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ORIGINAL PAPER

Physical mapping of chromosome 4J of *Thinopyrum bessarabicum* using gamma radiation-induced aberrations

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Abstract

Key message **Gamma radiation induced a series of structural aberrations involving *Thinopyrum bessarabicum* chromosome 4J. The aberrations allowed for deletion mapping of 101 4J-specific markers and fine mapping of blue-grained gene *BaThb*.**

Abstract Irradiation can induce translocations and deletions to assist physically locating genes and markers on chromosomes. In this study, a 12-Gy dosage of ^{60}Co-γ was applied to pollen and eggs of a wheat (*Triticum aestivum*) landrace Chinese Spring (CS)–*Thinopyrum bessarabicum* chromosome 4J disomic addition line (DA4J), and the gametes from irradiated plants were fertilized with normal CS eggs or pollen to produce M_1 seeds. Based on genomic in situ hybridization analysis of 261 M_1 plants, we identified 74 lines carrying structural aberrations involving chromosome 4J with the higher aberration rate in treated pollen (31.2 %) than in the treated eggs (21.3 %). We further identified 43 (53.8 %) lines with structural aberrations on chromosome 4J by analyzing another 80 M_1 plants with 74 4J-specific markers, indicating that combining molecular and cytological methods was more efficient for detecting chromosome aberrations. Marker analysis thus was performed prior to cytogenetic identification on M_2–M_4 seeds to detect chromosome structural aberrations. Sixty-eight M_3 lines with structural aberrations on chromosome 4J and six previously obtained chromosome 4J alien lines were then analyzed using 101 chromosome 4J-specific markers. After combining marker results with chromosome aberrations in each line, chromosome 4J was physically divided into 24 segmental blocks with 7 in the short arm and 17 in the long arm. The blue-grained gene *BaThb* was further mapped into the region corresponding to block 4JL-11. The chromosome aberrations and the physical map developed in this research provide useful stocks and tools for introgression of genes on chromosome 4J into wheat.

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Introduction

Beneficial genes from wheat (*Triticum aestivum* L., $2n = 6x = 42$, AABBDD) relatives can be used to improve end use quality, enhance resistance to biotic and abiotic stress, and broaden genetic diversity in wheat (Kazi et al. 2013; Ashraf and Foolad 2013). However, due to the presence of the *Ph1* gene, induction of homoeologous recombination between chromosomes of wheat and its relatives is difficult and there has, therefore, been limited utilization of alien genes in wheat (Jiang et al. 1994).

Development of translocation lines, particularly lines carrying small alien chromosome segments, is a useful

approach of utilizing alien genes in wheat. So far, several methods such as the *ph1b* mutant (Niu et al. 2011; Zhao et al. 2013), gametocidal chromosomes (Endo 2007), irradiation (Bie et al. 2007; Chen et al. 2013), and spontaneous translocations (Qi et al. 2010; Liu et al. 2013; Shen et al. 2013) have produced translocations for targeted transfer of alien genes into wheat. Due to lack of adequate markers, however, conventional cytological methods are difficult to use to identify the small segmental translocations, particularly the intercalary ones or introgressions. Additionally, cytological techniques are not flexible enough to screen large numbers of samples due to tedious work requirements of chromosome preparation and identification. The development and application of molecular markers provide new ways for fast and verifiable identification of small translocations or introgressions. The combination of molecular and cytological techniques has played an important role in introgression of alien genes into wheat (Qi et al. 2010; Niu et al. 2011; Zhao et al. 2013; Shen et al. 2013; Chen et al. 2013). Therefore, development, identification, and physically locating molecular markers specific for the alien genes will greatly improve the efficiency of alien gene introgression in wheat.

For physically locating markers and genes on the chromosomes, radiation hybrid (RH) mapping has been used in genetic studies of both animals (McCarthy 1996) and plants (Wardrop et al. 2002, 2004). In this method, the donor cells were first induced by irradiation, so they might involve different broken chromosome segments and were individually fused with normal cells of the recipient to produce a series of somatic hybrids. Different chromosome segments from different donor cells were maintained and could be used as a panel for physical mapping and genome analysis (Wardrop et al. 2002, 2004). RH mapping can determine marker order and distance without the requirement of marker polymorphism.

Somatic hybrid-based RH mapping is not a common method for plants because not all plants can be easily regenerated from cell culture. Alternatively, Riera-Lizarazu et al. (2000) developed a new way of RH mapping to generate aberrations containing different segments of chromosome 9 by irradiating the seeds of an oat-maize chromosome 9 monosomic addition line. They then used all the aberrations as a panel for physical mapping of maize chromosome 9. In wheat, a similar method was adopted in mapping chromosome 1D that was introgressed into durum wheat Langdon. A rough RH map of chromosome 1D including 39 molecular markers and the cytoplasm-specific gene (scs^{ae}) was developed (Hossain et al. 2004). Using the same panel, Kalavacharla et al. (2006) developed a high-resolution map of wheat chromosome 1D that included 378 markers. More recently, the scs^{ae} locus was fine mapped to a 1.1-Mb segment after RH mapping using 188 lines generated by gamma radiation followed by artificial pollination (Michalak de Jimenez et al. 2013), indicating RH mapping through seed irradiation can achieve a high marker density.

