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ORIGINAL PAPER

Chromosome engineering, mapping, and transferring of resistance to Fusarium head blight disease from *Elymus tsukushiensis* into wheat

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Abstract
Key message **This manuscript describes the transfer and molecular cytogenetic characterization of a novel source of Fusarium head blight resistance in wheat.**
Abstract Fusarium head blight (FHB) caused by the fungus *Fusarium graminearum* Schwabe [telomorph = *Gibberella zeae* (Schwein. Fr.) Petch] is an important disease of bread wheat, *Triticum aestivum* L. ($2n = 6x = 42$, AABBDD) worldwide. Wheat has limited resistance to FHB controlled by many loci and new sources of resistance are urgently needed. The perennial grass *Elymus tsukushiensis* thrives in the warm and humid regions of China and Japan and is immune to FHB. Here, we report the transfer and mapping of a major gene *Fhb6* from *E. tsukushiensis* to wheat. *Fhb6* was mapped to the subterminal region in the short arm of chromosome $1E^{ts}$#1S of *E. tsukushiensis*. Chromosome engineering was used to replace corresponding homoeologous region of chromosome 1AS of wheat with the *Fhb6* associated chromatin derived from $1E^{ts}$#1S of *E. tsukushiensis*. *Fhb6* appears to be new locus for wheat as previous studies have not detected any FHB resistance QTL in this chromosome region. Plant progenies homozygous for *Fhb6* had a disease severity rating of 7 % compared to 35 % for the null progenies. *Fhb6* has been tagged with molecular markers for marker-assisted breeding and pyramiding of resistance loci for effective control of FHB.

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Introduction

Fusarium head blight (FHB) caused by the fungus *Fusarium graminearum* Schwabe [telomorph = *Gibberella zeae* (Schwein. Fr.) Petch] is an important disease of bread wheat, *Triticum aestivum* L. ($2n = 6x = 42$, AABBDD) worldwide. FHB infection not only leads to reduced grain yield but also reduced quality because the grains are contaminated with the mycotoxin deoxynivalenol (DON). Host plant resistance is the most effective method of FHB control. In general, two major types of FHB resistance are recognized, resistance to initial infection (type-1) and resistance to spread of infection within the spike (type-2) (Bai and Shaner 1994; Mesterhazy 1995). There are limited sources of resistance to FHB, inheritance of resistance is often quantitative and complex, and resistance expression to FHB and DON levels is greatly affected by genetic background. Therefore, there is a constant need for evaluating and identifying new sources of resistance in alien germplasm as well as in wheat.

To date, five genes conferring resistance to FHB have been mapped and named. *Fhb1* and *Fhb2,* conferring type-2 FHB resistance, present in wheat cultivar Sumai 3

and were mapped to the short arms of wheat chromosomes 3B (Liu et al. 2006) and 6B (Cuthbert et al. 2007). *Fhb1* is being used intensely in wheat improvement. *Fhb3* was derived from a tetraploid wheat relative *Leymus racemosus* (Tein.) Tzvelev (syn *Elymus giganteus* Vahl.), and was transferred to wheat in the form of a compensating Robertsonian translocation T7AL·7Lr#1S (Qi et al. 2008). Like *Fhb1* and *Fhb2*, *Fhb3*, confers type-2 resistance. *Fhb4* and *Fhb5* conferring type-1 resistance were identified in the Chinese cultivar Wangshuibai and were mapped to the long arm of chromosome 4B (Xue et al. 2010) and the short arm of 5A (Xue et al. 2011), respectively.

Elymus tsukushiensis Honda ($2n = 6x = 42$, $S^{ts}S^{ts}H^{t\text{-}s}H^{ts}Y^{ts}Y^{ts}$, syn. *Roegneria kamoji* C. Koch) is a perennial cross-pollinating hexaploid species native to China, Korea, and Japan. *E. tsukushiensis* is a distant wild relative of bread wheat and a source of resistance to FHB (Weng and Liu 1989; Weng et al. 1995). Wang et al. (1999) reported the production and characterization of wheat-*E. tsukushiensis* chromosome addition lines; they also reported that the disomic addition having the group 1 *E. tsukushiensis* chromosome $1E^{ts}$#1S, added to the wheat genome, conferred resistance to FHB. Here, we report the production and characterization of novel FHB-resistant wheat-*E. tsukushiensis* recombinants with resistance to FHB, that may be employed in wheat improvement.

