HOMOEOLOGY OF THINOPYRUM JUNCEUM AND ELYMUS RECTISETUS CHROMOSOMES TO WHEAT AND DISEASE RESISTANCE CONFERRED BY THE THINOPYRUM AND ELYMUS CHROMOSOME IN WHEAT

A Paper
Submitted to the Graduate Faculty
of the
North Dakota State University
of Agriculture and Applied Science

By

Rachel Ione McArthur

In Partial Fulfillment of the Requirements
for the Degree of
MASTER OF SCIENCE

Major Department:
Plant Sciences

April 2015

Fargo, North Dakota

North Dakota State University
Graduate School

Title

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MASTER OF SCIENCE

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ABSTRACT

Thirteen common wheat ‘Chinese Spring’ (CS)-*Thinopyrum junceum* addition lines and three common wheat ‘Fukuhokomuji’(Fuku)-*Elymus rectisetus* addition lines were characterized and verified as disomic additions of a *Th. junceum* or *E. rectisetus* chromosome in the wheat backgrounds by fluorescent genomic *in situ* hybridization (FGISH). A1048 contained segregating *E. rectisetus* chromosomes. Seven partial CS-*Th. junceum* amphiploids were identified to combine *Th. junceum* chromosomes with CS chromosomes. Various CS-*Th. junceum* disomic addition lines were determined to contain *Th. junceum* chromosomes in homoeologous groups 1, 2, 4 and 5 by Restriction Fragment Length Polymorphism (RFLP) and storage protein analysis. The disomic addition lines A1026 and A1057 were identified to carry an *E. rectisetus* chromosome in group 1 and A1034 in group 5. A1048 contained *E. rectisetus* chromosomes from groups 1-6. Several *Th. junceum* chromosomes in the addition lines were found to contain genes for resistance to Fusarium head blight.

Keywords: Homoeology, wheat, *Thinopyrum junceum*, *Elymus rectisetus*, restriction fragment length polymorphism, storage protein analysis, and genomic *in situ* hybridization

ACKNOWLEDGMENTS

I would like to thank the members of the Cai and Xu labs, and Dr. Rebekah Oliver for their help in this research. Also I would like to thank Dr. Mark Sorrells, Dr. Shahryar Kianian, and Justin Hegsted for maintaining and generously providing the RFLP probes for this study. This research was supported by the US Wheat & Barley Scab Initiative and North Dakota Wheat Commission.

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FGISH…….....Fluorescent genomic *in situ* hybridization

FITC……..…Fluorescein isothiocyanate

RFLPs…….….Restriction fragment length polymorphisms

SDS-PAGE.…….…Sodium dodecyl sulfate polyacrylamide gel electrophoresis

A-PAGE……..Acid-polyacrylamide gel electrophoresis

HMW……High-molecular-weight glutenin subunits

LMW…….Low-molecular-weight glutenin subunits

FHB……...Fusarium head blight

PIS…….Percentage of infected spike

NIS…….Number of infected spike

SNB……Stagonospora nodorum blotch

INTRODUCTION

Cultivated wheats, including bread wheat (*Triticum aestivum*, 2n=6x=42, genome AABBDD) and durum wheat (T. *turgidium* ssp. *durum*, 2n=4x=28, genome AABB), are allopolyploids that originated from interspecific hybridization of three diploid ancestors followed by spontaneous chromosome doubling (Kihara 1944; Riley et al. 1958; Dvorak et al. 1993; Takumi et al. 1993; Huang et al. 2002). The nature of this specific allopolyploid origin led to a narrow genetic basis of cultivated wheat. This limits the genetic improvement of wheat due to the lack of genetic variability in the primary gene pool. However, allopolyploidy of cultivated wheats and homoeology between wheat A, B, and D genomes provides tremendous genetic flexibility for wheat improvement by exploiting the secondary and tertiary gene pools (Morris and Sears 1967). Over years, the wheat genome has been artificially reshaped and enriched in terms of genomic structure and gene content through chromosome engineering (Riley et al. 1968; Sears 1972, 1983; Shepherd and Islam 1988; Friebe et al. 1996; Xu et al. 2005; Bie et al. 2007; Qi et al. 2007; 2008; Chen et al. 2008; Niu et al. 2011). Genes conferring desirable traits, such as resistance to diseases and insects, tolerance to adverse conditions, and good quality, have been successfully transferred to wheat from its relatives (Riley et al. 1968; Shepherd and Islam 1988; Jiang et al. 1994; Friebe et al. 1996; Cox 1998; Cai et al. 2005; Oliver et al. 2005; Faris et al. 2008; Niu et al. 2011).

Wheat has a large number of relatives. Many genes have been transferred to wheat from its relatives through chromosome additions, substitutions, and translocations (Shepherd and Islam 1988). Disomic wheat-alien chromosome addition and substitution lines contain one pair of alien chromosomes in the wheat background. They usually cannot be used directly in wheat

production and breeding due to linkage drag (undesirable genes) on the alien chromosome and unstable transmission of alien chromosomes (Jiang et al. 1994; Cox 1998; Cai et al. 2005). They are, however, ideal genetic stocks for dissecting the genome of an alien species in the wheat genetic background to identify individual alien chromosomes containing the gene of interest for wheat improvement. Once an alien chromosome carrying the gene of interest is identified, the addition and/or substitution line involving the chromosome can be used as the starting material to incorporate the gene into the wheat genome through chromosome translocation (Friebe et al. 1996; Xu et al. 2005; Qi et al. 2007; 2008; Niu et al. 2011).

Integrating small amounts of alien chromatin containing the gene of interest into the wheat genome through chromosomal translocation is the most effective approach for alien gene transfer (Sears 1983; Jiang et al. 1994; Friebe et al. 1996; Cai et al. 2005; Chen PD et al. 2005; Chen S et al. 2008; Xu et al. 2005; Kuraparthy et al. 2007; Faris et al. 2008; Niu et al. 2011). Small compensating translocations between homoeologous wheat and alien chromosomes are more genetically stable than non-compensating translocations formed between wheat and alien chromosomes without homoeology (Friebe et al. 1994, 2005; Jiang et al. 1994; Cox 1998; Cai et al. 2005). Knowledge of the homoeology between alien and wheat chromosomes is essential to produce compensating translocations for gene transfer from alien species into the wheat genome.

