Theor Appl Genet (2008) 117:1155–1166
DOI 10.1007/s00122-008-0853-9

ORIGINAL PAPER

Molecular cytogenetic characterization of alien introgressions with gene *Fhb3* for resistance to *Fusarium* head blight disease of wheat

L. L. Qi · M. O. Pumphrey · Bernd Friebe · P. D. Chen · B. S. Gill

Received: 7 May 2008 / Accepted: 16 July 2008 / Published online: 20 August 2008

Abstract *Fusarium* head blight (FHB) resistance was identified in the alien species *Leymus racemosus*, and wheat-*Leymus* introgression lines with FHB resistance were reported previously. Detailed molecular cytogenetic analysis of alien introgressions T01, T09, and T14 and the mapping of *Fhb3*, a new gene for FHB resistance, are reported here. The introgression line T09 had an unknown wheat-*Leymus* translocation chromosome. A total of 36 RFLP markers selected from the seven homoeologous groups of wheat were used to characterize T09 and determine the homoeologous relationship of the introgressed *Leymus* chromosome with wheat. Only short arm markers for group 7 detected *Leymus*-specific fragments in T09, whereas 7AS-specific RFLP fragments were missing. C-banding and genomic in situ hybridization results indicated that T09 has a compensating Robertsonian translocation T7AL·7Lr#1S involving the long arm of wheat chromosome 7A and the short arm of *Leymus* chromosome 7Lr#1 substituting for chromosome arm 7AS of wheat. Introgression lines T01 ($2n = 44$) and T14 ($2n = 44$) each had two pairs of independent translocation chromosomes. T01 had T4BS·4BL-7Lr#1S + T4BL-7Lr#1S·5Lr#1S. T14 had T6BS·6BL-7Lr#1S + T6BL·5Lr#1S. These translocations were recovered in the progeny of the irradiated line Lr#1 (T5Lr#1S·7Lr#1S). The three translocation lines, T01, T09, and T14, and the disomic addition 7Lr#1 were consistently resistant to FHB in greenhouse point-inoculation experiments, whereas the disomic addition 5Lr#1 was susceptible. The data indicated that at least one novel FHB resistance gene from *Leymus,* designated *Fhb3*, resides in the distal region of the short arm of chromosome 7Lr#1, because the resistant translocation lines share a common distal segment of 7Lr#1S. Three PCR-based markers, BE586744-STS, BE404728-STS, and BE586111-STS, specific for 7Lr#1S were developed to expedite marker-assisted selection in breeding programs.

Communicated by J. Dubcovsky.

L. L. Qi · B. Friebe (✉) · B. S. Gill
Wheat Genetic and Genomic Resources Center,
Department of Plant Pathology, Kansas State University,
Manhattan, KS 66506-5502, USA
e-mail: friebe@ksu.edu

M. O. Pumphrey
ARS-USDA,
Plant Science and Entomology Research Unit,
Manhattan, KS 66506-5502, USA

P. D. Chen
The National Key Laboratory of Crop Genetics and Germplasm Enhancement, Nanjing Agricultural University,
210095 Nanjing, Jiangsu, People's Republic of China

Introduction

Fusarium head blight (FHB), caused by the fungus *Fusarium graminearum* Schwabe [telomorph = *Gibberella* zeae (Schw.) Petch], is a devastating disease in warm and humid wheat-growing areas worldwide with increasing importance in Europe and North America during the last 15 years (Johnson et al. 2003) . Host-plant resistance offers the most cost-effective and environmental friendly method of FHB disease control. Finding diverse sources of FHB resistance is critical for maintaining genetic diversity of resistance in wheat breeding-programs. Deployment of only one or a few sources of resistance over large crop production areas poses a danger of resistance breakdown and disease epidemics. Evaluation of thousands of accessions from the USDA germplasm collection, CIMMYT, and several other countries

revealed a potential hazard for current FHB-resistance breeding, because only a few sources, mainly Sumai 3 and its derivatives, are now being used widely around the globe (Bai and Shaner 2004). Thus, additional resistance sources are needed in order to broaden the genetic base of FHB resistance.

Tens of thousands of wheat land races and wild relatives were screened at Nanjing Agricultural University, Jiangsu Province, China, a major hotspot for FHB epidemics. The most potent levels of FHB resistance were identified in the alien species *L. racemosus* (Tein.) Tzvelev (syn *Elymus giganteus* Vahl.), *Roegneria kamoji* (Ohwi) Ohwi ex Keng (syn. *E. tsukushiensis Honda)*, and *R. ciliaris* (Trin). Nevski [syn. *E. ciliaris* (Trin) Tzvelev] (Weng and Liu 1989; Chen et al. 1993, 1995). A large number of disomic alien addition lines (DA) in wheat were developed, cytogenetically characterized, and screened for FHB resistance (Qi et al. 1997; Wang SL et al. 1999; Wang et al. 2001; Kishii et al. 2004). Chromosome engineering of these lines was attempted (Chen et al. 1998, 2005) by means of homoeologous recombination (Sears 1977), irradiation (Sears 1956), and gametocidal (Gc) gene activity (Endo 1988). Nine wheat-*Leymus* translocation lines were produced, five using irradiation, and four using the Gc gene (Chen et al. 2005). However, all translocations were of the non-compensating type, leading to duplications/deficiencies and, hence, may not be deployed in agriculture.

Induced homoeologous pairing and recombination is the method of choice for producing compensating translocations that have a much higher chance of being agronomically desirable. The first step in chromosome engineering by homoeologous recombination is the production of wheat-alien, whole-arm translocations by using the centric breakage-fusion mechanism of univalents at meiotic metaphase I (Sears 1952; Friebe et al. 1996). Although a large number of the wheat-alien translocations were produced, only a few were successfully used in wheat-breeding programs (Jiang et al. 1994; Friebe et al. 1996). Molecular cytogenetic analyses revealed that many of the transfers from the tertiary gene pool were between non-homoeologous chromosomes, and thus, non-compensating making them unsuitable for use in cultivar improvement (Jiang et al. 1994; Friebe et al. 1996). However, the wheat-rye T1BL·1RS and T1AL·1RS compensating translocations are present in the world's highest yielding wheats because they have a battery of genetic factors that combat both biotic and abiotic stresses and the entire 1RS arms are inherited as a single Mendelian units. Similarly, a wheat-*Agropyron* compensating translocation carrying *Lr19/Sr25* not only provides resistance to rusts, but also contributes to higher biomass (Sharma and Knott 1966; Friebe et al. 1996). Other compensating translocations from *Haynaldia villosa* L. (Qi et al. 1993; Zhang et al. 2005) and *Thinopyrom intermedium* (Host) Barkword and D.R. Dewey (Friebe et al. 1991, 1996) have been deployed in agriculture. In the present study, we report the discovery of a genetically compensating translocation involving the long arm of wheat chromosome 7A and the short arm of *Leymus* chromosome 7Lr#1 (T7AL·7Lr#1S) conferring FHB resistance. This translocation may be directly used in breeding programs.