Gamete irradiation is another effective way for inducing chromosome aberrations (Snape et al. 1983; Lyderson and Carlos 2002; Sanamyan 2003) and RH mapping in plants (Gao et al. 2004; Tiwari et al. 2012). Gao et al. (2004) obtained wide-cross whole-genome radiation hybrids (WWRH) after irradiation of cotton pollen and physically mapped 52 SSR markers in this panel. In wheat, Bie et al. (2007) irradiated the pollen of a durum wheat–*Haynaldia villosa* amphiploid and found 72.1 % of the M_1 plants with chromosome translocations involved chromosomes of *H. villosa* ($2n = 2x = 14$, VV). Chen et al. (2008) irradiated the eggs of a wheat–*H. villosa* T6VS·6AL translocation line and obtained 192 chromosome aberrations which led to physical mapping of 20 specific markers on arm 6VS and then located the powdery mildew resistance gene *Pm21* to a much smaller region on 6VS (Chen et al. 2013). Tiwari et al. (2012) used pollen irradiation to develop a novel approach of constructing RH-based physical maps of all seven D-genome chromosomes of hexaploid wheat rather than just focusing on one single chromosome. Gamete irradiation could be as effective as seed irradiation and may retain more types of chromosome aberrations in M_1 because the treated gametes are fertilized with normal eggs or pollen. In addition, at least half of the genetic materials in the M_1 are not irradiation-damaged, which increases the chance of survival in the M_1 plants that carry aberrations such as deletions.

Thinopyrum bessarabicum ($2n = 2x = 14$, JJ or E^bE^b) confers a high level of salinity tolerance and multiple disease resistance (King et al. 1997; Xu et al. 2009), and thus could be an important genetic resource for wheat improvement. In our previous study, the blue-grained gene *BaThb* of *Th. bessarabicum* was located in region 4JL C-FL0.52 on chromosome 4J and 85 4J-specific markers were physically mapped on this chromosome (Shen et al. 2013). In this research, pollen and egg irradiation was used to induce chromosome 4J aberrations. Cytological techniques and molecular marker analysis were then combined to detect, characterize, and align the induced aberrations, which allowed physical mapping of the blue-grained gene *BaThb*, along with the specific markers on chromosome 4J.

Materials and methods

Plant materials

Wheat landrace Chinese Spring (CS), a *Th. bessarabicum* accession PI 531711, and 14 CS–*Th. bessarabicum*

derivatives were used for determining markers specific for chromosome 4J and allocating the markers to the corresponding chromosomal location in this study. The CS–*Th. bessarabicum* derivatives included a CS–*Th. bessarabicum* amphiploid, three CS–*Th. bessarabicum* disomic substitutions [DS1J(1B), DS5J(5A) and DS6J(6A)], four CS–*Th. bessarabicum* disomic additions (DA4J, DA3J, DA6JS·2JL, and DA7J), one translocation line T2JS-2BS·2BL, and five CS–*Th. bessarabicum* chromosome 4J alien lines including DtA4JS (ditelosomic addition of chromosome 4JS), DtA4JL (ditelosomic addition of chromosome 4JL), i4JL·4JL (isoarm chromosome 4JL·4JL), DA3JS·4JL (disomic addition of translocated chromosome T3JS·4JL), and T4DS·4DL-4JL (translocation 4DS·4DL-4JL). The *Th. bessarabicum* accession PI 531711 was introduced from the United States via Sichuan Agricultural University, China, and the CS–*Th. bessarabicum* amphiploid was kindly provided by Dr. Mujeeb-Kazi of the International Maize and Wheat Improvement Center (CIMMYT, Mexico). All other CS–*Th. bessarabicum* alien lines were developed by Nanjing Agricultural University, China (Qi et al. 2010; Shen et al. 2013). The CS–*Th. bessarabicum* 4J addition line was also used to generate chromosome 4J aberrations through the gamete irradiation method.

Gamete irradiation treatment

Irradiation was performed on pollen and eggs of CS–*Th. bessarabicum* 4J addition line (DA4J). Pollen irradiation was conducted at flowering stage, and spikes of line DA4J were irradiated at 12 Gy using a ^{60}Co irradiator (Yangzhou Academy of Agricultural Sciences, Jiangsu, China). Following irradiation, fresh pollen was collected and used to pollinate emasculated spikes of non-irradiated CS plants. Egg irradiation was performed 2–3 days before flowering time, and plants of DA4J were irradiated with the same dose of radiation. Spikes were then emasculated and pollinated with fresh pollen of non-irradiated CS plants.

Cytogenetic analysis

Chromosome preparations followed the procedures described in Shen et al. (2013). Fluorescence in situ hybridization (FISH) was conducted according to procedures in Chen et al. (1995) and Shen et al. (2013). To precisely identify chromosome aberrations involving *Th. bessarabicum* chromatin, total genomic DNA and centromeric repeats of *Th. bessarabicum* (Wang 2013), simple sequence repeats (AAC)7, two tandem repeats pAs1 and pSc119.2, and one BAC clone 676D4 were used as probes. Of these, total genomic DNA of *Th. bessarabicum* was used to determine *Th. bessarabicum* chromatin and its segmental size. The centromeric repeat of *Th. bessarabicum* was amplified using primer pair Int-F (5-ATGATACTGATTTCAAAGAT-3) and Int-R (5-ACCATACACAATTTCAAAAGG-3) (Fukui et al. 2001) and was used to show centromere location on the chromosomes of both wheat and *Th. bessarabicum* (Wang 2013; Fig. 1). The tandem repeats pAs1 and pSc119.2 can specifically detect wheat chromatin from genomes D and B (Mukai et al. 1993; Fig. 1), and the BAC clone 676D4 can specifically detect wheat chromatin from the A genome (Zhang et al. 2004). The (AAC)*n* probe was also used to detect wheat chromatin (Cuadrado et al. 2008). Sequential GISH/FISH on the same preparation was used to determine the identity of chromosomes or segments from wheat or *Th. bessarabicum*. For sequential FISH/GISH, the preparations from the first FISH were washed in 2× SSC followed by 50 % formamide and were then used for the second FISH. The same preparation could be used no more than three times.