Homoeologous relationships between alien and wheat chromosomes can be determined using morphological and molecular markers (Gale and Miller 1987; Miller and Reader 1987; Forster et al. 1987; Sharp et al. 1989; Chen et al. 1994; Hart 1996; Qi et al. 1997; Kishii et al. 2004, Wang et al. 2010). Among the molecular markers, restriction fragment length polymorphisms (RFLPs) have been considered the most reliable approach to determine the

homoeology between wheat and alien chromosomes (Qi et al. 1997; Kishii et al. 2004). In addition, wheat seed storage proteins, including glutenins and gliadins, have been used as markers to identify wheat and alien chromosomes in homoeologous groups 1 and 6 (Payne et al. 1984; Gupta and Shepherd, 1990; Cai et al. 1998; Xu et al. 2004; 2005). The objectives of the present study were to identify and characterize the *Thinopyrum junceum* (2n=6x=42, genome $J_1J_1J_2J_2EE$) and *Elymus rectisetus* (2n=6x=42, genome StStYYWW) chromosomes added to the wheat genetic backgrounds by FGISH and to determine their homoeology with wheat chromosomes by RFLP and storage protein analyses. Also, we identified the chromosomes of these two wild species that contain genes for resistance to Fusarium head blight (FHB), stem rust (Ug99 races), tan spot, and Stagonospora nodorum blotch (SNB).

Table 1. Wheat-*Th. junceum*/*E. rectisetus* addition lines (AL), amphiploids, and their parents used in this study

Code	Pedigree/description	Chromosomes (2n)	References/sources
D3668	*Th. junceum*	42	France
PI 533028	*E. recticetus*	42	Australia
CS	*T. aestivum* 'Chinese Spring'	42	China
Fuku	*T. aestivum* 'Fukuhokomuji'	42	Japan
AJDAj1	CS-*Th. junceum* AL	44	Charpentier (1992)
AJDAj2	CS-*Th. junceum* AL	44	Charpentier (1992)
AJDAj3	CS-*Th. junceum* AL	44	Charpentier (1992)
AJDAj4	CS-*Th. junceum* AL	44	Charpentier (1992)
AJDAj5	CS-*Th. junceum* AL	44	Charpentier (1992)
AJDAj6	CS-*Th. junceum* AL	44	Charpentier (1992)
AJDAj7	CS-*Th. junceum* AL	44	Charpentier (1992)
AJDAj8	CS-*Th. junceum* AL	44	Charpentier (1992)
AJDAj9	CS-*Th. junceum* AL	44	Charpentier (1992)
AJDAj11	CS-*Th. junceum* AL	44	Charpentier (1992)
HD3505	CS-*Th. junceum* AL	44	Charpentier (1992)
HD3508	CS-*Th. junceum* AL	44	Charpentier (1992)
HD3515	CS-*Th. junceum* AL	44	Charpentier (1992)
AJAP1	Partial CS-*Th. junceum* amphiploid	54	Charpentier (1992)
AJAP2	Partial CS-*Th. junceum* amphiploid	56	Charpentier (1992)
AJAP3	Partial CS-*Th. junceum* amphiploid	56	Charpentier (1992)
AJAP4	Partial CS-*Th. junceum* amphiploid	58	Charpentier (1992)
AJAP7	Partial CS-*Th. junceum* amphiploid	56	Charpentier (1992)
AJAP8	Partial CS-*Th. junceum* amphiploid	58	Charpentier (1992)
AJAP9	Partial CS-*Th. junceum* amphiploid	54	Charpentier (1992)
A1026	Fuku/*E. rectisetus* AL	44	Xue & Wang (1999)
A1034	Fuku/*E. rectisetus* AL	44	Xue & Wang (1999)
A1048*	Fuku/*E. rectisetus* AL	41-44	Xue & Wang (1999)
A1057	Fuku/*E. rectisetus* AL	44	Xue & Wang (1999)

* This line involved multiple *E. rectisetus* chromosomes as addition, substitution, and translocation.

MATERIALS AND METHODS

Plant materials and FHB disease screening

Thirteen disomic common wheat 'Chinese Spring' (CS)-*Th. junceum* addition lines (Charpentier 1992), seven partial CS-*Th. junceum* amphiploids (Charpentier 1992), four common wheat 'Fukuhokumuji' (Fuku)-*E. rectisetus* addition lines (Xue and Wang 1999), and their respective parents CS, *E. recticetus* (PI 533028), and Fuku were included in this study (Table 1). PI 533028 is the original *E. recticetus* accession used for the development of the Fuku-*E. rectisetus* addition lines. The original *Th. junceum* accession used to develop the CS-*Th. junceum* amphiploids and addition lines was not available and the accession D3668 from France was used in this study. Plant materials were grown in the greenhouse over three seasons to evaluate Type II FHB resistance in a randomized complete block design with three replicates. Plants were grown in pots with two plants per genotype in each pot at approximately 27^{o}C, with a photoperiod of 16 hours. Approximately 10 spikes per genotype were inoculated according to the procedure described by Stack et al. (2002). The common wheat varieties CS and Russ were used as susceptible controls and Sumai 3 as a resistant control. The FHB severity was scored in a spike at 14 and 21 days post inoculation. The disease data was analyzed using SAS 8.2 and Fishers protected LSD was used for mean separation between genotypes.