Materials and methods

Plant material

The material analyzed consisted of the wheat-*E. tsukushiensis* disomic chromosome addition line DA1E^{ts}#1 (TA7684), the derived wheat-*E. tsukushiensis* disomic addition/translocation line DATW·1E^{ts}#1S (TA5655), and the wheat cultivars 'Chinese Spring', 'Everest', 'Karl 92', and 'Overley'. The small metacentric wheat-*E. tsukushiensis* TW·1E^{ts}#1S translocation chromosome was recovered in the progeny of DA1E^{ts}#1. Line TA5655 with TW·1E^{ts}#1S added to the wheat complement ($2n = 44$) was crossed twice with the *ph1b* mutant stock (TA3809) and plants with $2n = 43$ and homozygous *ph1b/ph1b* and hemizygous for DATW·1E^{ts}#1S were selected and their progenies screened by molecular markers to identify putative recombinants. Chromosome designations follow the nomenclature guidelines proposed by Raupp et al. (1995) where 'T' indicates a terminal translocation, 'Ti' indicates an interstitial translocation, '·' marks the centromere, '-' marks an interstitial translocation breakpoint, the number indicates the homoeologous group, followed by the genome symbol, and the chromosome arm designation 'S' for short and 'L' for long arms, the '#' sign is used to distinguish between the same alien chromosome transferred from different donor accessions.

Cytological procedures

Genomic in situ hybridization (GISH) and fluorescence in situ hybridization (FISH) were performed according to Zhang et al. (2001). Oligonucleotide probe pAs1 (Danilova et al. 2012) hybridized to all D-genome chromosomes of wheat permitting their unambiguous identification (Rayburn and Gill 1986). The BAC clone 676D4, isolated from *T. monococcum,* paints all A-genome chromosomes of wheat over their entire lengths (Zhang et al. 2004a, b). Squash preparations were made after staining with acetocarmine. After hybridization at 37 °C overnight, the slides were washed in 2X SSC twice at room temperature for 5 min, twice at 42 °C for 10 min, 5 min at 42 °C, and once at room temperature for 5 min. A drop (25–30 µl) of Vectashield mounting medium containing 1 µg/ml of PI (Cat. No. H-1400, Vector laboratories Inc., Burlingame, CA) was added to each slide, and then covered with a 24 × 30 cm glass cover slip. Images were captured with a SPOT2.1 charge-coupled device (CCD) camera (Diagnostic Instruments, Sterling Heights, MI) using an epifluorescence Zeiss Axioplan 2 microscope. Images were processed with Adobe Photoshop CS3 (Version 10.0.1) (Adobe Systems Incorporated, San Jose, CA). C-banding and chromosome identification was according to Gill et al. (1991).

Marker development and screening

A total of 96 wheat expressed sequence tags (ESTs) previously mapped from the centromere to the telomere in the short arms of wheat group 1 were selected from data in the wheat EST Mapping Project (http://wheat.pw.usda.gov/NSF/project/mapping_data.html). The sequences of these ESTs were used to design primers using Primer 3 software (Rozen and Skaletsky 2000). DNA from all samples was isolated using a Qiagen Biosprint 96® robot with a Biosprint 96 DNA plant kit® (Qiagen, Valencia, CA) according to the manufacturer's instructions. DNA was quantified on a Nano-drop® and normalized to 25 ng/µl for PCR. The conditions for PCR amplification and enzyme digestion of the post-PCR products were according to Qi et al. (2007). Four polymorphic EST–STS markers were developed to screen progenies of plants homozygous for *ph1b* and heterozygous for TW·1E^{ts}#1S for the presence of putative recombinants (Table 1). Dissociation of these markers identified putative recombinants that were further characterized by GISH.

For tagging the *E. tsukushiensis* segment, we developed markers by comparative sequence analysis of wheat with rice chromosome 5, Brachypodium chromosome 2