Molecular marker analysis and physical map development

A total of 101 markers specific for chromosome 4J (Table S1) were used for analyzing M_1 plants and their progenies. Of these, 87 markers were reported in Shen (2011) and Shen et al. (2013). The remaining 14 markers were developed in this study with seven produced by primers designed based on wheat EST sequences, one on *Brachypodium* and six were generated from primers based on transcriptomic sequences of *Th. bessarabicum*.

PCR reactions were conducted in a total volume of 10 μL that contained 2 μL (ca.25 ng μL^{-1}) of the template DNA, 0.2 μL left primer (10 μmol L^{-1}), 0.2 μL the right primer (10 μmol L^{-1}), 0.15 μL Taq polymerase (5U μL^{-1}), 0.8 μL $MgCl_2$ (25 mmol L^{-1}), 0.8 μL dNTP (2.5 mmol L^{-1}), 1.0 μL 10× buffer, and 4.85 μL ddH_2O. PCR was conducted in a thermocycler programmed as predenaturation at 94 °C for 3 min, followed by 34–38 cycles of denaturation at 94 °C for 30 s, annealing at 45–58 °C (adjusted according to the primer annealing temperature) for 50 s, and extension at 72 °C for 1 min and 10 s, with a final extension at 72 °C for 10 min. PCR products were separated using 8 % polyacrylamide gel (Acrylamide:*N*′*N*′-methylene-bis-Acrylamid = 39:1 or 19:1 based on the size of PCR products) and then stained with 0.1 % $AgNO_3$ and 2 % NaOH (contains 1 % formaldehyde), and visualized under an automatic gel imager (Peiqing Science and Technology, Shanghai, China). The sizes of the PCR amplicons were determined by a gel image analysis system, Sensi-Ansys 1.0.3 (Peiqing Science and Technology, Shanghai, China).

To develop the physical map of chromosome 4J, the 4J-specific markers were first allocated to the short arm and long arm using chromosome 4J alien lines including DA4J,

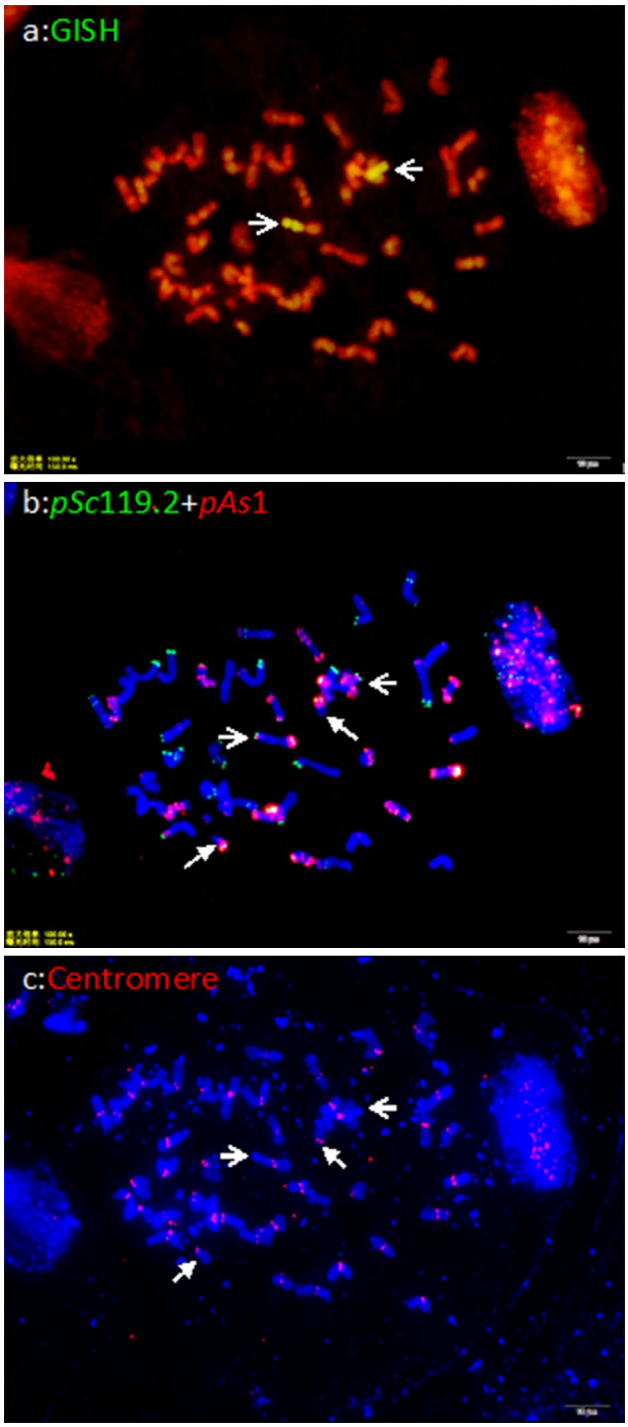

Fig. 1 Chromosomes of wheat–*Th. bessarabicum* translocation line 12PJ212-4 after GISH and sequential multi color (mc)-FISH. *Arrows* indicate the translocated chromosomes and telosomes of 7DS. **a** Total genomic DNA of *Th. bessarabicum* labeled with Fluorescein-12-dUTP (*green*) as a probe; chromosomes were stained with PI (*red*). **b** The repeat sequences pAs1 labeled with Digoxigenin-11-dUTP (*red*), pSc119.2 labeled with Biotin-11-dUTP (*green*) as probes; chromosomes were stained with DAPI (*blue*). **c** The centromeric repeat of *Th. bessarabicum* labeled with Digoxigenin-11-dUTP (*red*) as a probe; chromosomes were stained with DAPI (*blue*) (color figure online)