Materials and methods

Plant materials

The wheat-*Leymus* introgression lines (T01, T09 and T14) analyzed in the present study were selected from the progenies of radiation-treated backcrosses of wheat × *Leymus* (Table 1). Other plant materials used in the study included the *Triticum aestivum* cultivars Chinese Spring (CS), Sumai 3, Yangmai 158, Wheaton (Busch et al. 1984), and Roblin (CN 43847); and the *L. racemosus* accession TA10298. A BC_1 population between introgression line T09 and CS *ph1b* (T09 / 2* CS *ph1b*) was developed in order to initiate a further round of chromosome engineering. Other genetic stocks included: six nullisomic-tetrasomics (in NTs a missing pair of chromosomes is compensated by four doses of a homoeologous chromosome); one monotelosomic (Mt); nine ditelosomic (Dt) stocks (Sears 1954, 1966); and four deletion lines of the short arm of chromosome 7A (Table 2). The fraction length

Table 1 Wheat-*Leymus* introgression lines used in the study

TA#	Original plant	Chromosome no. (2*n*)	Previous designation of translocation chromosome[a]	New designation of translocation chromosome in this study	Pedigree
5607	02G543 T01	44	T4BS·4BL-7Lr#1S	T4BS·4BL-7Lr#1S T4BL-7Lr#1S·5Lr#1S	Yangmai 5 × (C.S/*L.r*//C.S)^{1000R}
5608	04G666 T09	42	TW?·Lr.7	T7AL·7Lr#1S	(C.S/*L.r*//C.S)^{600R}
5609	04G874 T14	44	T6BL·6BS-5Lr#1L	T6BS·6BL-7Lr#1S T6BL·5Lr#1S	Mianyang 11 x (C.S/*L.r*//C.S)^{1000R}

[a] P. D. Chen, personal communication

Table 2 Genetic stocks used in the study. All lines are in the Chinese Spring background unless otherwise indicated

TA no.[a]	Genetic stocks	Description	Reference
TA3063	N5AT5D	Nullisomic 5A tetrasomic 5D	Sears (1954)
TA3065	N5BT5D	Nullisomic 5B tetrasomic 5D	Sears (1954)
TA3067	N5DT5B	Nullisomic 5D tetrasomic 5B	Sears (1954)
	Mt5AS[b]	Monotelosomic 5AS	Qi unpublished data
TA3107	Dt5AL	Ditelosomic 5AL	Sears and Steinitz-Sears (1978)
TA3118	Dt5BL	Ditelosomic 5BL	Sears and Steinitz-Sears (1978)
TA3127	Dt5DL	Ditelosomic 5DL	Sears and Steinitz-Sears (1978)
TA3281	N7AT7D	Nullisomic 7A tetrasomic 7D	Sears (1954)
TA3284	N7BT7D	Nullisomic 7B tetrasomic 7D	Sears (1954)
TA3286	N7DT7B	Nullisomic 7D tetrasomic 7B	Sears (1954)
TA3108	Dt7AS	Ditelosomic 7AS	Sears and Steinitz-Sears (1978)
TA3109	Dt7AL	Ditelosomic 7AL	Sears and Steinitz-Sears (1978)
TA3119	Dt7BS	Ditelosomic 7BS	Sears and Steinitz-Sears (1978)
TA3120	Dt7BL	Ditelosomic 7BL	Sears and Steinitz-Sears (1978)
TA3130	Dt7DS	Ditelosomic 7DS	Sears and Steinitz-Sears (1978)
TA3069	Dt7DL	Ditelosomic 7DL	Sears and Steinitz-Sears (1978)
TA4519, 2	7AS-1	Deletion 7AS-1, FL 0.89	Endo and Gill (1996)
TA4511, 5	7AS-5	Deletion 7AS-5, FL 0.59	Endo and Gill (1996)
TA4546, 8	7AS-8	Deletion 7AS-8, FL 0.45	Endo and Gill (1996)
TA4546, 6	7AS-6	Deletion 7AS-6, FL 0.21	Endo and Gill (1996)
TA7646	DA5Lr#1	Wheat-*Leymus* disomic addition	Qi et al. (1997)
TA7648	DA7Lr#1	Wheat-*Leymus* disomic addition	Qi et al. (1997)
TA7652-2	DA?Lr#1	Wheat-*Leymus* disomic addition	Qi et al. (1997)

[a] WGGRC collection accession number

[b] Mt5AS was selected from a cross between ditelo 5AS monotelo 5AL and N5AT5D

FL fraction length

(FL) value of a deletion stock identifies the position of the breakpoint from the centromere relative to the length of the complete arm (Endo and Gill 1996). All materials are maintained at the Wheat Genetic and Genomic Resources Center, Kansas State University, Manhattan, Kansas, USA.

RFLP analysis

Procedures used for genomic DNA isolation, restriction endonuclease digestion, gel electrophoresis, and DNA gel blot hybridization were as described in Qi et al. (2003). Genomic DNAs were digested with restriction enzymes *Dra*I, *Eco*RI, *Eco*RV, and *Hin*dIII. The RFLP and EST clones used are listed in Table 3. These clones were kindly provided by Dr. M. E. Sorrells, Cornell University, Ithaca, NY, USA (designated BCD, CDO, and WG), Dr. M. D. Gale, John Innes Centre, Norwich, UK (designated PSR), Dr. A. Kleinhofs, Washington State University, Pullman, WA, USA (designated ABG), and Dr. O. Anderson, USDA-ARS-WRRC Albany, California, USA (designated BE).

STS primer design and analysis

We developed user-friendly STS markers to expedite selection of critical genotypes in a wheat-breeding program. A sample of 82 wheat expressed sequence tags (ESTs) previously mapped from the centromere to the telomere in the short arm of chromosome 7A were selected from data in the

Table 3 Molecular markers used in the study

Probe	Group	Probe	Group	SSR marker	Group
PSR596	1S	BE443187	5L	GWM493	3BS
PSR544	1L	BE443610	5L	BARC133	3BS
BE446010	1L	PSR580	5L	GWM533	3BS
BCD433	2S	PSR627	6S	GWM233	7AS
PSR388	2L	CDO497	6L	GWM471	7AS
PSR909	3S	BE443120	7S	GWM350	7AS
BCD589	3L	BE586111	7S	BARC127	7AS
PSR584	4S	BE489090	7S	GWM130	7AS
PSR141	4S	BE585543	7S	GWM635	7AS
PSR920	4L	BE443596	7S	CFD62	7AS
BCD873	5S	BCD385	7S	CFA2049	7AS
ABG705	5S	CDO595	7S	GWM60	7AS
BE444720	5S	BE585744	7S	BARC105	7AS
PSR945	5S	BE442797	7S	CFA2174	7AS
PSR628	5S	BE445222	7L	CFD242	7AS
PSR360	5L	KSUE9	7L	GWM260	7AS
WG909	5BL	PSR129	7L	GWM666	7AS
BE443021	5L	PSR311	7L	CFA2028	7AS

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 the Primer 3 software (Rozen and Skaletsky 2000). The conditions for PCR amplification were according to Qi et al. (2007). To achieve higher rates of polymorphism, the amplified products were digested with four-base cutter enzymes *Alu*I, *Hae*III, *Msp*I, and *Rsa*I, fractionated on 1% agarose gels, and visualized by ethidium bromide staining and UV irradiation. The PCR products for microsatellites (SSRs) were separated in 2.3% agarose gels and visualized under UV light (Röder et al. 1998).

C-banding and genomic in situ hybridization (GISH)

C-banding and chromosome identification were according to Gill et al. (1991). The procedure for GISH was as described in Zhang et al. (2001) with some modifications. *Leymus* genomic DNA was isolated using DNeasy Plant Mini Kit following the manufacturer's instructions (Qiagen, Valencia, CA, USA). One microgram of genomic DNA was labeled with fluorescein-12-dUTP (FITC, yellow and green fluorescein, Enzo Life Sciences Inc, Farmingdale, NY, USA) using nick translation. The hybridization mixture containing 50% deionized formamide, 2× SSC, 10% dextran sulfate, 0.3 mg/ml of sheared salmon testes DNA, and ~1 μg/ml of labeled genomic DNA and was denatured by boiling for 7 min. Thirty microliter of denatured hybridization mixture was applied to each slide and allowed to hybridize overnight in a humidity chamber at 37°C. No wheat genomic DNA was used as blocking DNA in the present study. Post-hybridization washes were in 2× SSC at 42°C for 10 min, 50% formamide in 2× SSC at 42°C for 10 min (equivalent to 82% stringency), 2× SSC at 42°C for 10 min, and 1× PBS at room temperature for 5 min. Chromosomes were counterstained with propidium iodide (PI) and fluorescein red. Fluorescent images were captured using a SPOT 2.1 charge-coupled device (CCD) camera (Diagnostic Instruments) attached to an epifluorescence Zeiss Axioplan 2 microscope and processed with Photoshop v5.5 (Adobe Systems, Inc, San Jose, CA, USA).