Fluorescent genomic *in situ* hybridization (FGISH)

Fluorescent genomic *in situ* hybridization was performed on mitotic chromosomes to identify *Th. junceum* and *E. recticetus* chromosomes/chromatin in the addition lines and amphiploids as described by Cai et al. (1998). Total genomic DNAs of *Th. elongatum* (2n=14, genome EE) and *E. recticetus* were labeled with Biotin-16-dUTP via nick translation

(Diagnostics Nick Translation Kit, Enzo Diagnostics, Inc., NY, USA) and used as probes for FGISH. The *Th. elongatum* genomic DNA probe was used to detect *Th. junceum* chromosomes/chromatin because both *Th. elongatum* and *Th. junceum* share E genome and J genome in *Th. junceum* is closely related to E genome. Total genomic DNA of CS was sheared by boiling in 0.4 M NaOH for 40–50 min, and used as blocking DNA for FGISH. Fluorescein isothiocyanate (FITC)-conjugated avidin (Vector Laboratories, Inc., CA, USA) was used to detect hybridization of the biotin-labeled probe with *Th. junceum* and *E. recticetus* chromatin (yellow-green fluorescence). Wheat chromatin was counterstained with propidium-iodide (red fluorescence). Slides were mounted in VECTASHIELD antifading medium (Vector Laboratories, Inc.) containing 1 µg/ml propidium iodide for counterstaining. Fluorescein isothiocyanate-conjugated avidin and propidium iodide were excited at 450–490 nm. Photographs were taken with a CCD camera (SPOT RT, Diagnostic Instruments, Inc., MI, USA) under an Olympus BX-51 fluorescence microscope.

DNA extraction, electrophoresis, and Southern blotting

Restriction fragment length polymorphism (RFLP) probes identifying each of the 14 homoeologeous chromosome arms were selected based on previously published linkage data in *Triticeae* species. The RFLP probes used in this study, which are maintained by Dr. Mark Sorrells at Cornell University, were kindly provided by Dr. Shahryar Kianian at North Dakota State University (Table 2). DNA extraction, restriction digestion, and Southern blotting were according to Gill et al. (1991). For each sample, 20-40 ng of genomic DNA was digested individually to completion with one of the restriction enzymes *Eco*RI, *Eco*RV, *Hind*III, *Bam*HI, or *Dra*I. Digested samples were fractionated on a 0.8% agarose gel in NEB buffer and then

transferred to a nylon membrane (Hybond-N+, Amersham). Approximately 15 ng of probe DNA was labeled using the Rediprime II labeling kit (GE Biosciences), with 30 μCi of α^{32}P dCTP in a 20 μL reaction volume. All other procedures including pre-hybridization, hybridization, and washing were carried out according to Gill et al. (1991). Hybidization was performed using 35 x 300 mm glass bottles containing 15 mL of hybridization buffer, incubated at 65^{o}C for 12-16 hours in a hybridization oven. Blots were washed at 65^{o}C in 2X SSPE and 0.5% SDS for 60 minutes, and then exposed on X-ray film for 14 days at -80 ^{o}C. RFLP films were scored for the presence or absence of the bands of interest between parents and the addition lines/amphiploids.

Protein extraction and electrophoresis

The high-molecular-weight (HMW) and low-molecular-weight (LMW) glutenin subunits of the addition lines were analyzed by sodium dodecyl sulfate polyacrylamide gel electrophoresis (SDS-PAGE) as modified by Khan et al. (1989), using a Hoefer SE600 vertical gel apparatus (G.E. Biosciences). Gels were prepared 0.75-mm thick, with the separating gel consisting of 8% total acrylamide, and the stacking gel layer at 5%. Samples were extracted with reducing sample buffer(1% Dithiothreitol, DTT) in 0.0625 M Tris-HCl, pH 6.8, 20% glycerol, 2% SDS, and 0.02% Pyronin-Y tracking dye, and each gel lane was loaded with a 5 μl sample size (Xu et al. 2004). After electrophoresis the SDS-PAGE gels were stained with Coomassie Brilliant Blue G-250 as described by Neuhoff et al. (1988). Gliadin proteins were separated by acid-polyacrylamide gel electrophoresis (A-PAGE) following the procedures of Khan et al. (1989) and described by Xu et al. (2004). Briefly, gliadin proteins were extracted in 70% ethanol and an aliquot of the extract was mixed with an equal volume of loading buffer. Samples were run on 1.5mm A-PAGE gels composed of 7% acrylamide and 0.25% bis-acrylamide dissolved in tank

buffer. The tank buffer was 0.25% aluminum lactate with pH adjusted to 3.1 by addition of lactic acid. Following electrophoresis, gels were stained with Blue Silver stain (Candiano et al. 2004) and photographed.

RESULTS

Chromosome constitutions of the addition lines and partial amphiploids

Fluorescent genomic *in situ* hybridization was performed to detect *Th. junceum* and *E. rectisetus* chromatin in the addition lines and partial amphiploids. Previously, Charpentier (1992) identified the thirteen CS-*Th. junceum* addition lines involved in this study as disomic additions with one pair of *Th. junceum* chromosomes by meiotic chromosome pairing analysis. Here we confirmed the presence of one pair of *Th. junceum* chromosomes in each of the thirteen CS-*Th. junceum* addition lines by FGISH (Fig. 1). Seven partial CS-*Th. junceum* amphiploids contained varied numbers of *Th. junceum* chromosomes in addition to CS chromosomes (Fig. 2). AJAP2 and AJAP3 both had 14 *Th. junceum* and 42 CS chromosomes, making their total chromosomes 2n=56 (Fig. 2b, 2e). However, we were unable to determine the homology of the *Th. junceum* chromosome sets in these two partial amphiploids even though they had the same wheat chromosome set of CS by FGISH. AJAP7 also had 56 chromosomes in total, but it contained 13 *Th. junceum* chromosomes and one whole arm wheat-*Th. junceum* translocated chromosome (Fig. 2c). This partial amphiploid may not be genetically stable because of the heterozygous condition of the translocated chromosome. AJAP1 and AJAP9 comprised 54 chromosomes with 12 from *Th. junceum* and 42 from CS (Fig. 2a, 2d). The other two partial amphiploids, AJAP4 and AJAP8, were identified to have 16 *Th. junceum* and 42 CS chromosomes with 2n=58 (Fig. 2f, 2g). Also, we were unable to determine whether the *Th. junceum* chromosome sets in these two partial amphiploids were different or not according to their FGISH patterns.