Table 1 Primer sequences for CAPS and SNP used the present study

Marker	Forward primer 5′-3′	Reverse primer 5′-3′	Annealing temper. (°C)	Enzyme producing polymorphic PCR product	EST/flcDNA	Rice locus	Brachypodium locus	Chromosome location[a]
BE591682	TGTTTGGGGTGTTTCACTCA	AGCAATTTGGATGACGAACC	55.0	*Alu*I or *Hae*III	BE591682	Os05g02490	Bradi2g37440	1AS3-0.86-1.00
BG607503	AAACATTGCGCCCTATAACG	TCCCAATTCCTCTCCAACTG	55.0	*Rsa*I	BG607503	Os05g03820	Bradi2g38340	1DS1-0.59-0.70
BE426771	TCCGCACTACTGGGTGAAGT	ATATTGTGCTTTGCGCTTCC	55.0	*Rsa*I	BE426771	Os05g03540	Bradi2g38140	1DS3-0.48-0.59
BF202643	GTCCTAGCGCTTCACCTCAC	TCTTTCCCGACTCATTGTCC	55.0	*Hae*III	BF202643	Os10g27340	Bradi3g26660	C-1DS3-0.48
tplb0017E15	CACCTTTGGAGACGGTTCAT	CACAAGCGATCTGGAGAACA	55.0	*Hae*III	tplb0017e15	Os05g01610	Bradi2g39600	–
tplb0029J02	ATGGCAAATGGATTGCTGAT	GCATACGAAGGAGAGGCAAG	55.0	*Rsa*I	tplb0029j02	Os05g02530	Bradi2g37480	–
AK357509	ATTGACGTTTACGGCTTTGG	TGCAAGTAGTCTTGGCATCG	55.0	*Hae*III	AK357509	Os05g07420	–	–
wg1S_snp1[b]	CATACATGGAAAGCCAGTAAGCAACT ATACATGGAAAGCCAGTAAGCAACC	TGCAAGCTCATCCCTGCATCTCTT	55.0	–	–	–	–	–

[a] Chromosome location was derived from http://wheat.pw.usda.gov/NSF/project/mapping_data.html

[b] Wg1s_snp1 is KASPar marker with two allele-specific forward primer and one common reverse primer allele FAM T, allele HEX C

and barley chromosome 1. Further sorted chromosome arm sequence of wheat chromosome 1AS was also used. Wheat ESTs/flcDNA from the terminal end of wheat group 1 chromosomes was used to design 50 conserved primers from the exons amplifying the introns. Amplicons from Chinese Spring, Everest and TW·1E^{ts}#1S (TA5655) were digested with seven enzymes, *Hae*III, *Alu*I, *EcoR*I, *Rsa*I, *Msp*I, *Mse*I, *Mbo*I, and the cleaved products were separated on 2 % agarose gel to identify polymorphic markers. The polymorphic markers were used to screen the recombinant progenies. SNP based KASPar™ markers were designed from 20 SNPs from the 10 K infinium chip developed by Luo et al. (2013). All samples were genotyped for the SNP markers using KASPar technology by KBioscience® (http://www.kbioscience.co.uk/). DNA from the parents and recombinants was amplified using two allele-specific primers and a common primer as described in Cuppen (2007) on a CFX96 Touch™ Real-Time PCR detection system (Bio-Rad, Hercules, CA). Normalized signals from each SNP allele (*x* and *y*) were plotted in two dimensions using Biorad CFX Manager software v3.1 under the allelic discrimination mode.

FHB resistance screening

For entries that displayed spring habit, 5 seeds were sown in a 15 cm diameter plastic pot with four replications, and incubated in a greenhouse (25 ± 4 °C) with supplemental light. For entries that displayed winter habit, 3 seeds were sown in a plastic cone (2.5 × 13 cm) with four replications and the resulting seedlings were grown in the greenhouse for 10 days. The seedlings were then vernalized at 4 °C for 7 weeks and transplanted into 15 cm diameter pots, 3 tubes per pot with four replications. Transplanting was timed to synchronize, as much as possible, heading dates between the winter and spring habits. Pots were arranged in a randomized design on the greenhouse bench and plants were watered and fertilized as needed. About 10 heads per pot, 40 heads per entry, were inoculated soon after emergence. A single floret on the tenth spikelet from the bottom of each head was inoculated. A conidial suspension (10 µl) with about 10^5 spores per ml of *Fusarium graminearum* culture 3639 was introduced between the lemma and palea with a pipette. Spores were produced by inoculating sterile mung bean broth with 2–3 cubes cut from a fresh fungal culture growing on homemade potato-dextrose agar. Mung bean broth (100 ml in a 250 ml flask) was produced by adding 40 g mung beans to 1 L of boiling distilled water, boiling for 8 min, filtering through cheesecloth, re-adjusting to 1 L, and autoclaving. The homemade potato-dextrose agar consisted of 250 g peeled potato pieces (2.5 cm cubes) boiled until soft (20–25 min) in 1 L distilled water, filtered through two layers of cheesecloth, readjusted to 1 L, augmented