FHB resistance evaluation

All experimental lines, the FHB-resistant line Sumai 3, and the FHB-susceptible lines Roblin and Wheaton were screened for FHB response with three replications per experiment in three greenhouse seasons, with a total of approximately 15 plants per entry per experiment. At anthesis, 10 μl macroconidia (1×10^5/ml) of *F. graminearum* isolate Z-3639 (NRRL accession 29169) were placed in a central floret and plants transferred to a humidity tent. Humidity was applied overnight (15 h) by mist irrigation for 15 s every 12 min for three nights following inoculation. Pots were returned to the greenhouse and evaluated for disease spread at 14 and 21 day post-inoculation. The numbers of symptomatic spikelets and total spikelets were recorded for each spike (Dill-Macky 2003). Analysis of variance and least significant differences were calculated based on a randomized complete block design using Proc GLM of SAS v.8.1 (SAS Institute, Cary, NC, USA).

Results

Identification of a wheat-*Leymus* compensating translocation T7AL·7Lr#1S in T09

A total 58 wheat-*Leymus* introgression lines from 14 siblings were introduced from Nanjing Agricultural University. These lines were repeatedly tested for scab resistance in China. We further screened these lines for resistance to spread of infection within spikes under controlled conditions in the greenhouses at Kansas State University. Among 24 lines with high levels of resistance to FHB, we determined that three lines (T01, T09, and T14) lacked Sumai 3-type alleles at three marker loci linked to *Fhb1* (Liu and Anderson 2003), indicating that the FHB resistance in these lines was likely derived from *L. racemosus* (Table 4). The line T09 had 42 chromosomes with a pair of unknown wheat-*Leymus* translocation chromosomes. A total of 36 RFLP markers from all seven homoeologous groups of wheat were used to screen this line. Only the markers located in the short arm of group-7 chromosomes detected *Leymus* polymorphic fragments in T09 (Table 5) indicating that the translocated chromosome in this line belonged to homoeologous group 7. These markers also detected missing 7AS-specific RFLP fragments. All 7AL-specific RFLP fragments were present. We concluded that the short arm of the *Leymus* chromosome was translocated to the long arm of wheat chromosome 7A. The RFLP pattern in line T09 was the same as that in disomic addition 7Lr#1 (Table 5). C-banding and GISH analyses using *Leymus* genomic DNA as probe further indicated that the translocation chromosome in T09 was a whole-arm Robertsonian translocation (Fig. 1) designated as T7AL·7Lr#1S.

For shortening the 7Lr#1S arm we crossed the T7AL·7Lr#1S translocation stock with the CS*ph1b* mutant stock. The F_1 was backcrossed as a male and female with *ph1b* and the chromosome constitution of the backcross progeny was determined by molecular marker analysis. When the F_1 was used as the female parent (T7AL·7Lr#1S X CS*ph1b*) × CS*ph1b*) 13 plants had the translocation chromosome (54%) and 11 plants were lacking it (46%). On the contrary, when the F_1 was used as the male parent [CS*ph1b* X (T7AL·7Lr#1S X CS*ph1b*)], 125 plants had the translocation chromosome (96%) and 5 plants were lacking

Table 4 Evaluation and genotyping of alien introgressions and control wheat lines for *Qfhs.ndsu-3BS* QTL and FHB disease severity

Line	GWM493	BARC133	GWM533	Disease Severity (%)[b]
DA5L#1	N/A	N/A	N/A	62
DA7Lr#1	N/A	N/A	N/A	17[a]
Sumai 3	1[c]	1	1	11
T4BS·4BL-7Lr#1S, TW-7Lr#1S·5Lr#1S	2	2	2	16[a]
T6BS·6BL-7Lr#1S, TW·5Lr#1S	2	2	2	22[a]
T7AL·7Lr#1S	2	2	2	23[a]
CS	2	2	2	48
Wheaton	2	2	2	95
Roblin	2	2	2	100
$LSD_{0.05}$				13

N/A not available

[a] Disease severity means not significantly different from Sumai 3

[b] Disease severity means calculated using SAS LSMEANS based on replicated inoculation experiments conducted from 2004-2007

[c] Sumai 3-type alleles designated as 1, all other alleles as 2

Table 5 RFLP clones from homoeologous group-5 and -7 used to detect *Leymus*-specific fragments in wheat-*Leymus* introgression lines

Clone/enzyme	Chromosome bin location[a]	DA5Lr#1	T6BS·6BL-7Lr#1S T6BL·5Lr#1S (T14)	T4BS·4BL-7Lr#1S T4BL-7Lr#1S·5Lr#1S (T01)	T7AL-7Lr#1S (T09)	DA7Lr#1
BCD873/*Dra*I	5S-0.78-1.00	+	+	+	–	–
ABG705/*Hind*III	5S-0.67-0.78	+	+	+	–	–
BE444720/*Eco*RI	5S-0.63-0.67	+	+	+	–	–
PSR945/*Hind*III	5S-0.63-0.67	+	+	+	–	–
PSR628/*Hind*III	5S-0.63-0.67	+	+	+	–	–
BE443426/*Hind*III	C-5S-0.40	+	+	+	–	–
BE443446/ *EcoR*I	C-5S-0.40	+	+	+	–	–
PSR360/*Dra*I	C-5L-0.29	+	–	–	–	–
WG909/*Hind*III	C-5L-0.29	+	–	–	–	–
BE443021/*Dra*I	C-5L-0.29	+	–	–	–	–
BE443187/*Hind*III	5L-0.29-0.55	+	–	–	–	–
BE443610/*Eco*RI	5L-0.61-0.67	+	–	–	–	–
PSR580/*Eco*RV	5L-0.82-1.00	+	–	–	–	–
BE443120/*Eco*RV	7S-0.89-1.00	–	+	+	+	+
BE586111/*Dra*I	7S-0.89-1.00	–	+	+	+	+
BE489090/*Hind*III	7S-0.59-0.89	–	+	+	+	–
BE585543/*Dra*I	7S-0.59-0.89	–	+	+	+	+
BE443596/*Dra*I	7S-0.45-0.59	–	+	+	+	+
BCD385/*Hind*III	7S-0.29-0.45	–	–	+	+	+
CDO595/*Hind*III	7S-0.29-0.45	–	–	+	+	+
BCD349/*Dra*I	7S-0.21-0.26	–	–	+	+	+
BE585744/*Eco*RV	C-7S-0.36	–	–	+	+	+
BE442797/*Eco*RI	C-7S-0.21	–	–	+	+	+

+ and -: presence and absence of *Leymus* polymorphic fragments

[a] Chromosome bin locations of RFLP clones were from Hohmann et al. 1994 and Qi and Gill 2001. The bin locations of EST clones were from http://wheat.pw.usda.gov/NSF/project/mapping_data.html