A1026, A1034, and A1057 were reported to be disomic Fuku-*E. rectisetus* addition lines by Xue and Wang (1999). In the present study, we identified one pair of *E. rectisetus* chromosomes in these three addition lines by FGISH and confirmed the disomic condition of the *E. rectisetus* chromosome in these addition lines (Fig. 3a-3c). The other one, A1048, apparently segregated for several *E. rectisetus* chromosomes. Out of 34 individual plants analyzed of this line, we identified plants without any *E. rectisetus* chromatin (Fig. 3d) as well as the plants with 1-4 *E. rectisetus* chromosomes by FGISH (Fig. 3e-3h). A telocentric *E. rectisetus* chromosome was observed in some of the individuals investigated (Fig. 3e-3f). One of the 34 plants analyzed had a wheat- *E. rectisetus* translocated chromosome (Fig. 3h). In addition, some of the plants with *E. rectisetus* chromosomes were found to contain 38-40 wheat chromosomes, instead of 42 (Fig. 3e-3h). Thus, A1048 was a mixture of Fuku-*E. rectisetus* addition, substitution, and translocation lines involving multiple *E. rectisetus* chromosomes. Disomic addition and substitution lines with a single *E. rectisetus* chromosome and homozygous Fuku-*E. rectisetus* translocation lines could be identified from the progeny of this segregating line. Speckled hybridizations were observed on some of the Fuku chromosomes in A1048 (Fig. 3e-3h). We performed FGISH in the wheat controls Fuku and CS to determine whether the signals on the Fuku chromosomes in A1048 resulted from cross hybridizations or wheat-*E. rectisetus* translocations. Similar hybridization patterns were observed on the Fuku and CS chromosomes (data not shown), demonstrating cross hybridizations of the *E. rectisetus* genomic DNA probe to wheat chromosomes. These cross hybridizations probably occurred primarily between the repetitive DNA sequences of the wheat and *E. rectisetus* genomes. Increasing the ratio of blocking DNA to probe DNA reduced or eliminated cross hybridizations (Fig. 3a-3d).

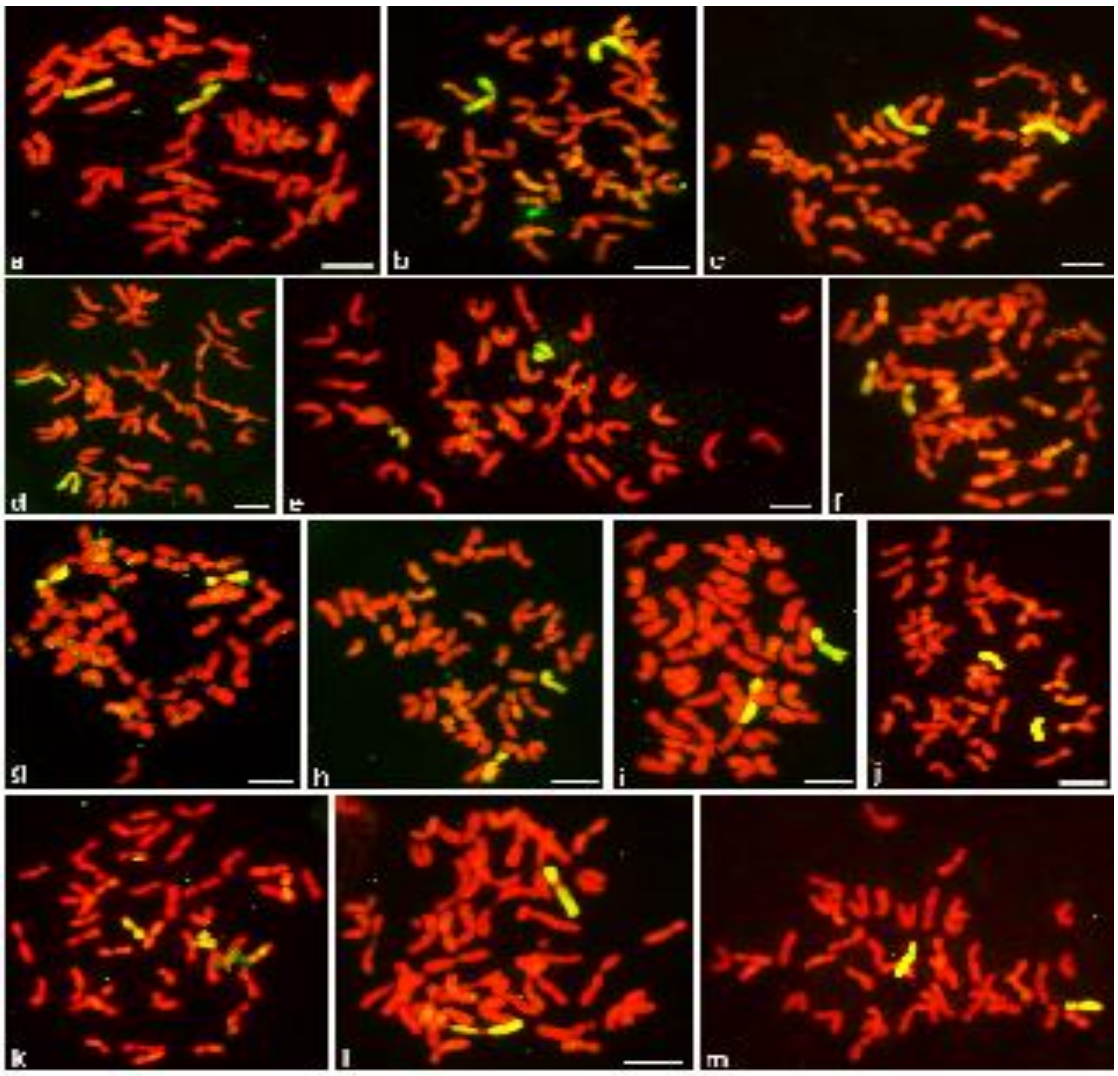

Fig. 1. FGISH patterns of the CS-*Th. junceum* addition lines. **a)** AJDAj1; **b)** AJDAj2; **c)** AJDAj3; **d)** AJDAj4; **e)** AJDAj5; **f)** AJDAj6; **g)** AJDAj7; **h)** AJDAj8; **i)** AJDAj9; **j)** AJDAj11; **k)** HD3505; **l)** HD3508; and **m)** HD3515. Chromosomes in red and yellow-green are wheat and *Th. junceum* chromosomes, respectively. Scale bar = 5 µm.

