (863 Grant no. 2011AA100101), the National Natural Science Foundation of China (Grant No. 31071416).

Conflict of interest The authors declare that they have no conflict of interest.

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