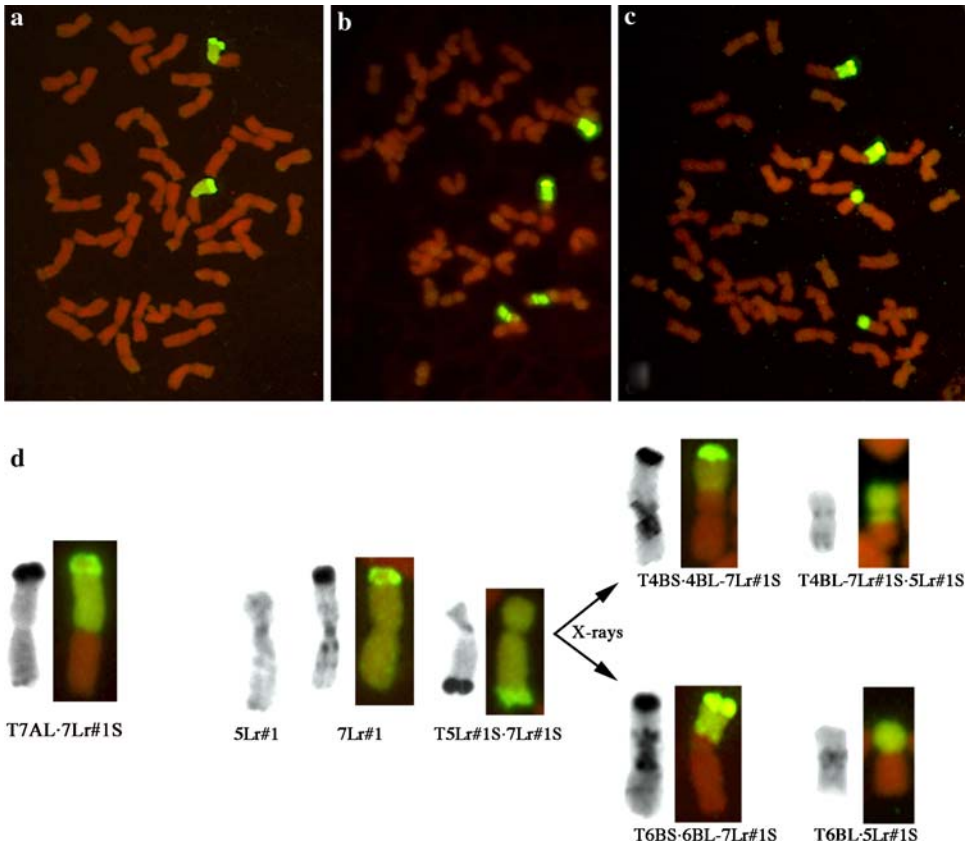

Fig. 1 C-banding and GISH patterns of wheat-*Leymus* addition and translocation lines. **a–c** *from left to right* GISH pattern of T09 ($2n = 42$), T01 ($2n = 44$), and T014 ($2n = 44$); **d** *from left to right* C-banding and GISH patterns T7AL·7Lr#1S in T09; C-banding pattern of 5Lr#1; C-banding and GISH patterns 7Lr#1, T5Lr#1S·7Lr#1S, and derived translocations following irradiation. Both arms of 5Lr#1S and 7Lr#1S are present as a part of T4BS·4BL-7Lr#1S + T4BL-7Lr#1S·5Lr#1S in T01 (*top*); but in T14 (T6BS·6BL-7Lr#1S + T6BL·5Lr#1S, *bottom*) proximal part of 7Lr#1S has been lost (see also molecular marker data in Table 5). The translocation chromosomes were visualized by yellow-green FITC florescence using GISH

it. The data show that T7AL·7Lr#1S translocation is transmitted at about 50% through the female gametes, whereas its transmission frequency through the male side is almost 100%.

We evaluated the scab responses of 61 BC_1 progeny from the cross T09/2*CS *ph1b* and grouped them based on molecular marker classification as homozygous for T7AL·7Lr#1S, heteromorphic for chromosomes 7A/T7AL·7Lr#1S, and those with 7A (CS). The mean disease severity in plants with only one copy of T7AL·7Lr#1S (heteromorphic) was not significantly different (LSD0.05) than CS, whereas progeny homozygous for T7AL·7Lr#1S had significantly lower disease (Fig. 2); similarly, homozygous T7AL·7Lr#1S progeny (disease severity mean = 34%) had significantly lower disease severity than those heteromorphic for chromosomes 7A/T7AL·7Lr#1S (disease severity mean = 52%) in this experiment based on the LSD 0.05 of 8.3%.

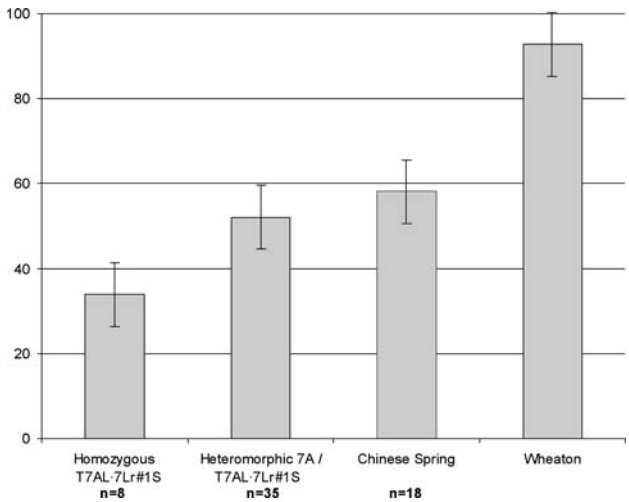

Fig. 2 Disease severity means for genotypes segregating for translocation chromosome, T7AL·7Lr#1S, scored 21 d after central floret inoculation of *F. graminearum*. Error bars represent the $LSD_{0.05}$ for disease severity measurements in this experiment (8.3%) based on ANOVA with SAS PROC GLM. Spring wheat cultivar 'Wheaton' is included as a susceptible check

Double wheat-*Leymus* translocation lines T01 and T14

RFLP analysis

Previous cytogenetic data revealed that T01 and T14 had non-compensating translocations T4BS·4BL-7Lr#1S and T6BL·6BS-5Lr#1L, respectively (PD Chen, personal communication). A total of 13 group-5 specific and 12 group-7S specific RFLP markers providing full chromosome coverage for 5Lr#1 and 7Lr#1S were used to test the two lines. T09 (T7AL·7Lr#1S), DA5Lr#1, and DA7Lr#1 were used as controls. The short arm markers of group-5 chromosomes detected *Leymus* polymorphic fragments in

both T01 and T14 with the same pattern as in DA5Lr#1 (Fig. 3); these were absent in T09 and DA7Lr#1 (Table 5). The *Leymus* specific group-5 long arm polymorphic fragments were absent in T01 and T14 and only observed in DA5Lr#1. These data indicated that both lines contained the 5Lr#1S arm in the form of a non-compensating translocation with a wheat chromosome.

Testing of lines T01 and T14 with the chromosome group-7 short arm markers indicated that T01 was polymorphic for all the tested markers similar to T7AL·7Lr#1S. However, in T14 only four of ten markers covering the distal 50% of 7Lr#1S were polymorphic (Table 5) indicating complex introgression of whole arms or parts of *Leymus* chromosome arms 5Lr#1S and 7Lr#1S and wheat chromosomes 6B. Therefore, C-banding and GISH were employed to further resolve the chromosome composition and the nature of the alien introgression in these two lines (Fig. 1).

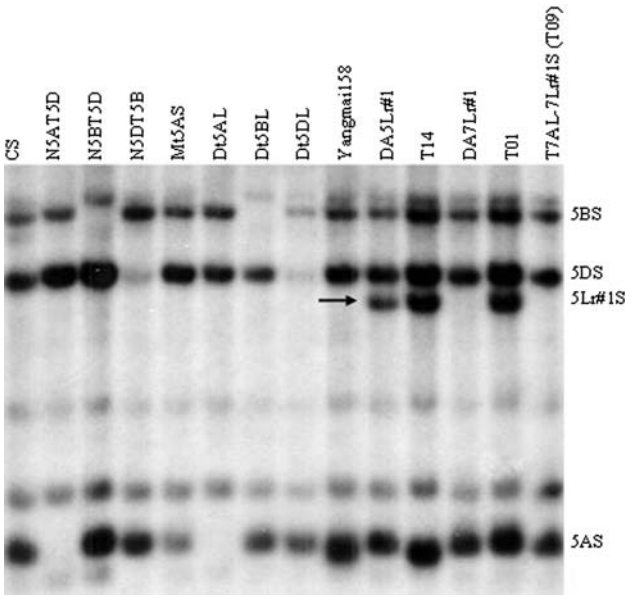

Fig. 3 An autoradiograph of the Southern hybridization of clone PSR628 to genomic DNAs digested with *Hin*dIII of nullisomic-tetrasomic (*NT*) and ditelosomic (*Dt*) lines of group-5 chromosomes and wheat-*Leymus* addition and translocation lines. The arrow indicates the *Leymus* 5Lr#1S-specific fragment that is present in DA5Lr#1 and lines T14 and T01

C-banding and GISH analysis

C-banding and GISH data indicated both T01 and T14 each harbor two pairs of wheat-alien translocations (Fig.1b and c). In addition to T4BS·4BL-7Lr#1S, line T01 has a reciprocal translocation T4BL-7Lr#1S·5Lr#1S. Because T01 has all the 7Lr#1S-specific polymorphic fragments, the distal half of the 7Lr#1S arm must be present in T4BS·4BL-7Lr#1S as indicated by the distal C-band which is an excellent cytological marker. The proximal part of 7Lr#1S is present at the interstitial position in 4BL-7Lr#1S·5Lr#1S. Because T01 was polymorphic for 5Lr#1S-specifc markers, the short arm of T4BL-7Lr#1S·5Lr#1S must come from 5Lr#1S.