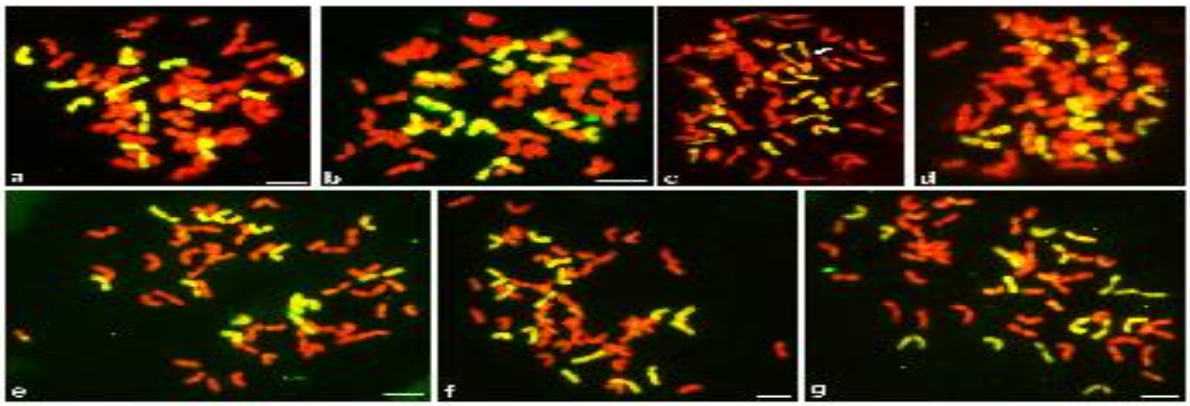

Fig. 2. FGISH patterns of the partial CS-*Th. junceum* amphiploids. **a)** AJAP1; **b)** AJAP2; **c)** AJAP7; **d)** AJAP9; **e)** AJAP3; **f)** AJAP4; and **g)** AJAP8. The arrow indicates a wheat-*Th. junceum* transclocated chromosome. Chromosomes in red and yellow-green are wheat and *Th. junceum* chromosomes, respectively. Scale bar = 5 µm.

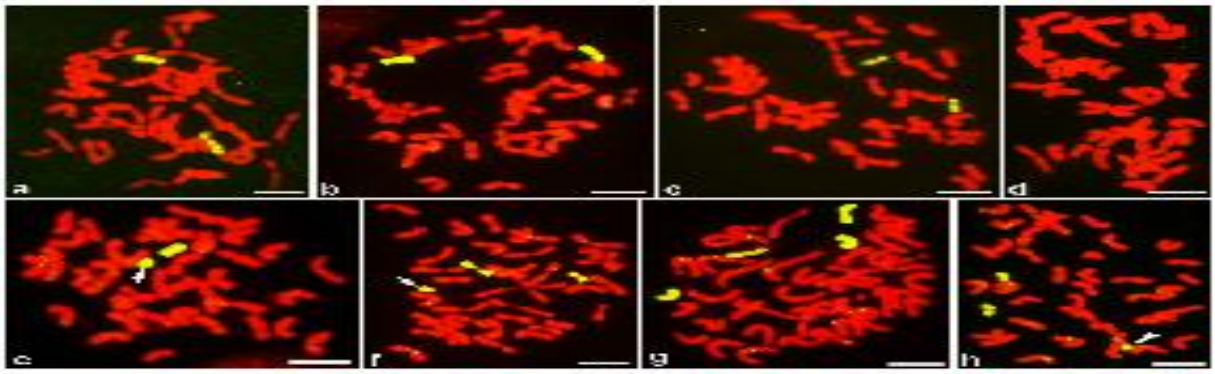

Fig. 3. FGISH patterns of the Fuku-*E. rectisetus* addition/substitution/translocation lines. **a)** A1026; **b)** A1034; **c)** A1057; **d-h)** A1048. Chromosomes in red and yellow-green are wheat and *E. rectisetus* chromosomes, respectively. Arrows point to the *E. rectisetus* telocentric chromosomes and arrow head to the wheat-*E. rectisetus* translocated chromosome. Scale bar = 5 µm.

Homoeology of *Th. junceum* and *E. recticetus* chromosomes to wheat

Restriction fragment length polymorphism analysis was first performed to determine the homoeology of the *Th. junceum* and *E. recticetus* chromosomes in the addition lines and partial amphiploids to wheat. Initially, parental blots with the genomic DNAs of *Th. junceum*, CS, *E. recticetus*, and Fuku digested by five restriction enzymes (*Eco*RI, *Eco*RV, *Hind*III, *Bam*HI, or *Dra*I) were screened with 86 RFLP probes selected from various locations along the 14 homoeologous chromosome arms for polymorphisms between the parental pairs, i.e. CS *vs. Th. junceum* and Fuku *vs. E. recticetus*. Polymorphic probes were then used to screen the blots of the CS-*Th. junceum* and Fuku-*E. recticetus* addition lines, partial amphiploids, and their respective parents. The probes selected for analysis had insert sizes ranging from 600 bp to 1800 bp. Unique fragments present or absent specifically in the *Th. junceum* and *E. recticetus* parents were analyzed to determine the homoeology of the individual *Th. junceum* and *E. recticetus* chromosomes in the addition lines and partial amphiploids with wheat.

Bread wheat, *Th. junceum*, and *E. recticetus* are all allo-hexaploids with three homoeologous sub-genomes. Generally, a RFLP probe detects three fragments in the wheat, *Th. junceum, E. recticetus* genome. Six of the 29 probes used in this study, including BCD110 (4L), BCD1087 (5L), BCD1381 (5L), CDO270 (6S), BCD276 (6L), and CDO551 (7L), detected a *Th. junceum*- or *E. recticetus*-specific fragment only in one of the addition lines. The rest of the 29 probes each detected a *Th. junceum*- or *E. recticetus*-specific fragment in more than one addition line, suggesting those addition lines contain a *Th. junceum* or *E. recticetus* chromosome or chromosomal fragment from the same homoeologous group (Table 2).