with 2 % dextrose and 2 % agar, and autoclaved for 20 min. Flasks were incubated on a rotary shaker for 2–5 days to produce suitable suspensions of macroconidia. Inoculated heads were immediately bagged with a 7.5 × 13 cm "zip-lock" plastic bag that had been misted with water on the inside with a hand-operated plant mister (spray bottle). The bags were removed 48 h after inoculation and heads rated 14 days after inoculation for percentage spikelets blighted by the fungus. The following rating scale was used to rate FHB severities: no symptoms = 0 %; only the inoculated floret blighted = 3 %; two of the three florets in the inoculated spikelet blighted = 7 %; only the inoculated spikelet blighted = 10 %; the inoculated spikelet and the spikelet immediately below the inoculated one blighted = 20 %; three spikelets blighted = 30 %; etc. if the inoculated spikelet and all 9 spikelets below the inoculated one were blighted, severity = 100 %. Spikelets above the inoculation site were ignored because the fungus can girdle the rachis and blight all distal spikelets without actually colonizing them. The above scale rates the ability of the fungus to run down the rachis and blight the head (type-2 resistance).

Results

Two PCR-based markers, WPG90 and PSR2120, previously developed and mapped within the *ph1b* deletion (Sehgal et al. 1997; Roberts et al. 1999) and two EST–STS markers, BF202643/*Hae*III and BE591682/*Hae*III specific for TW·1E^{ts}#1S developed in this study (Table 1), were used to screen 96 BC_1 plants derived from the cross DATW·1E^{ts}#1S translocation x the *ph1b* mutant: 23 plants were identified as homozygous *ph1b/ph1b* and hemizygous for the TW·1E^{ts}#1S translocation.

Meiotic metaphase I pairing was analyzed in pollen mother cells (PMCs) of $2n = 43$ chromosome plants homozygous for *ph1b* and hemizygous for TW·1E^{ts}#1S by GISH. Chromosome TW·1E^{ts}#1S was univalent in 152 PMCs (57 %; Fig. 1j) and was paired in the wheat arm in 117 PMCs (43 %; Fig. 1k, l) but no pairing was observed between the 1E^{ts}#1S segment and a wheat chromosome arm in the 269 PMCs analyzed. Because meiotic metaphase I pairing is triggered by homology at the chromosome ends and the wheat segment in TW·1E^{ts}#1S was paired in 43 % of the PMCs with a normal complete wheat chromosome, this suggests that this segment was derived from the telomeric region of a wheat chromosome. However, no pairing was observed between the *E. tsukushiensis* chromosome segment and a wheat chromosome indicating that the recovery of wheat-*E. tsukushiensis* recombinants will be difficult.

Figure 1a shows the GISH pattern of chromosome 1E^{ts}#1 and Fig. 1b shows the GISH pattern of the derived small metacentric TW·1E^{ts}#1S chromosome. The unidentified wheat chromosome segment in TW·1E^{ts}#1S is very small, suggesting that this wheat segment suffered from a large deletion and in addition is also likely to be rearranged. We identified one plant that was homozygous for TW·1E^{ts}#1S and had $2n = 42$ chromosomes. This plant was highly sterile further indicating that TW·1E^{ts}#1S is highly rearranged as it did not compensate for the missing wheat chromosome.

One polymorphic proximal group 1 short arm marker BF202643 previously mapped to deletion bin C-1DS3-0.48, and one polymorphic distal group 1 short arm marker, BE591682 previously mapped to deletion bin 1AS3-0.86-1.00 (http://wheat.pw.usda.gov/NSF/project/mapping_data.html) were used to screen 488 progenies of plants homozygous for *ph1b* and heterozygous for TW·1E^{ts}#1S for the presence of putative recombinants. Two plants, #74 and #107, were identified to be recombinants. Plant #74 had the 1E^{ts}#1S allele of *BF202643*, but was missing the 1E^{ts}#1S allele of *BE591682*, indicating that this plant was a proximal recombinant. Plant #107 was missing the 1E^{ts}#1S allele of *BF202643*, but had the 1E^{ts}#1S allele of *BE591682*, indicating that it was a distal recombinant. The recombinant chromosomes in both plants #74 and #107 were further characterized by GISH. Plant #74 was heterozygous for an interstitial wheat-*E. tsukushiensis* translocation, consisting of the long arm of an unidentified wheat chromosome, part of the short arm of a wheat chromosome, a small segment derived from 1E^{ts}#1S and a distal part of a wheat chromosome, which can be described as TiWL·WS-1E^{ts}#1S-WS (Fig. 1d). Plant #107 was heterozygous for a distal wheat-*E. tsukushiensis* translocation, consisting of the long arm of a wheat chromosome, part of the short arm of a wheat chromosome and a distal part of 1E^{ts}#1S, designated as TWL·WS-1E^{ts}#1S (Fig. 1c), consistent with the marker data. The size and arm ratios of the wheat-*E. tsukushiensis* recombinant chromosomes suggest that the wheat chromosomes involved in these translocations are either chromosomes 1A or 1D. The identity of the wheat chromosomes involved was determined using simultaneous FISH and GISH on the same metaphase spreads.