In addition to T6BS·6BL-7Lr#1S previously identified as T6BS·6BL-5Lr#1L (Table 1; Fig. 1), line T14 has a reciprocal translocation T6BL·5Lr#1S. The RFLP mapping data and GISH results indicated that the T6BS·6BL-7Lr#1S has only 50% of the distal portion of 7Lr#1S including the landmark terminal C-band. Because this line was polymorphic for 5Lr#1S-specific markers, T6BL·5Lr#1S must have the complete arm 5Lr#1S.

Development of alien-chromatin-specific PCR-based markers

Polymerase chain reaction based molecular markers specific for 7Lr#1S were developed to expedite marker-assisted selection in breeding programs. A total of 82 EST-based primers were used to screen the two parents, CS and translocation line T7AL·7Lr#1. The 7Lr#1S-specific fragment was amplified in the translocation line with primer BE585744. Two additional polymorphic primers, BE404728 and BE586111, were found after amplified PCR products were digested with the four-base cutter restriction enzymes *Alu*I, *Hae*III, *Msp*I, or *Rsa*I (Table 6). BE585744 and BE404728 were previously mapped to the proximal regions of group-7 chromosomes and BE586111 to the distal regions (http://wheat.pw.usda.gov/NSF/project/mapping_data.html). Three polymorphic PCR markers were tested in line T01, line T14, and a set of NT and Dt stocks of the group-7 chromosomes to identify 7AS-specific

Table 6 Primer sequences of the STS markers derived from wheat ESTs on the short arm of wheat chromosome 7A and primer/enzyme combinations producing polymorphic PCR products

Marker	EST	Forward primer 5′–3′	Reverse primer 5′–3′	Annealing temperature (°C)	Enzyme producing polymorphic PCR product
BE585744-STS	BE585744	GCTATGGCATTCCTCAGCTC	GCCCAAGCCATATCTATCCA	58.0	–
BE404728-STS	BE404728	GGTGGTGCCTGTCAAGATTT	TTGATGGATCCTGGCTTAGG	58.0	*Msp*I
BE586111-STS	BE586111	GGCAAAGAACTTGACCTGCT	CTGTCGGTAACAAGGCGAAT	57.0	*Rsa*I

fragments. All produced dominant markers where the 7AS fragment co-migrated with those of 7B and 7D, but 7Lr#1S was polymorphic (Fig. 4). Line T14 lacked the BE585744-specific *Leymus* fragment but retained the BE586111 fragment (Fig. 4), consistent with the RLFP results indicating that only a distal portion of 7Lr#1S was present in the translocation chromosome T6BS·6BL-7Lr#1S.

The PCR-based dominant STS markers developed in the present study cannot be used to distinguish between homozygous and heterozygous wheat-alien translocation lines. To solve this problem, we selected 15 SSR markers covering the entire short arm of chromosome 7A (Table 3, Sourdille et al. 2004) to screen for T7AL·7Lr#1S. Seven SSR markers detected missing 7AS-specific fragments in 7AL·7Lr#1S (Table 7; Fig. 5). The chromosome locations of these SSR markers were further confirmed with NT, Dt, and deletion lines of group-7 chromosomes (Table 7; Fig.5). The combination of SSR and 7Lr#1S-specific STS markers can be used to select homozygous translocation carriers during cultivar development.

Discussion

Isolation of a FHB-resistant T7AL·7Lr#1S line is the culmination of a long-term breeding effort spanning 25 years

Because of its recent origin, common wheat has a narrow genetic base. Interspecific and intergeneric hybridizations have been used for over a century to enrich and broaden the genetic base of cultivated wheat (reviewed in Sharma and

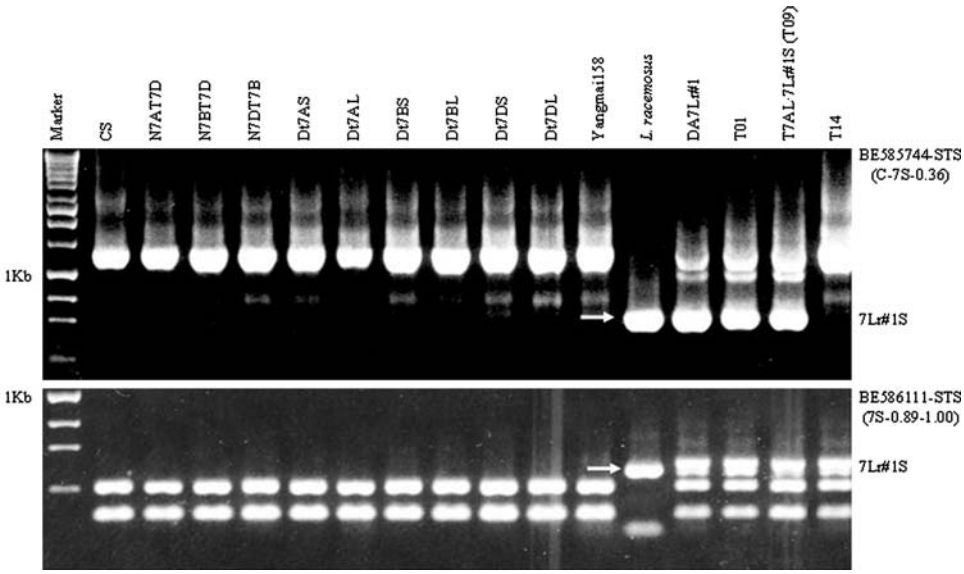

Fig. 4 PCR patterns of primers BE585744 (proximal marker) and BE586111 (distal marker). a, *Leymus* 7Lr#1S-specific fragment amplified by primer BE585744 is present in *L. racemosus*, DA7Lr#1S, and translocation lines T01 and T09 but absent in line T14. b, *Leymus* 7Lr#1S-specific fragment amplified by primer BE586111 and digested with enzyme *Rsa*I is present in *L. racemosus*, and DA7Lr#1S, translocation lines T01, and T09, as well as in line T14, indicating that only a distal portion of 7Lr#1S was transferred to the translocation chromosome in line T14. The arrows indicated 7Lr#1S-specific fragments

Table 7 SSR markers detected missing 7AS in the translocation T7AL·7Lr#1S and their locations in 7AS

Line	GWM233	GWM471	BARC127	CFA2049	GWM60	CFA2174	GWM260
TA10298, *L.r*	–	–	–	–	–	–	–
T7AL·7Lr#1S	–	–	–	–	–	–	–
CS	+	+	+	+	+	+	+
N7AT7D	–	–	–	–	–	–	–
N7BT7D	+	+	+	+	+	+	+
N7DT7B	+	+	+	+	+	+	+
Dt7AS	+	+	+	+	+	+	+
Dt7AL	–	–	–	–	–	–	–
del7AS-6	–	–	–	–	–	–	–
del7AS-8	–	–	–	–	–	–	+
del7AS-5	–	–	–	–	–	–	+
del7AS-1	–	–	+	+	+	+	+
Location	7AS1-0.89-1.00	7AS1-0.89-1.00	7AS5-0.59-0.89	7AS5-0.59-0.89	7AS5-0.59-0.89	7AS5-0.59-0.89	7AS6-0.21-0.45