Table 2. RFLP analysis of the *Th. junceum* and *E. rectisetus* chromsomes in the addition lines*

Probes	Group	AJDAj1	AJDAj2	AJDAj3	AJDAj4	AJDAj5	AJDAj6	AJDAj7	AJDAj8	AJDAj9	AJDAj11	HD3505	HD3508	HD3515	A1026	A1034	A1048	A1057
BCD98	1S	-	-	-	-	-	-	-	-	-	-	-	-	-	+	-	+	+
BCD371	1S	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-
BCD1072	1S	-	-	-	-	+	-	+	-	+	-	-	+	-	-	-	+	+
BCD386	1L	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-
BCD446	1L	-	-	-	-	-	-	-	+	-	-	-	-	-	-	-	+	+
BCD454	1L	-	-	-	-	-	-	+	+	+	-	-	-	-	~	~	~	~
CDO393	1L	-	-	-	-	-	-	+	+	+	-	-	-	-	+	-	-	-
BCD120	2S	-	+	+	-	-	-	-	-	-	-	-	-	-	~	~	~	~
BCD433	2S	-	+	+	+	-	-	-	-	-	-	-	-	-	-	-	-	-
BCD855	2S	-	+	+	+	-	-	-	-	-	-	-	-	-	-	-	-	-
BCD111	2L	-	+	-	-	-	-	-	-	-	-	-	-	+	-	-	+	-
BCD1278	3S	-	-	-	-	-	-	-	+	-	-	-	-	-	-	-	+	-
BCD147	3L	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	+	-
BCD589	3L	-	+	+	-	-	-	-	-	-	-	-	-	-	~	~	~	~
BCD110	4L	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	+	-
BCD734	4L	+	+	-	-	-	-	-	-	-	-	-	-	-	~	~	~	~
BCD1262	4L	-	-	-	-	-	-	-	-	-	+	+	-	-	-	-	+	-
CDO542	4S	-	-	-	-	-	-	+	+	+	-	-	-	-	-	-	-	-
BCD9	5S	~	~	~	~	~	~	~	~	~	~	~	~	~	+	+	-	-
CDO344	5S	-	-	-	-	+	+	-	-	-	+	-	-	+	~	~	~	~
BCD1087	5L	-	-	-	-	-	-	-	-	-	+	-	-	-	~	~	~	~
BCD1381	5L	~	~	~	~	~	~	~	~	~	~	~	~	~	-	-	+	-
BCD1821	6S	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-
CDO270	6S	~	~	~	~	~	~	~	~	~	~	~	~	~	-	-	+	-
BCD276	6L	+	-	-	-	-	-	-	-	-	-	-	-	-	~	~	~	~
BCD1860	6L	~	~	~	~	~	~	~	~	~	~	~	~	~	-	-	-	-
BCD385	7S	~	~	~	~	~	~	~	~	~	~	~	~	~	-	-	-	-
BCD1338	7S	~	~	~	~	~	~	~	~	~	~	~	~	~	-	-	-	-
CDO551	7L	-	-	-	-	-	-	-	-	-	-	-	+	-	-	-	-	-

* Note: "+" refers to the presence of the *Th. junceum*- or *E. rectisetus*-specific band; "-" absence of the *Th. junceum*- or *E. rectisetus*-specific band; "~" no data.

Seed storage proteins of the addition lines were analyzed to obtain additional evidence for a better understanding of the homoeology of the *Th. junceum* and *E. recticetus* chromosomes with wheat. Results from the protein analysis consistently supported the RFLP data and provided

new evidence to uncover the homoeology of the alien chromosome with wheat. Following are the detailed homoeology results of the alien chromosomes in the individual addition lines and partial amphiploids revealed by the RFLP and storage protein analyses.

Disomic addition lines AJDAj4, AJDAj6, HD3505, A1034, and A1057

The Southern blot hybridization results of the 17 addition lines with 29 probes localized on 14 homoeologous chromosome arms are included in Table 2. Three of the 13 disomic CS-*Th. junceum* addition lines, i.e. AJDAj4, AJDAj6, and HD3505, were found to contain a *Th. junceum* chromosome detected by the probe from only one homoeologous group (Table 2). The RFLP and FGISH results consistently indicated that AJDAj4, AJDAj6, and HD3505 contained one pair of *Th. junceum* chromosomes in group 2, 5, and 4, respectively. Also, the Fuku-*E. recticetus* addition lines A1034 and A1057 had an *E. recticetus* chromosome detected by the probe from only one homoeologous group, i.e. group 5 for A1034 and group 1 for A1057 (Table 2). Thus, A1034 and A1057 were determined to contain one pair of *E. recticetus* chromosomes within group 5 and 1, respectively. In addition, an *E. rectisetus*-specific LMW subunit of glutenin was identified in A1057, confirming the presence of a group 1 *E. rectisetus* chromosome in this disomic addition line (Fig. 4).

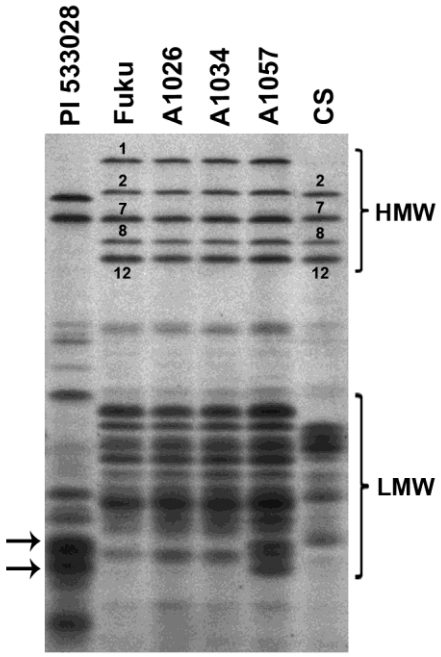

Fig. 4. SDS-PAGE patterns of the HMW and LMW subunits of glutenin. Arrows point to *E. rectisetus*-specific subunits.

All other addition lines, except A1048, were found to contain a *Th. junceum* or *E. recticetus* chromosome detected by the probes from 2-3 different homoeologous groups. FGISH analysis indicated that each of these addition lines contained only one pair of *Th. junceum* or *E. recticetus* chromosomes (Figs. 1 and 3). Thus, non-homologous recombination and/or interchromosomal DNA sequence duplication (Qi et al. 2004) might occur with these *Th. junceum* or *E. recticetus* chromosomes within these two wild species or during the development of the addition lines (Table 2).