Two-color GISH with the D-genome-specific oligonucleotide probe pAs1 and total genomic DNA of *E. tsukushiensis* did not detect any pAs1 hybridization site in either TiWL·WS-1E^{ts}#1S-WS or TWL·WS-1E^{ts}#1S indicating that both recombinants did not involve a D-genome chromosome of wheat (data not shown). Simultaneous detection using A-genome-specific probe BAC676D4 labeled with Texas Red and total genomic *E. tsukushiensis* DNA labeled with fluorescein in green revealed that the distal recombinant rec107, TWL·WS-1E^{ts}#1S, involved an A-genome chromosome, which was painted over its entire length in red except for a small distal region of the short arm, which strongly hybridized with total genomic *E. tsukushiensis*

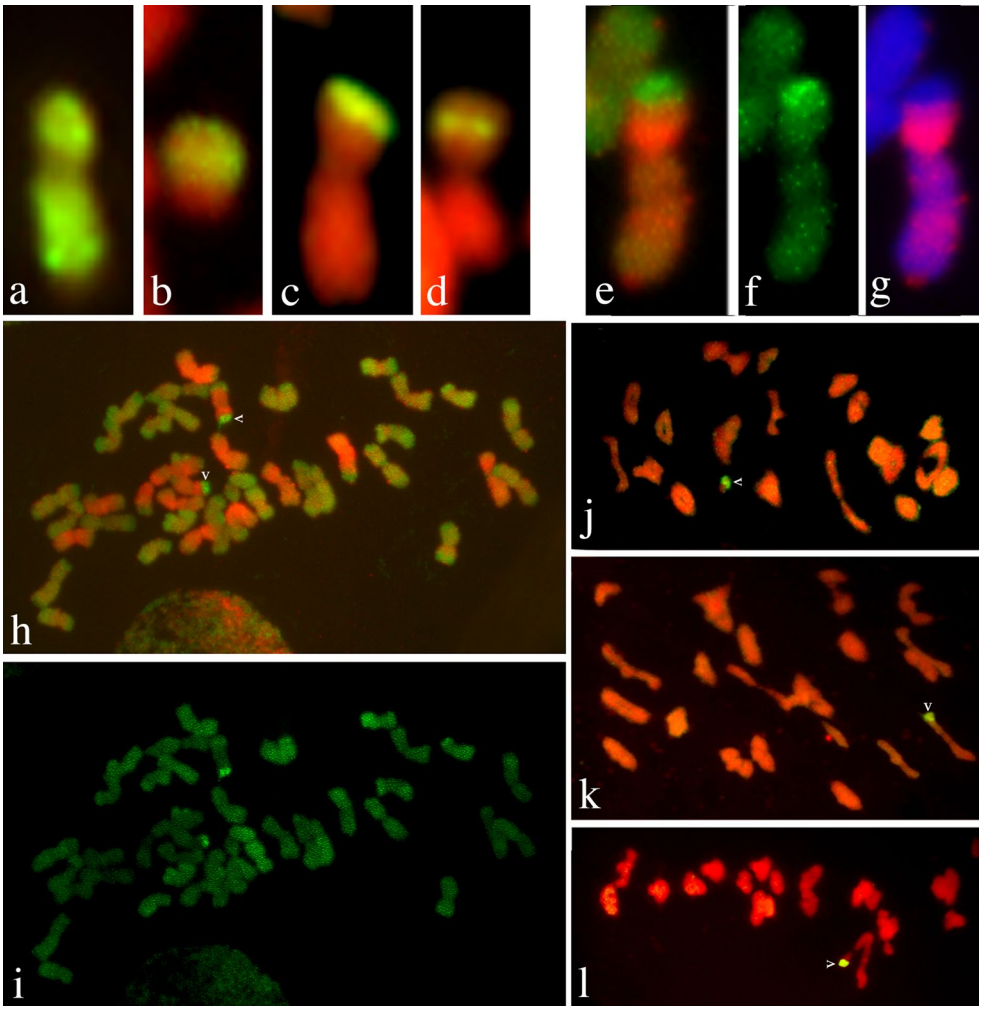

Fig. 1 Genomic in situ hybridization patterns of mitotic (**a**–**i**) and meiotic (**j**–**l**) metaphase chromosomes involved in the FHB-resistant wheat-*E. tsukushiensis* transfer, **a**–**d** GISH using total genomic *E. tsukushiensis* DNA labeled with fluorescein-12-dUTP and detected by *yellow-green* fluorescence and wheat chromosomes were counterstained with propidium iodide and fluoresce *red*, 1E^{ts}#1(**a**), TW·1E^{ts}#1S (**b**), rec107 (**c**), rec74 (**d**); **e**–**i** simultaneous FISH and GISH of rec107 critical chromosome T1AL·1AS-1E^{ts}#1S (**e**–**g**) and mitotic cells (**h**, **i**) using the A-genome specific BAC676D4 labeled with Texas Red and detected by *red* fluorescence, total genomic *E. tsukushiensis* DNA labeled with fluorescein-12-dUTP and detected by yellow-green fluorescence (**h**, **i**) and wheat chromosomes were counterstained with DAPI and fluoresce blue (**g**); **j**–**l** PMCs of $2n = 43$ chromosome plants homozygous for *ph1b* and heterozygous for TW·1E^{ts}#1S, TW·1E^{ts}#1S unpaired as a univalent (**j**), TW·1E^{ts}#1S paired as a rod in the wheat arm (**k**), TW·1E^{ts}#1S paired as a trivalent in the wheat arm (**l**), *E. tsukushiensis* chromatin is labeled with fluorescein-12-dUTP and fluoresce *green* and wheat chromosomes are counterstained with propidium iodide and fluoresce *red*; *arrowheads* point to the translocated *E. tsukushiensis* segment

DNA and was painted in green (Fig. 1e–i). The size and arm ratio of the A-genome chromosome involved in rec107 identified this chromosome as 1A of wheat and, thus, this recombinant chromosome can be described as T1AL·1AS-1E^{ts}#1S. Further attempts to identify the wheat chromosome involved in rec74 failed, suggesting that the interstitial recombinant chromosome is a rearranged chromosome.