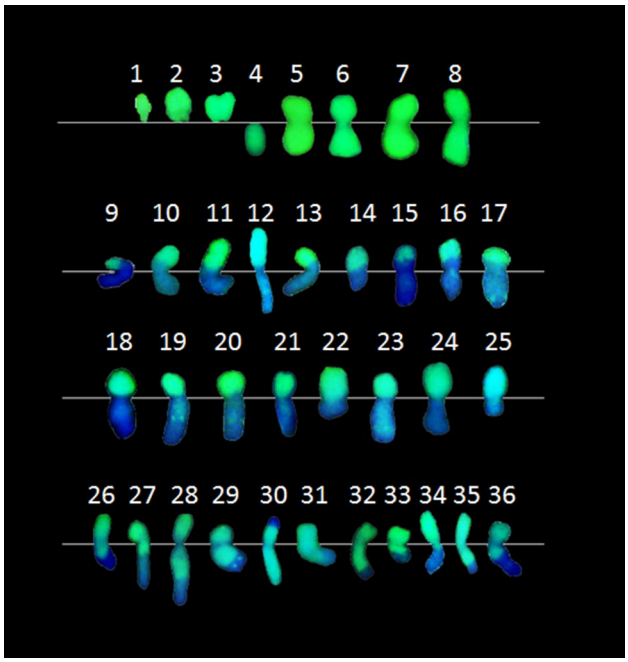

Fig. 2 GISH analysis on structural aberrations of *Th. bessarabicum* chromosome 4J in M_1 plants induced by ^{60}Co-γ radiation. Total genomic DNA of *Th. bessarabicum* labeled with Fluorescein-12-dUTP (*green*) as a probe, and total genomic DNA of CS as block. The chromosomes were counterstained with DAPI (*blue*). *1–8* deletions; *9–17* small segmental translocations (SST, the alien chromatin was smaller than one arm); *18–25* Robertsonian translocations (RT); *26–36* large segmental translocations (LST, the alien chromatin was larger than one arm) (color figure online)

DtA4JS, DtA4JL, i4JL·4JL, and DA3JS·4JL. The translocation line T4DS·4DL-4JL along with all plants carrying chromosome 4J aberrations obtained in this research were used to align the chromosome 4J segments based on their size and chromosomal position revealed through GISH and multi-color FISH. The specific markers were then allocated to the corresponding regions accordingly based on their presence or absence in the corresponding lines.

Results

Chromosome 4J aberrations from gamete irradiation

After pollinating normal wheat flowers with the treated pollen, a total of 432 M_1 seeds were obtained but only 295 (68.3 %) germinated. Cytological analysis was performed on 186 M_1 plants and identified 58 (31.2 %) that carried aberrations involving *Th. bessarabicum* chromosome 4J. The aberrations included deletions (Del), small segmental translocations (SST, smaller than one arm), Robertsonian translocations (RT), large segmental translocations (LST, larger than one arm), and intercalary translocations (IT) (Fig. 2; Table 1).

Table 1 Type and frequency of chromosome aberrations detected in M_1 Plants derived from $^{60}C_O$-γ radiation treatment on gametes of CS–*Th. bessarabicum* 4J addition line

Irradiated gamete	Chromosome aberration type					
	Del	SST	RT	LST	IT	Total
Pollen	9 (9.7 %)	38 (40.9 %)	9 (9.7 %)	36 (38.7 %)	1 (1.1 %)	93
Egg	0 (0 %)	11 (45.8 %)	3 (12.5 %)	9 (37.5 %)	1 (4.2 %)	24
Total	9 (7.7 %)	49 (41.9 %)	12 (10.3 %)	45 (38.5 %)	2 (1.7 %)	117

Del deletion, *SST* small segmental translocation, smaller than one arm, *RT* Robertsonian translocation, *LST* large segmental translocation, larger than one arm, *IT* intercalary translocation