Segregating addition, substitution, and translocation line A1048

A1048 was identified to contain multiple *E. recticetus* chromosomes segregating in the Fuku background by FGISH (Fig. 3d-3h). The probes from six homoeologous groups (group 1

through 6) detected *E. recticetus*-specific fragments in A1048, confirming the presence of multiple *E. recticetus* chromosomes in this line (Table 2).

Disomic addition lines AJDAj5, AJDAj7, AJDAj8, AJDAj9, and HD3508

The probe BCD1072 (1S) detected a *Th. junceum*-specific fragment in AJDAj5, AJDAj7, AJDAj9, and HD3508. The fragment detected in AJDAj7, AJDAj9, and HD3508 was different from the one in AJDAj5 (Fig. 5a). These results suggested that AJDAj7, AJDAj9, and HD3508 might contain the same *Th. junceum* chromosome or chromosomal fragment harboring the same *Th. junceum* allele at the *BCD1072* locus within homoeologous group 1. AJDAj5 might contain a *Th. junceum* chromosome or chromosomal fragment from a different sub-genome within the same homoeologous group. However, an additional *Th. junceum*-specific fragment was detected in AJDAj5, AJDAj7, AJDAj9, and HD3508 by the probes from other homoeologous groups, including CDO344 (5S), CDO542 (4S), and CDO551 (7L). Both AJDAj7 and AJDAj9 contained the same *Th. junceum*-specific fragment detected by CDO542 (4S). In addition, AJDAj7 and AJDAj9 showed the same hybridization pattern for all seven probes (BCD98, BCD371, BCD1072, BCD386, BCD446, BCD454, and CDO393) in homoeologous group 1 (Table 2). BCD454 and CDO393 each detected the same *Th. junceum*-specific fragment in AJDAj7 and AJDAj9. Moreover, these seven RFLP markers distribute across the entire chromosome within homoeologous group 1 (Sourdille et al. 2004). Therefore, AJDAj7 and AJDAj9 probably contain the same *Th. junceum* chromosome in homoeologous group 1. The *Th. junceum*-specific fragment detected by the probe CDO542 (4S) in AJDAj7 and AJDAj9 might result from non-homologous recombination between the *Th. junceum* chromosomes in groups 1 and 4 or interchromosomal duplication of the CDO542 locus on the *Th. junceum*

chromosome in group 1. Evidently, the *Th. junceum* chromosomes in AJDAj5 and HD3508 were different from each other and also different from the one in AJDAj7 and AJDAj9. Most likely, AJDAj8 also carried a *Th. junceum* chromosome within group 1 because three group 1 probes (BCD446, BCD454, and CDO393) detected *Th. junceum*-specific fragments in this addition line. The additional *Th. junceum*-specific fragments detected by the probes in group 3 (BCD1278) and group 4 (CDO542) in AJDAj8 could result from non-homologous recombination or interchromosomal duplication of the *Th. junceum* chromosomes involved (Table 2) (Qi et al. 2004).

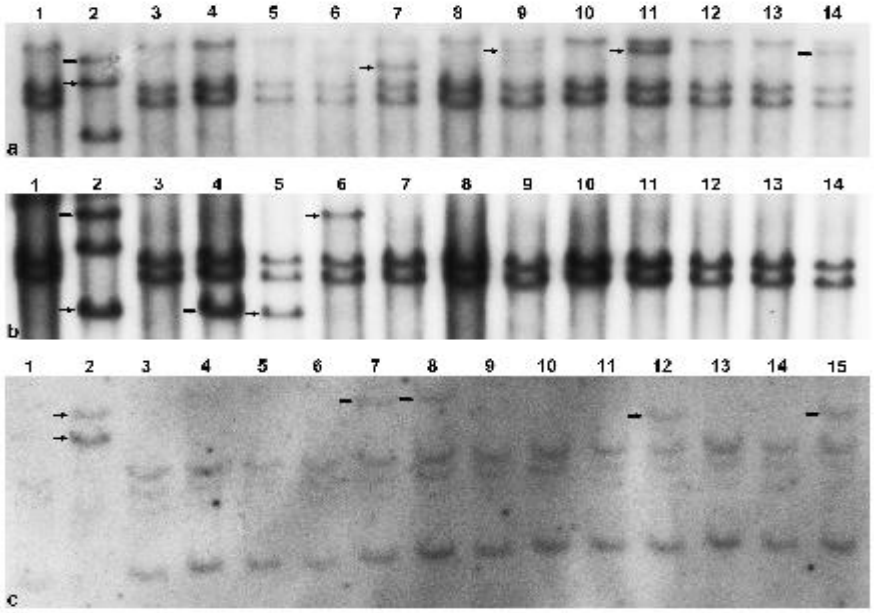

Fig. 5. Southern blot hybridization patterns with the probe/restriction enzyme. **a)** BCD1072 (1S)/*Eco*RV; **b)** BCD855 (2S)/*Eco*RV; **c)** CDO344 (5S)/*Hind*III. **1** – CS; **2** – D3668 (*Th. junceum*); **3** – AJDAj1; **4** – AJDAj2; **5** – AJDAj3; **6** – AJDAj4; **7** – AJDAj5; **8** – AJDAj6; **9** – AJDAj7; **10** – AJDAj8; **11** – AJDAj9; **12** – AJDAj11; **13** – HD3505; **14** – HD3508; **15** – HD3515. Arrows point to the *Th. junceum*-specific bands.