The FHB ratings of the wheat-*E. tsukushiensis* introgression lines together with the susceptible (Overley) and moderately resistant (Everest, Karl 92, and Chinese Spring) controls are shown in Table 2. Everest, Karl 92, and Chinese Spring had average FHB ratings ranging from 27.7 to 35.1 % and Overley had a FHB rating of 54.6 %. The parental *E. tsukushiensis* derived lines DA1E^{ts}#1 and DATW·1E^{ts}#1S had average FHB ratings of 12.5 and 6.2 %, respectively, ratings that were lower than those of the recipient wheat cultivar Chinese Spring. Three rec74 families homozygous for the interstitial translocation TiWL·WS-1E^{ts}#1S-WS had FHB ratings ranging from 12.5 to 14.7 %, whereas the family 2011-55-13 in the same genetic background had an average FHB rating of 39.3 %, similar to that of Chinese Spring. Similarly, the four rec107 families homozygous for the distal translocation T1AL·1AS-1E^{ts}#1S had FHB ratings of 4.2–13.3 %, whereas the families

Table 2 Fusarium head blight ratings of wheat-*Elymus tsukushiensis* introgression lines

Name	Chromosome constitution	Cultivar or genetic background	Average FHB rating (%)	No. of heads in inoculated
Everest	–	Everest	27.7	40
TA2923	–	Karl 92	32.7	40
TA9107	–	Overley	54.6	40
TA3008	–	Chinese Spring	35.1	42
TA7684-2	DA1E^{ts}#1	Chinese Spring	12.5	41
TA5655	TW·1E^{ts}#1S	Chinese Spring	6.2	51
2011-55-5	rec74, hom TiWL·WS-1E^{ts}#1S-WS	Chinese Spring	13.3	53
2011-55-12	rec74, hom TiWL·WS-1E^{ts}#1S-WS	Chinese Spring	14.7	41
2011-55-14	rec74, hom TiWL·WS-1E^{ts}#1S-WS	Chinese Spring	12.5	39
2011-55-13	no *E. tsukushiensis* chromatin	Chinese Spring	39.3	41
2011-56-3	rec107: hom T1AL·1AS-1E^{ts}#1S	Chinese Spring	4.2	40
2011-56-10	rec107: hom T1AL·1AS-1E^{ts}#1S	Chinese Spring	13.3	42
2011-56-13	rec107: hom T1AL·1AS-1E^{ts}#1S	Chinese Spring	8.9	51
2011-60-5-1	rec107: hom T1AL·1AS-1E^{ts}#1S	Chinese Spring	8.6	40
2012-56-4	no *E. tsukushiensis* chromatin	Chinese Spring	31.7	40
2012-60-5-2	no *E. tsukushiensis* chromatin	Chinese Spring	42.5	39

LSD ($P = 0.05$) 10.85

The FHB rating is the percentage blighted spikelets from plants inoculated under greenhouse conditions

2012-56-4 and 2012-60-5-2 lacking T1AL·1AS-1E^{ts}#1S that had FHB ratings between 31.7 and 42.5 %, similar to those of Chinese Spring. These data show that both wheat-*E. tsukushiensis* recombinant chromosomes harbor a novel type-2 FHB resistance gene that reduces infection spread after point inoculation.