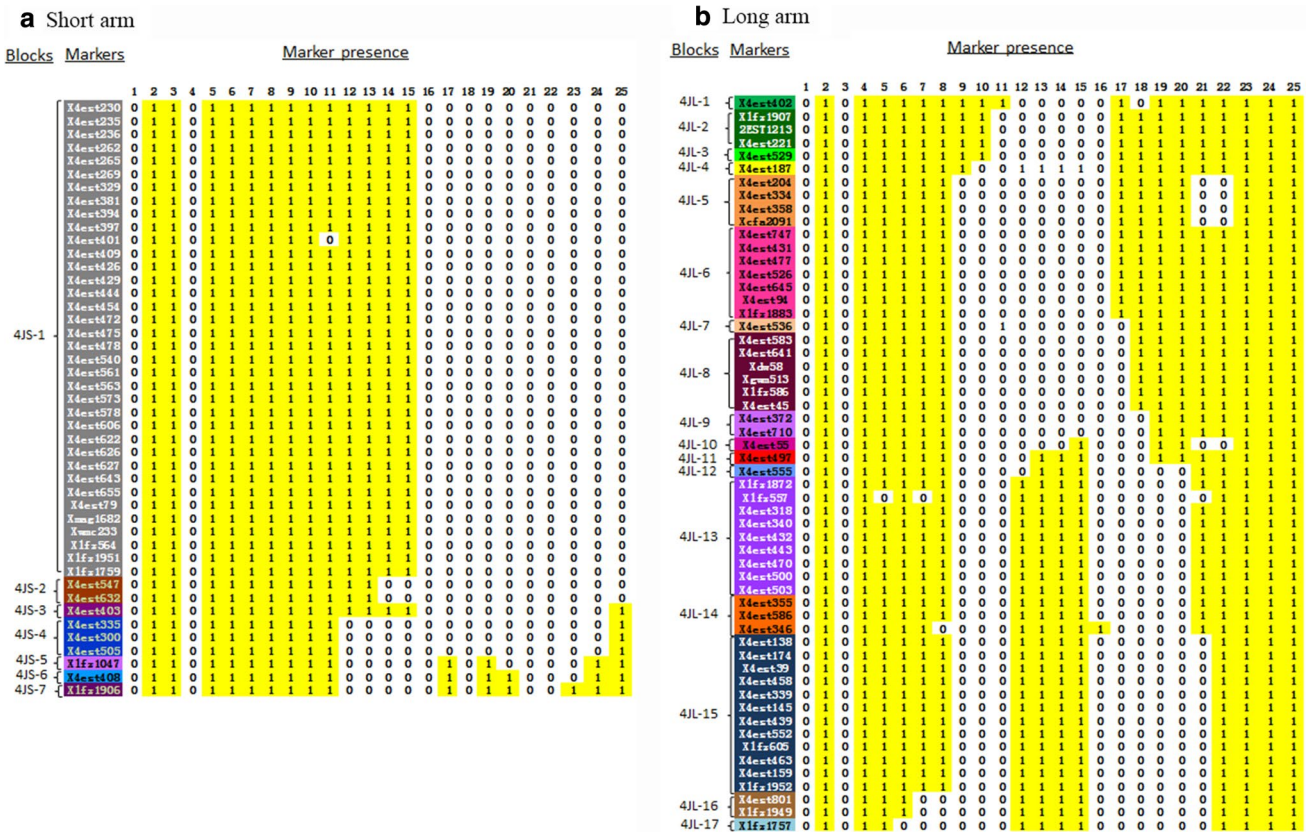

Fig. 3 Physical mapping of chromosome 4J of *Th. bessarabicum* based on analysis of 101 specific markers on CS–*Th. bessarabicum* chromosome 4J alien lines and 68 M_3 plants carried different aberrations of chromosome 4J. **a** Short arm; **b** long arm. The figure shows marker results in 25 lines/plants to reduce the figure size, and the lines are *1* CS; *2* DA4J; *3* DtA4JS; *4* DtA4JL; *5–25* M_3 plants that were labeled as 12PJ168-3, 12PJ167-2, 12PJ168-2, 12PJ242-1, 12PJ162-6, 12PJ164-4, 12PJ157-7, 12PJ183-1, 12PJ183-2, 12PJ183-3, 12PJ183-4, 12PJ245-2, 12PJ159-1, 12PJ158-7, 12PJ184-9, 12PJ184-20, 12PJ182-2, 12PJ182-1, 12PJ187-4, 12PJ212-4, 12PJ185-1, accordingly. The segmental block names are on the *left side* of the chromosome, "*1*" and "*0*" means the presence and absence of the marker, respectively. Markers within the same segmental block are randomly listed but not in genetic order (color figure online)

Of M_1 plants carrying chromosome aberrations, 23 carried only a single variation of SST, LST or Del, while 35 involved two or more aberrations. Overall, the observed frequency of the aberrations were 40.9 % for SST, 38.7 % for LST, 9.7 % for RT, 9.7 % for Del, and 1.1 % for IT (Fig. 2; Table 1).

Pollination of the treated eggs using normal wheat pollen yielded 168 M_1 seeds of which 149 (88.7 %) germinated. Cytological analysis on 75 plants identified 16 plants (21.3 %) that carried aberrations similar to those found in pollen irradiation except for deletion. The observed frequency of aberrations in the treated egg method was 45.8 % for SST, 37.5 % for LST, 12.5 % for RT, 4.2 % for IT, and 0 % for Del (Fig. 2; Table 1). This result was similar to the result seen in the pollen irradiation.