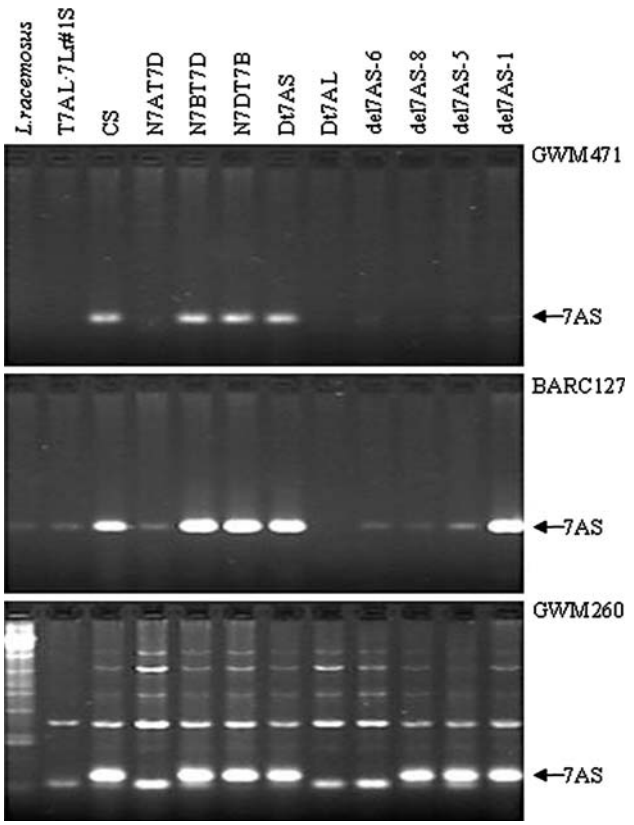

Fig. 5 Polymerase chain reaction patterns of three SSR markers, GWM471, BARC127, and GWM260, in *L. racemosus*, translocation T7AL·7Lr#1S, nullisomic 7A-tetrasomic 7D (N7AT7D), N7BT7D, N7DT7B, ditelosomic 7AS (Dt7AS), Dt7AL, deletion 7AS-6 (del7AS-6), del7AS-8, del7AS-5, and del7AS-1. 7AS-specific fragments detected by these SSR markers are missing in the translocation T7AL·7Lr#1S

Gill, 1983; Jiang et al. 1994). The identification of a compensating T7AL·7Lr#1S translocation line with high levels of resistance to FHB is the culmination of a long-term breeding effort that began in the 1980s. *L. racemosus* is an allotetraploid species ($2n = 4\times = 28$, genomically JJNN) (Dewey 1984). The first wheat-*L. racemosus* hybrids reported by Mujeeb-Kazi and Rodriguez (1981) were produced to tap genes for salt and drought tolerance and disease resistance present in this species. Further wheat-*L. racemosus* hybrids and backcross derivatives with scab resistance were reported by Wang et al. (1986, 1991). Early attempts were made to produce a set of wheat-*Leymus* addition lines (Chen et al. 1993, 1995; Kishii et al. 2004). The homoeologous relationship of the added *Leymus* chromosomes with wheat were determined by RFLP analyses (Qi et al. 1997; Kishii et al. 2004). Several wheat-*Leymus* translocation lines with FHB resistance were developed using radiation treatment or gametocidal gene action (Chen et al. 2005). These studies showed that the FHB resistance in *Leymus* may be located on three different chromosomes, because translocation lines involved *Leymus* chromosomes 5Lr#1, 7Lr#1, and Lr.7 had FHB resistance (Chen et al. 2005). Our study revealed that a previously unknown translocation chromosome involving *Leymus* chromosome Lr.7 in line T09 is a compensating translocation, T7AL·7Lr#1S. Backcross derivatives of T7AL·7Lr#1S in an elite wheat background are fully fertile (our unpublished results). Similar to other whole-arm translocations such as T1BL·1RS (Rajaram et al. 1983), T1AL·1RS (Wood et al. 1974), T6AL·6VS (Qi et al. 1993), and T4DL·4Ai#1S (Friebe et al. 1991) that have been deployed in agriculture, T7AL·7Lr#1S may have agronomic potential, and has often been the experience, the wider use of this translocation in breeding programs may reveal additional desirable or undesirable features. In anticipation of this outcome, we have initiated further engineering of the alien arm to isolate recombinant chromosomes.

The FHB resistance conferred by translocation T7AL·7Lr#1S in the CS background is novel and the level of resistance is similar to that of Sumai 3 (Table 4). However, our screening results of a population segregating for T7AL·7Lr#1S showed that the resistance gene in 7Lr#1S is likely hemizygous ineffective (Fig. 2). We are presently transferring the T7AL·7Lr#1S translocation to commercial cultivars, which will allow us to evaluate the effect in other genetic backgrounds.

The translocated chromosome in T01 was previously designated as T4BS·4BL-7Lr#1S (PD Chen, pers. commun.). However, our data show that, in addition to T4BS·4BL-7Lr#1S, T01 has a second translocation designated T4BL-7Lr#1S·5Lr#1S. The chromosome constitution in T14 was previously reported as T6BL·6BS-5Lr#1L. However, our analysis indicated that wheat chromosome 6B was translocated to 7Lr#1S to form T6BS·6BL-7Lr#1S. In addition, T14 has a reciprocal translocation, T6BL·5Lr#1S. Thus, both T01 and T14 are double translocation lines with four translocation chromosomes of independent origin (Fig. 1d). The distal 50% of the short arm of chromosome 7Lr#1 is a common segment present in all three lines (Table 5). FHB tests indicated that the resistance level in DA7Lr#1 was similar to those in the three translocations, whereas, DA5Lr#1 was susceptible (Table 4). These data indicate that a novel FHB-resistance gene, designated as *Fhb3*, is located in the distal 50% region of the short arm of chromosome 7Lr#1.

Origins of T7AL·7Lr#1S and the double wheat-*Leymus* translocations

Origin of T7AL·7Lr#1S: The process of alien-crop plant hybridization may be described as evolutionary breeding where spontaneous chromosomal changes generate unique genotypes that have selective advantages. Wheat-*Leymus*

derivatives were subjected to scab screening and fertile derivatives with resistance were repeatedly selected (Chen et al. 1993, 1995, 1998, 2005). We hypothesize that T7AL·7Lr#1S conferring a high level of scab resistance, fertility, and stable meiotic behavior is the end product of this evolutionary breeding process. Chromosome 7Lr#1 (and other group-7 chromosomes, see Marais 1990) has meiotic drive and is preferentially transmitted to progeny, creating double monosomics during backcross breeding, and hence opportunities for generating compensating translocations through breakage-fusion (Sears 1952; Friebe et al. 2005). Thus, T7AL·7Lr#1S probably arose in the progeny of a plant double monosomic for chromosomes 7A and 7Lr#1. We have evidence that the meiotic drive gene is located on the short arm of 7Lr#1, because T7AL·7Lr#1S is preferentially transmitted through male gametes. Thus, we speculate that the presence of both FHB resistance and meiotic-drive genes was responsible for generating and selecting this chromosome during breeding efforts.