We further crossed the wheat-*E. tsukushiensis* recombinants in Chinese Spring background with Everest to develop agronomically superior winter habit germplasm. BC_1F_1 plants were screened for *E. tsukushiensis* segment using GISH. The BC_1F_1 plants were then selfed to obtain BC_2F_2 progenies homozygous for the *E. tsukushiensis* segment.

We developed molecular markers to monitor the transfer of the *E. tsukushiensis* segment conferring FHB resistance in wheat breeding. Comparative genomic analysis of wheat with rice, Brachypodium and barley was used to identify ESTs/flcDNAs from the telomeric region of wheat group 1 chromosomes. Further flcDNAs (wheat/barley) based on the sorted chromosome arm sequence of chromosome 1AS were also identified. A set of 50 conserved primers representing 40 unique genes was designed. Ten markers between Chinese Spring and TW·1E^{ts}#1S (TA5655) and 14 markers between Everest and TW·1E^{ts}# 1S were polymorphic when the amplicons were digested with enzymes. Ten CAPS markers polymorphic with both CS/Everest and TW·1E^{ts}#1S were used to screen the BC_1F_1 and BC_1F_2 progenies. Three of the 10 CAPS markers, tplb0017E15, tplb0029J02, and AK357509, tagged the *E. tsukushiensis* segment in the homozygous recombinants. The additional 20 KASPar SNP markers developed from the 10 K Infinium chip were also screened to identify polymorphisms between Chinese Spring/Everest and TW·1E^{ts}#1. Two polymorphic KASPar SNPs were used to screen the BC_1F_1 and BC_1F_2 progenies and KASPar SNP (wg1S_snp1) tagged the *E. tsukushiensis* segment in the homozygous recombinants (Fig. 2; Table 1). Homozygous recombinants (2014-77-8, 2014-77-9, 2014-77-10) were further confirmed by GISH.

Discussion

The successful chromosome engineering and transfer to wheat homoeologous chromosome 1A of a small *E. tsukushiensis* segment harboring *Fhb6*, specifying resistance to FHB, is a culmination of a long-term experiment aimed at improving the resistance of wheat to this devastating disease. Extensive screening of wheat genetic resources, both in China (Weng and Liu 1989) and Japan (Ban 1997) identified *E. tsukushiensis* as a potent source of type 1 and type 2 resistance to FHB. Wide hybridization was used to produce a set of addition lines and one unique chromosome TW·1E^{ts}#1S as an addition to wheat genome provided a level of type 2 resistance to wheat similar to Sumai 3. Several aspects of this study provide unique insights into chromosome engineering of alien resistance for crop improvement and the potential of this source of resistance for wheat breeding and are discussed below.

In designing the homoeologous recombination experiment, an attempt is made to target transfers to specific

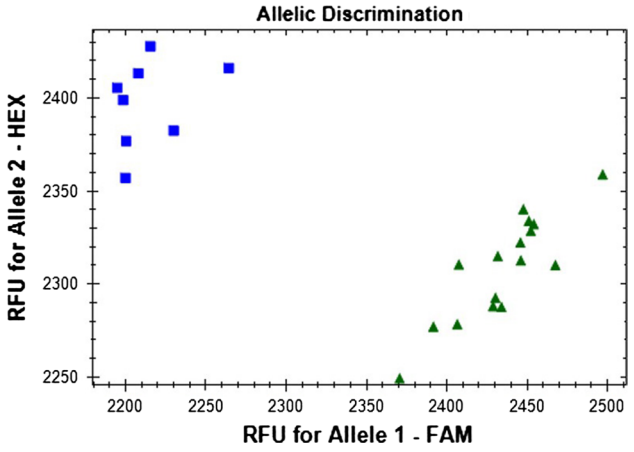

Fig. 2 KASPar SNP assay (wg1S_snp1) for Chinese Spring and Everest in *blue* and *E. tsukushiensis* (TA5655), the source of FHB resistance, and homozygous recombinant lines 201477-8, 2014-77-9, 2014-77-10) in *green* with four replications