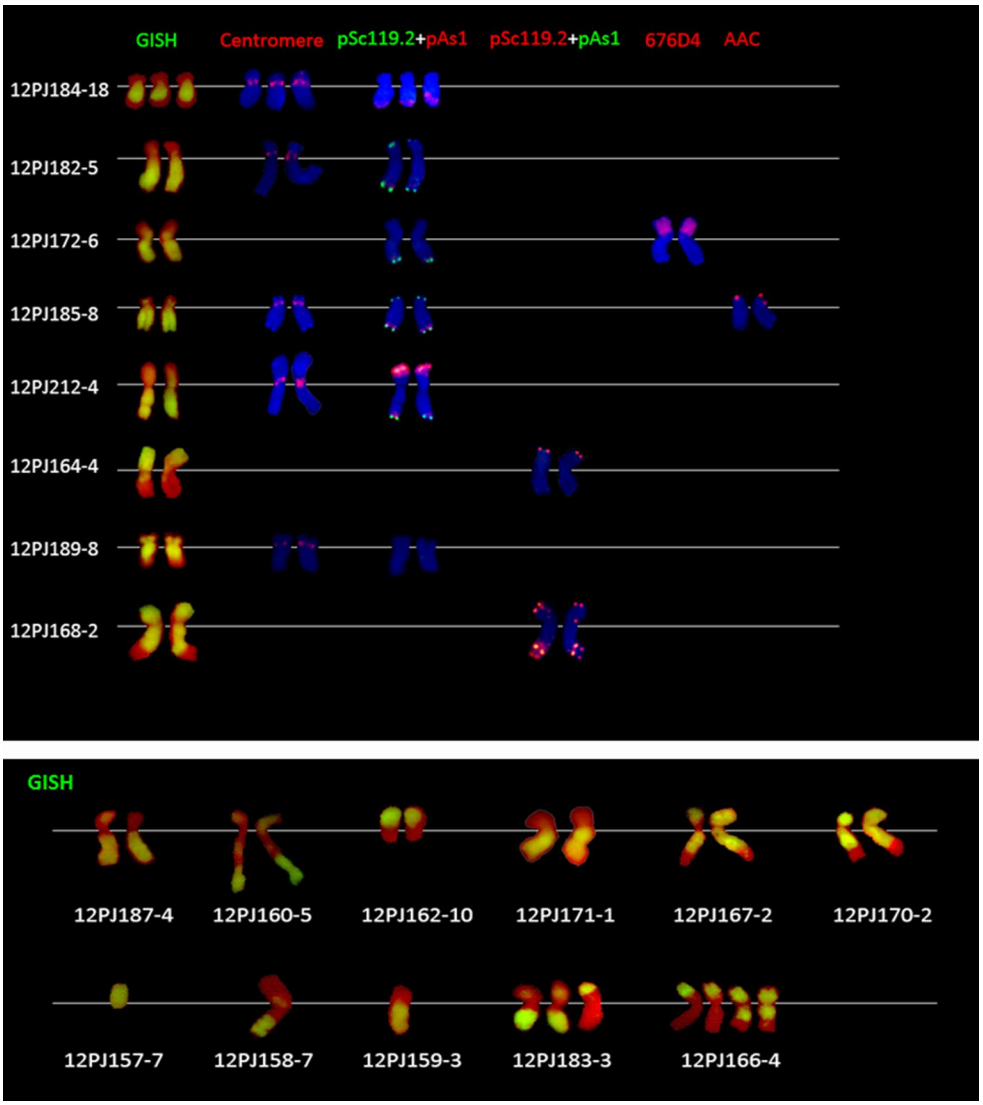

Fig. 4 Images of individual aberrant chromosomes detected in 19 M_4 lines after GISH and sequential FISH analysis and which had steady transmissible to progeny. The name of *each line* is shown in *white* accordingly (color figure online)

Other than the above mentioned cytologically analyzed M_1 plants, a subset of 80 plants were only investigated using 74 markers specific for *Th. bessarabicum* chromosome 4J (Shen et al. 2013), and these markers identified 43 (53.8 %) plants carrying aberrations. Of these, four carried aberrations on arm 4JS only, 19 had aberrations on arm 4JL only, and 20 had aberrations involving both arms; indicating the aberrations occurred across the centromere. In all, 117 M_1 plants that carried chromosome aberrations were identified.

Molecular and cytological characterization of chromosome 4J aberrations induced by gamete irradiation

The set of 117 M_1 plants that carried chromosome aberrations were self-pollinated to produce M_2 and then M_3 seeds. During the process, marker analysis combined with GISH and phenotypic observations were used to identify and track the aberrations. A total of 68 M_3 plants carried aberrations on chromosome 4J. Among them, 50 plants carried part of the 4J-specific markers and GISH analysis indicated that they carried different translocations and deletions (Fig. 3). Two plants carried only one marker specific to chromosome 4J, but GISH analysis failed to detect 4J chromatin, indicating they may carry a 4J segment too small to be revealed through cytological methods. Sixteen plants carried all chromosome 4J-specific markers, and GISH indicated that two plants were disomic substitutions, two had translocations that involved a large 4J segment and a small piece of wheat chromosome, and 12 were disomic or monosomic additions (Fig. 3).

The 68 M_3 plants carrying aberrations on chromosome 4J were self-pollinated to produce M_4 seeds. Multi-color

Table 2 List of 19 M_4 lines that carried steady transmissible aberrations on chromosome 4J of *Th. bessarabicum*

Line name	Chromosome number	Aberrations[a]	Zygosity [b]	Grain color
12PJ182-5	$2n + 2 = 44$	TW·W-4JL	Homo	Blue
12PJ172-6	$2n = 42$	T4AS-4JS·4JL	Homo	Blue
12PJ185-8	$2n + 2 = 44$	T2BS-4JS·4JL	Homo	Blue
12PJ187-4	–	TW·4JL	Homo	Blue
12PJ160-5	$2n = 42$	TW·W-4JL	Homo	Blue
12PJ212-4	$2n + 2 = 44$	T7DL-4JS·4JL	Homo	Blue
12PJ162-10	$2n + 2 = 44$	T4JS·4JL-W	Homo	White
12PJ164-4	$2n + 2 = 44$	T4JS·4JL-W	Homo	White
12PJ184-18	$2n = 42$	Tdel1DS-4JS·4JL-1DL	Hetero	Blue
12PJ171-1	$2n + 2 = 44$	T4JS·4JL-W	Homo	Blue
12PJ183-3	$2n = 42$	TW·W-4JL	Hetero	Blue
12PJ170-2	$2n + 2 = 44$	T4JS·4JL-W	Homo	Blue
12PJ166-4	$2n + 2 = 44$	T4JS·4JL-W and TW·W-4JL	Homo	Blue
12PJ189-8	$2n + 2 = 44$	Tdel4JS·4JL-W	Homo	White
12PJ167-2	$2n + 2 = 44$	T4JS·4JL-W	Homo	Blue
12PJ168-2	$2n = 42$	T4JS·4JL-W	Homo	Blue
12PJ157-7	–	del4J-1	Hetero	White
12PJ158-7	–	TW·W-4JL	Hetero	White
12PJ159-3	–	TW-4JS·4JL-W	Hetero	White

[a] In chromosome aberrations "T" means translocation, and "W" means wheat chromatin where its chromosome identity had not been determined. "–" indicates data not available

[b] Homo = homozygous, hetero = heterozygous

FISH and the sequential FISH/GISH revealed that 19 M_4 lines contained different structural aberrations such as large segmental translocations, disomic additions of large segmental translocations, intercalary translocations, small segmental translocations, Robertsonian translocations, deletions, disomic additions of deletion and translocated chromosomes, and disomic substitutions of LST with seed color blue or white as expected (Figs. 1, 4; Table 2). These 19 M_4 lines could be considered stable 4J alien lines since the aberrations can be stably transmitted among generations and 15 of them carried homozygous aberrations.