Origin of double translocations: group-5 chromosomes also harbor meiotic-drive genes (Okamoto 1957; Riley and Chapman 1958; Sears and Okamoto 1958; Sears 1977; Jiang and Gill 1998; Kumar et al. 2007), and the same evolutionary process with some variations may have led to the production and selection of the more complex translocations lines T01 and T14. Both *Leymus* chromosomes 5Lr#1 and 7Lr#1 were isolated as individual addition lines in wheat and are structurally conserved in relation to wheat group-5 and -7 chromosomes (Qi et al. 1997). These two chromosomes did not show cross hybridization patterns between markers for groups 5 and 7. In a previous study, however, we identified one line (DA?Lr#1, $2n = 44$, 96-1012 = TA7652-1) that hybridized with short arm markers of both groups 5 and 7, and another line, 96-1021 ($2n = 44$, TA7652-2) with a hybridization pattern similar to that of line 96-1012. None of the long-arm markers from wheat homoeologous chromosome groups 5 and 7 detected polymorphic *Leymus* fragments in these two lines (Qi et al. 1997). It now appears that chromosome Lr#1 originated from a Robertsonian translocation event involving *Leymus* chromosomes 5Lr#1 and 7Lr#1. These two chromosomes broke at the centromere and their short arms fused to form a new chromosome now designated as T5Lr#1S·7Lr#1S (Fig. 1d). We do not know if this translocation chromosome existed in the *L. racemosus* accession used as a parent or occurred during selection among wheat × *L. racemosus* hybrid progenies because the original *Leymus* accession has been lost. However, we did see one pair of C-banded chromosomes in *L. racemosus* accession TA12096 that is similar to chromosome Lr#1 (Qi et al. 1997). Line 96-1021, with chromosome T5Lr#1S·7Lr#1S, was used for further chromosome engineering by the use of irradiation. Thus, the double translocations present in lines T01 and T14 trace their origin to this chromosome (see Fig. 1e). Because both 5Lr#1 and 7Lr#1 have meiotic drive and resistant lines were selected during backcrossing, they provided an opportunity for the selection of these translocation chromosomes.

EST-based STS markers vs. SSR markers

Developing PCR-based molecular markers for a specific alien chromosome can accelerate selection in a breeding program. The high level of informativeness and co-dominance of microsatellite markers make SSRs markers of choice in many species. Many wheat SSR markers have been developed, their primer sequences published, and their chromosomal locations determined (Ma et al. 1996; Röder et al. 1998; Somers et al. 2004; Sourdille et al. 2004). However, most SSR markers are genome-specific, and their transferability across related species is low (Mullan et al. 2005). We screened 64 SSR markers mapped in the short arms of wheat group-7 chromosomes in the two parents, CS and translocation T7AL.7Lr#1S, as well as in *Leymus*. None detected polymorphic fragments in *Leymus* and the translocation line (data not shown). Most of the wheat SSR primers failed to amplify fragments in *Leymus* and some others amplified PCR products in *Leymus*, but were not polymorphic.

The US wheat EST Mapping Project has mapped nearly 7,000 ESTs in chromosome bins covering all 21 wheat chromosomes, providing an excellent resource for marker development for specific chromosome regions (Qi et al. 2004; http://wheat.pw.usda.gov/NSF). Alternatively, EST-based PCR products can be digested with frequent-cutting restriction enzymes to increase polymorphism. We developed three EST-STS markers specific for 7Lr#1S from 58 selected EST primers. However, all are dominant markers that cannot distinguish between homozygous and heterozygous translocations. This problem was solved by selecting 7AS-specific SSR markers that detect a missing 7AS in 7AL·7Lr#1S homozygous translocation lines. Among 15 7AS-specific SSRs, seven detected the homozygous translocation. The advantage of engineered alien segments is their large phenotypic effect and simple inheritance. 7Lr#1S-specific STS markers combined with selected 7AS-specific SSR markers will facilitate their rapid deployment in wheat-breeding programs. Alternately, we could identify SNPs and use them to develop 7AS and 7Lr#1S-specific markers.

Acknowledgments This project was supported by the McKnight Foundation, WGGRC, and the US Department of Agriculture under agreement no. 59-0790-7-075. This was a cooperative project with the US Wheat and Barley Scab Initiative (USBWI). Any opinions, findings, conclusions, or recommendations expressed in this publication

are those of the authors and do not necessarily reflect the view of the US Department of Agriculture. We thank Dr. R. A. McIntosh and W. J. Raupp for critical reading of the manuscript and D. L. Wilson for excellent technical assistance. This is contribution number 08-339-J from the Kansas Agricultural Experiment Station, Kansas State University, Manhattan, KS 66506-5502, USA.

References

Bai GH, Shaner G (2004) Management and resistance in wheat and barley to Fusarium head blight. Annu Rev Phytopathol 42:135–161

Busch RH, McVey DV, Rauch T, Baumer J, Elsayed F (1984) Registration of 'Wheaton' wheat. Crop Sci 24:622

Chen PD, Wang ZT, Wang SL, Huang L, Wang YZ, Liu DJ (1993) Transfer of scab resistance from *Elymus gigantenus* into common wheat. In: Li ZS, Xin ZY (eds) Proceedings of 8th international wheat genetic symposium, China Agricultural Scien-tech Press, Beijing 1:153–157

Chen PD, Wang ZT, Wang SL, Huang L, Wang YZ, Liu DJ (1995) Transfer of scab resistance from *Elymus gigantenus* into common wheat. III. Development of addition lines with wheat scab resistance (in Chinese with English abstract). Acta Genet Sin 22:206–216

Chen PD, Sun WX, Liu WX, Yuan JH, Liu ZH, Wang SL, Liu DJ (1998) Development of wheat-*Leymus racemosus* translocation lines with scab resistance. In: Slinkard AE (ed) Proceedings of 9th international wheat genetic symposium, University Extension Press, Saskatoon, Canada 2:32–34

Chen PD, Liu WX, Yuan JH, Wang XE, Zhou B, Wang SL, Zhang SZ, Feng YG, Yang BJ, Liu GX, Liu DJ, Qi LL, Zhang P, Friebe B, Gill BS (2005) Development and characterization of wheat-*Leymus racemosus* translocation lines with resistance to Fusarium head blight. Theor Appl Genet 111:941–948

Dewey DR (1984) The genome system of classification as a guide to intergeneric hybridization with perennial Triticeae. In: Gustafson JP (ed) Gene manipulation in plant improvement. Plenum Press, New York, pp 209–279

Dill-Macky R (2003) Inoculation methods and evaluation of Fusarium head blight resistance in wheat. In: Leonard KJ, Bushnell WR (eds) Fusarium head blight of wheat and barley. Am Phytopathol Soc, St Paul, pp 184–210

Endo TR (1988) Induction of chromosomal structural changes by a chromosome from *Aegilops cylindrica* L. in common wheat. J Hered 79:366–370

Endo TR, Gill BS (1996) The deletion stocks of common wheat. J Hered 87:295–307

Friebe B, Mukai Y, Dhaliwal HS, Martin TJ, Gill BS (1991) Identification of alien chromatin specifying resistance to wheat streak mosaic and greenbug in wheat germ plasm by C-banding and in situ hybridization. Theor Appl Genet 81:381–389

Friebe B, Raupp Jiang J, WJ McIntosh RA, Gill BS (1996) Characterization of wheat-alien translocations conferring resistance to diseases and pests: current status. Euphytica 91:59–87

Friebe B, Zhang P, Linc G, Gill BS (2005) Robertsonian translocations in wheat arise by centric misdivision of univalents at anaphase I and rejoining of broken centromeres during interkinesis of meiosis II. Cytogenet Genome Res 109:293–297

Gill BS, Friebe B, Endo TR (1991) Standard karyotype and nomenclature system for description of chromosome bands and structural aberrations in wheat (*Triticum aestivum)*. Genome 34:830–839

Hohmann U, Endo TR, Gill KS, Gill BS (1994) Comparison of genetic and physical maps of group 7 chromosomes from *Triticum aestivum* L. Mol Gen Genet 245:644–653

Jiang J, Friebe B, Gill BS (1994) Recent advances in alien gene transfer in wheat. Euphytica 73:199–212

Jiang J, Gill BS (1998) Preferential male transmission of an alien chromosome in wheat. J Hered 89:87–89