wheat chromosomes (see the scheme presented by Qi et al. 2007). This is done by creating homozygous *ph1/ph1* genotypes, which are double monosomic for the target chromosome and a homoeologous wheat chromosome. The expectation is that because the monosomic wheat chromosome is missing its homologous partner, it will preferably recombine with the homoeologous alien chromosome. At the same time, non-target wheat homoeologous chromosomes pairs will not be available for homoeologous recombination. However, in practice homoeologous recombination has been observed among non-target homoeologous chromosomes but the frequency of recombination may vary. Qi et al. (2011) observed an extreme case of bias where the target chromosomes 6D of wheat and 6V of *Dasypyrum villosum* did not recombine, instead all translocations involved chromosomes 6A and 6V. In the present experiment, TW·1E^{ts}#1S was monosomic and homoeologous wheat chromosomes 1A, 1B and 1D were disomic, and, of the two recombinants, one involved wheat 1AS and 1E^{ts}#1S arms. Although the data are not sufficient, it is tempting to speculate that 1AS and 1E^{ts}#1S may have greater genetic affinity resulting in preferential recombination as well as fitness (in terms of functioning of gametes) and hence, may result in a more desirable agronomic transfer. Now that extensive molecular markers are available and large progenies may be screened for rare recombinants, it may no longer be necessary to target specific homoeologus chromosomes. Instead, one may allow competitive homoeologous recombination between wheat and an alien chromosome for the recovery of most desirable recombinants involving one or all wheat homoeologus chromosomes. Although markers are readily available, there is considerable cost to the screening of large segregating populations. In this regard, monitoring of potential homoeologous recombination by meiotic analysis allows an estimation of the size of the progeny to be screened. The 1E^{ts}#1S arm of TW·1E^{ts}#1S did not show any pairing with a wheat chromosome in 269 PMCs indicating that a larger progeny need to be screened. A total of 488 progeny were analyzed and two recombinants were recovered providing an estimate of 0.4 % recombination frequency. The frequency of recombination may vary from less than 1–20 % and in general short arms show much reduced homoeologous recombination compared to long arms (reviewed in Qi et al. 2007). It is not known if other chromosomes of *E. tsukushiensis* will show similar low levels of homoeologous recombination. Nevertheless, this is the first demonstration of a successful transfer of an important gene from *E. tsukushiensis* and because most chromosomes of this species are available as alien additions in wheat (Wang et al. 1999), this species can be exploited for wheat improvement.

Of the two recombinants isolated, one is a compensating distal wheat-*E. tsukushiensis* recombinant chromosome consisting of the long arm of wheat chromosome 1A, a proximal part of the 1AS arm and a small distal segment derived from 1E^{ts}#1S, that can be described as T1AL·1AS-1E^{ts}#1S. Because the chromosomes involved in this translocation belong to the same homoeologous group, the resulting translocation is of compensating type and, thus, agronomically useful. In addition, a noncompensating interstitial wheat-*E. tsukushiensis* recombinant was identified; it involved a rearranged unidentified wheat chromosome, TiWL·WS-1E^{ts}#1S-WS. The origin of this recombinant chromosome is unknown. However, we previously observed that wheat-alien recombinant chromosomes also originated from the production of wheat-alien dicentric chromosomes although the transfer was targeted by *ph1b*-induced homoeologous recombination and often the resulting recombinants are rearranged (Liu et al. 2011, 2013). Apparently, both recombinants harbor *Fhb6* because they showed resistant reaction upon infection (Table 2). Because the recombinant #74 consists of the proximal half of 1E^{ts}#1S and the recombinant #107 consists of the distal half of 1E^{ts}#1S, the *Fhb6* gene must be located in the overlapping subterminal region of the 1E^{ts}#1S arm.

We further developed molecular markers tagging the *E. tsukushiensis* segments, which can be used as a perfect marker to monitor the *E. tsukushiensis* segment in wheat backgrounds thus, making *Fhb6* amenable to marker-assisted breeding. The *Fhb6* line was released as germplasm KS14WGRC61 in Chinese Spring background (TA5660) (Friebe et al. 2013) and was also transferred to the Kansas winter wheat cultivar Everest (TA5093) and will be evaluated for FHB response and DON accumulation under field conditions. Small quantities of TA5093 are available for distribution upon request.

Author contribution statement JCC, TVD, LQ, SKS, TVD, and D-HK performed the cytogenetic and molecular marker analyses. WWB evaluated the lines for their resistance to Fusarium head blight. YF and PC provided seeds of the wheat-*E. tsukushiensis* chromosome addition/translocation line. BF and BSG designed the project and provided guidance through all steps of the experiments. All authors contributed to, have read, and approved the final manuscript.

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

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