Physical mapping of chromosome 4J and the blue-grained gene

One hundred and one chromosome 4J-specific markers were used to analyze CS, CS–*Th. bessarabicum* amphiploid, the above mentioned 68 M_3 plants that contained chromosome 4J aberrations, and six previously obtained chromosome 4J alien lines including DA4J, DtA4JS, DtA4JL, i4JL·4JL, DA3JS·4JL, and T4DS·4DL-4JL (Shen et al. 2013). From the analysis, we were able to determine that 4J can be physically divided into 24 segmental blocks with 7 blocks on the short arm (labeled as 4JS-1–7) and 17 blocks on the long arm (4JL-1–17). The blocks on the short arm contained 45 chromosome 4J-specific markers while those on the long arm included 56 specific markers (Fig. 3).

In previous studies, the blue-grained gene *BaThb* of *Th. bessarabicum* was allocated in region 4JL C-FL0.52 (Shen et al. 2013). The region corresponds to segmental blocks 4JL-1–14. The M_4 line 12PJ184-18 has blue grains and carried an IT translocation (Figs. 4, 5). Marker analysis revealed that the chromosome 4J segment in this line corresponds to segmental blocks 4JS-5–4JL-11 (Fig. 5), indicating that *BaThb* should be within the region corresponding to blocks 4JL-1–11, which comprised of the overlapping region of the 4J segment in 12PJ184-18 with the region 4JL C-FL0.52. The white-grained M_4 line 12PJ189-8 carried a pair of translocated chromosomes with a deletion on the 4JS distal end (Fig. 5). The 4J segment in this line corresponds to segmental blocks of 4JS-5–4JL-6 (Fig. 5), suggesting that *BaThb* should be located in the region corresponding to blocks 4JL-7–11 since there is no blue-grained gene in line 12PJ189-8. Another blue-grained M_4 line, 12PJ183-3, carried heterozygous reciprocal translocations and the two 4J segments in this line correspond to blocks 4JS-1–3 on the short arm and 4JL-11–17 on the long arm, respectively (Figs. 4, 5). The common region of 4J segment in this line with blocks 4JL-7–11 is the block 4JL-11; therefore, *BaThb* should be located in the region corresponding to this block (Fig. 5). *X4est497* is the only marker in segmental block 4JL-11, so it could be used for tagging *BaThb* during introgression of the blue-grained gene into wheat.