Johnson DD, Flaskerud GK, Taylor RD, Satyanayana V (2003) Quantifying economic impacts of *Fusarium* head blight in wheat. In: Leonard KJ, Bushnell WR (eds) *Fusarium* head blight of wheat and barley. APS press, St Paul, pp 461–483

Kishii M, Yamada T, Sasakuma T, Tsujimoto H (2004) Production of wheat-*Leymus racemosus* chromosome addition lines. Theor Appl Genet 109:255–260

Kumar S, Gill BS, Faris JD (2007) Identification and characterization of segregation distortion loci along chromosome 5B in tetraploid wheat. Mol Genet Genomics 278:187–196

Liu S, Anderson JA (2003) Marker assisted evaluation of Fusarium head blight resistant wheat germplasm. Crop Sci 43:760–766

Ma ZQ, Röder M, Sorrells ME (1996) Frequencies and sequence characteristics of di-, tri-, and tetra-nucleotide microsatellites in wheat. Genome 39:123–130

Marais GF (1990) Preferential transmission in bread wheat of a chromosome segment derived from *Thinopyrum distichum* (Thumb.) Löve. Plant Breed 104:152–159

Mujeeb-Kazi A, Rodriguez R (1981) An intergeneric hybrid of *Triticum aestivum* L X *Elymus giganteus*. J Hered 72:253–256

Mullan DJ, Platteter A, Teakle NL, Appels R, Colmer TD, Anderson JM, Francki MG (2005) EST-derived SSR markers from defined regions of the wheat genome to identify *Lophopyrum elongatum* specific loci. Genome 48:811–822

Okamoto M (1957) Asynaptic effect of chromosome V. Wheat Inf Serv 5:6

Qi LL, Chen PD, Liu DJ, Zhou B, Zhang SZ et al (1993) Development of translocation lines of *Triticum aestivum* with powdery mildew resistance introduced from *Haynaldia villosa*. In: Li ZS, Xin ZY (eds) Proceedings of 8th international wheat genetic symposium. Chinese Agricultural Scientech Press, Beijing, China, pp 333–337

Qi LL, Wang SL, Chen PD, Liu DJ, Friebe B, Gill BS (1997) Molecular cytogenetic analysis of *Leymus recemosus* chromosomes added to wheat. Theor Appl Genet 95:1084–1091

Qi LL, Gill BS (2001) High-density physical maps reveal that the dominant male-sterile gene *Ms3* is located in a genomic region of low recombination in wheat and is not amenable to map-based cloning. Theor Appl Genet 103:998–1006

Qi LL, Echalier B, Friebe B, Gill BS (2003) Molecular characterization of a set of wheat deletion stocks for using in chromosome bin mapping of ESTs. Funct Integr Genomics 3:39–55

Qi LL, Echalier B, Chao S, Lazo GR, Butler GE, Anderson OD et al (2004) A chromosome bin map of 16, 000 expressed sequence tag loci and distribution of genes among the three genomes of polyploid wheat. Genetics 168:701–712

Qi LL, Friebe B, Zhang P, Gill BS (2007) Homoeologous recombination, chromosome engineering and crop improvement. Chromosome Res 15:3–19

Rajaram S, Mann CE, Ortis Ferrara G, Mujeeb-Kazi A (1983) Adaptation, stability and high yield potential of certain 1B/1R CIMMYT wheats. In: Sakamoto S (ed) Proceedings of 6th international wheat genetic symposium. Plant Germ-Plasm Institute, Kyoto, pp 613–621

Riley R, Chapman V (1958) Genetic control of cytologically diploid behavior of hexaploid wheat. Nature 182:713–715

Röder MS, Korzum V, Wendehake K, Plaschke J, Tixier M-H, Leroy P, Ganal M (1998) A microsatellite map of wheat. Genetics 149:2007–2023

Rozen S, Skaletsky HJ (2000) Primer3 on the WWW for general users and for biologist programmers. In: Krawetz S, Misener S (eds) Methods and protocols: methods in molecular biology. Bioinformatics Humana Press, Totowa, pp 365–386

Sears ER (1952) Misdivision of univalents in common wheat. Chromosoma 4:535–550

Sears ER (1954) The aneuploids of common wheat. Univ Mo Agric Exp Stn Bull 572:1–58

Sears ER (1956) The transfer of leaf rust resistance from *Aegilops umbellulata* to wheat. Brookhaven Symp Biol 9:1–22

Sears ER (1966) Nullisomic-tetrasomic combinations in hexaploid wheat. In: Riley R, Lewis KR (eds) Chromosome manipulations and plant genetics. Oliver and Boyd, Edinburgh, pp 29–45

Sears ER (1977) An induced mutant with homoeologous pairing in common wheat. Can J Genet Cytol 19:585–593

Sears ER, Okamoto M (1958) Intergenomic chromosome relationships in hexaploid wheat. In: Proc of 10th Int Congr Genet, Montreal, Canada 2:258–259

Sears ER, Steinitz-Sears LM (1978) The telocentric chromosomes of common wheat. In: Ramanujam S (ed) Proceedings of 5th international wheat genetics symposium. Ind Soc Gen Plt Breed. New Delhi, India, pp 389–407

Sharma D, Knott DR (1966) The transfer of leaf rust resistance from *Agropyron* to *Triticum* by irradiation. Can J Genet Cytol 8:137–143

Sharma HC, Gill BS (1983) Current status of wide hybridization in wheat. Euphytica 32:17–31

Somers DJ, Isaac P, Edwards K (2004) A high-density microsatellite consensus map of bread wheat (*Triticum aestivum* L.). Theor Appl Genet 109:1105–1114

Sourdille P, Singh S, Cadalen T, Brown-Guedira GL, Gay G, Qi LL, Gill BS, Dufour P, Murigneux A, Bernard M (2004) Microsatellite-based deletion bin system for the establishment of genetic-physical map relationships in wheat (*Triticum aestivum* L.). Funct Int Genomics 4:12–25

Wang SL, Qi LL, Chen PD, Liu DJ, Friebe B, Gill BS (1999) Molecular cytogenetic identification of wheat-*Elymus tsukushiense* introgression lines. Euphytica 107:217–224

Wang XE, Chen PD, Liu DJ, Zhang P, Zhou B, Friebe B, Gill BS (2001) Molecular cytogenetic characterization of *Roegneria ciliaris* chromosome additions in common wheat. Theor Appl Genet 102:651–657

Wang YN, Chen PD, Wang ZT, Liu DJ (1986) Transfer of useful germplasm from *E. giganteus* to common wheat. I. Production of wheat X *E. giganteus* hybrids. J Nanjing Agric Univ 1:10–14

Wang YN, Chen PD, Wang ZT, Liu DJ (1991) Transfer of useful germplasm from *E. giganteus* to common wheat. II. Cytogenetics and scab resistance of backcross derivatives. J Nanjing Agric Univ 14:1–5

Weng YQ, Liu DJ (1989) Morphology, scab resistance and cytogenetics of intergeneric hybrids of *Triticum aestivum* L. with *Roegneria* C. Koch (*Agropyron* species) (in Chinese with English abstract). Scentia Agric Sin 22:1–7

Wood EA, Sebesta EE, Stark KJ (1974) Resistance of 'Gaucho' triticale to *Schizaphis graminum*. Environ Entomol 3:720–721

Zhang P, Friebe B, Lukaszewski AJ, Gill BS (2001) The centromere structure in Robertsonian wheat-rye translocation chromosomes indicates that centric breakage-fusion can occur at different positions within the primary constriction. Chromosome 110:335–344

Zhang QP, Li Q, Wang XE, Wang HY, Lang SP et al (2005) Development and characterization of a *Triticum aestivum-Haynaldia villosa* translocation line T4VS·4DL conferring resistance to wheat spindle streak mosaic virus. Euphytica 145:317–320

Web site References

http://wheat.pw.usda.gov/NSF/project/mapping_data.html