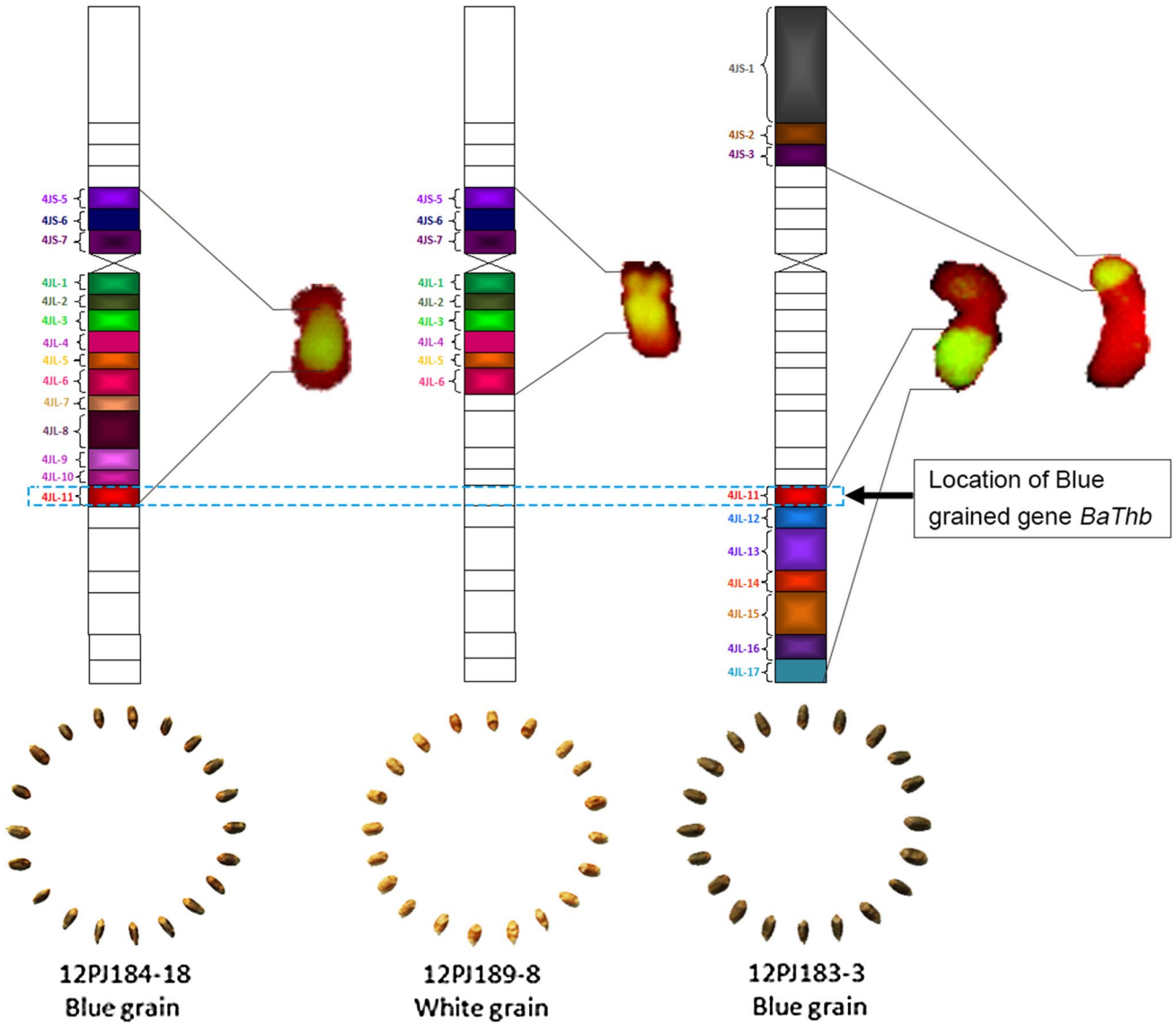

Fig. 5 Physical localization of *BaThb*, the blue-grained gene of *Th. bessarabicum*, to a region corresponding to segmental block 4JL-11 on chromosome arm 4JL (color figure online)

Discussion

In this study, we successfully generated a series of wheat lines carrying different segments of *Th. bessarabicum* chromosome 4J using gamete irradiation. The aberration ratio in the treated pollen (31.2 %) was higher than that of the treated eggs (21.3 %). This not only confirmed that gametes treated on alien chromosome lines by ^{60}Co-γ radiation is an effective way of inducing aberrations on alien chromosomes, but also suggested that treated pollen will be more effective than treated eggs as described in Bie et al. (2007) and Chen et al. (2008). Additionally, marker and GISH analysis in this study revealed the aberrations almost covered the entire chromosome 4J, but no translocation hot spot could be identified (Fig. 3). This indicated that the radiation-induced aberration likely occurred randomly, and this further validated that radiation-induced aberrations could be suitable for physical mapping of alien chromosomes.

In gamete irradiation for RH mapping, the treated gametes were immediately fertilized with the normal pollen or eggs, and the M_1 plants would have at least half of the genetic materials coming from the normal pollen or eggs with no irradiation damage. The non-damaged genetic materials could provide compensatory effects for their homologous parts that were irradiation-damaged. This would increase the chance of survival in M_1 plants even though they may carry chromosome aberrations such as

large deletions. So gamete-irradiated M_1s and their self-pollinated progenies could retain more different types of chromosome aberrations than in spontaneous method as described in *Th. bessarabicum* chromosome 4J (Shen et al. 2013), and thus they could have more potential for increasing the resolution in physical mapping.

In this research, we used 68 M_3 and 19 M_4 lines that carried aberrations for mapping of chromosome 4J. A higher resolution physical map of chromosome 4J was thus developed where we divided chromosome 4J into 24 segmental blocks, which further located the blue-grained gene *BaThb* into the region only corresponding to the block 4JL-11 (Figs. 3, 4). During cytogenetic analysis of plants in this study, we also found that irradiation-induced aberrations occurred on wheat chromosomes (data not shown). The M_2 plants derived through self-pollination of M_1s showed many kinds of variations such as partial or complete male sterility, very short plant height, later maturity, and branches on spikes, or even plant death. Therefore, the continuous backcrossing of M_1 plants (or M_2 and M_3 plants later on) with normal non-irradiated wheat lines using the gamete irradiation method will not only maintain more types of chromosome aberrations, but also can create genetic stocks by carrying small 4J segments in different genetic backgrounds. Actually, we are currently backcrossing the M_2–M_4 plants with the elite breeding lines to develop new germplasm, as well as maintain the chromosome aberrations identified.

Previously, the GISH technique was the commonly used method for identifying aberrations of alien chromosomes. However, GISH sometimes failed to detect aberrations that involved very small alien chromosome segments, mainly because the small segment either had an undetectable, weak signal due to small size, or the segment was blocked from producing signal if it was inserted into wheat chromosomes and wheat genomic DNA was used as a blocker. In this study, we detected 43 out of 80 M_1 plants that carried aberrations by only using the markers specific for the 4J chromosome, indicating that marker analysis can be more efficient than cytological methods, particularly for detecting aberrations involving tiny pieces of segment. Using markers alone, however, makes it difficult to determine the marker location on the chromosome. Therefore, combining marker and cytological methods will not only increase the possibility of detecting chromosome aberrations, but also assist in determining chromosome identity and marker order.

In summary, this research used ^{60}Co-γ radiation to treat gametes of CS–*Th. bessarabicum* 4J addition line and produced aberrations involving different segments of chromosome 4J. Marker analysis combined with cytological assay physically divided chromosome 4J into 24 segmental blocks and physically located 101 4J-specific markers onto corresponding blocks. The blue-grained gene *BaThb* thus was mapped to an even smaller region corresponding to block 4JL-11. The lines contained different 4J chromosome aberrations and the 4J physical map developed in this research will be very useful for utilizing beneficial genes on chromosome 4J in wheat.

Author contribution statement J P and QW performed experiment in M_2–M_4 plants, preparing the manuscript. YS performed experiment in M_1 plants. LZ contributed to development of chromosome aberrations and suggestions to the project and manuscript. CL designed primers and developed markers based on transcriptome data. TF in silico mapped the primer sequences in wheat and model plants. TB contributed to development of chromosome aberrations via gamma irradiation and suggestions to the manuscript. CC, a collaborator, contributed to manuscript development and suggestions to the project. ZQ, the corresponding author, oversaw all activities related to the project implementation and manuscript development. All the authors reviewed and approved this manuscript.

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