An additional HMW subunit of glutenin was identified in AJDAj7, AJDAj8, and AJDAj9 and an additional LMW subunit was identified in AJDAj5, AJDAj7, AJDAj9, and HD3508. The wheat parent of these addition lines, CS, did not have either of these two subunits (Fig. 6). Both HMW and LMW glutenin subunits are encoded by the genes on the chromosomes in homoeologous group 1 (Payne et al. 1984). Thus, the additional glutenin subunits should be encoded by the genes on the *Th. junceum* chromosome in these five addition lines and the *Th. junceum* chromosomes in these addition lines should belong to homoeologous group 1. These results confirmed the presence of a group 1 *Th. junceum* chromosome in AJDAj5, AJDAj7, AJDAJ8, AJDAj9, and HD3508. Also, the SDS-PAGE patterns of these addition lines suggested that the *Th. junceum* chromosomes in AJDAj7 and AJDAj9 might be the same, but different from the one in AJDAJ5, AJDAj8, and HD3508 (Fig. 6). The homoeologous group of the *Th. junceum* chromosome in AJDAj5 and HD3508 could not be explicitly determined based on the RFLP results because two *Th. junceum*-specific DNA fragments from different homoeologous groups were detected in each of these two lines. Both lines were detected to have a *Th. junceum*-specific DNA fragment from group 1 by RFLP analysis. Here glutenin subunit analysis provided additional evidence for the presence of a group 1 *Th. junceum* chromosome in these two addition lines. Therefore, AJDAj5 and HD3508 probably contain a *Th. junceum* chromosome in group 1. However, the *Th. junceum* chromosome in AJDAj5 was different from the one in HD3508 according to the RFLP results with the probe BCD1072 (Fig. 5a).

Gliadin analysis of the CS-*Th. junceum* addition lines by A-PAGE confirmed the presence of a group 1 *Th. junceum* chromosome in AJDAj5, AJDAj7, AJDAj9, and HD3508. Also, AJDAj7 and AJDAj9 showed the same gliadins encoded by the genes on a group 1 *Th.*

junceum chromosome, confirming the same genomic origin of the *Th. junceum* chromosome in these two addition lines (Fig. 7; Metakovsky 1991). Two gliadins encoded by the *Gli-D1* allele of CS were missing in AJDAj9 (Fig. 7; Metakovsky 1991). This might result from the structural alteration, such as deletion or non-homologous recombination, of the *Gli-D1* locus in CS during the development of this addition line. *Th. junceum*- and *E. rectisetus*-specific gliadins encoded by the genes on the group 6 chromosomes were not identified from all the addition lines.

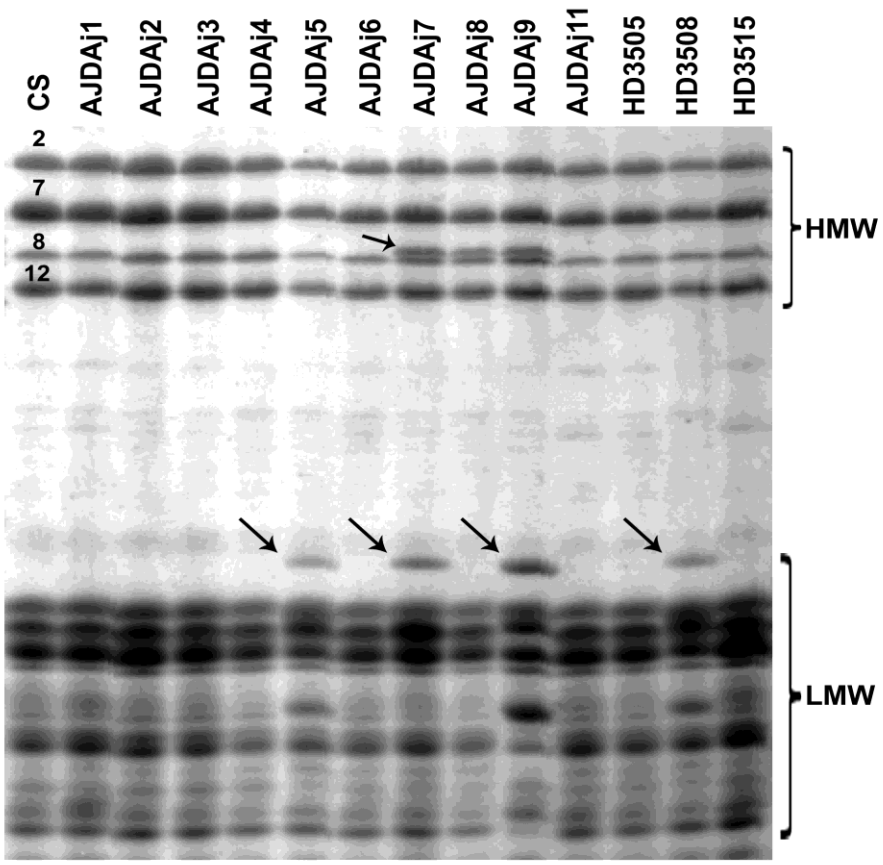

Fig. 6. SDS-PAGE patterns of the HMW and LMW subunits of glutenin. Arrows point to *Th. junceum*-specific subunits.

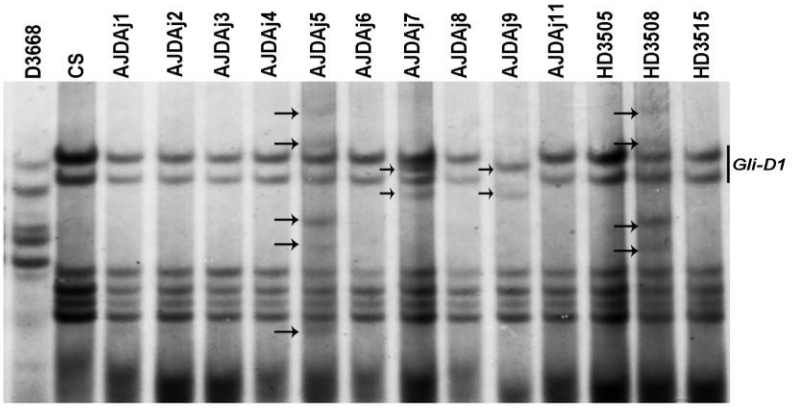

Fig. 7. A-PAGE patterns of gliadins. Arrows point to *Th. junceum*-specific gliadins.

Disomic addition lines AJDAj2, AJDAj3, and AJDAj4

Two probes from homoeologous group 2, BCD433 and BCD855, each detected a *Th. junceum*-specific fragment in AJDAj2, AJDAj3, and AJDAj4. AJDAj2 and AJDAj3 were found to contain the same fragment at both loci, which was different from the one in AJDAj4 (Table 2; Fig. 5b). In addition, all four probes from group 2 (BCD120, BCD433, BCD855, and BCD111) detected a *Th. junceum*-specific fragment in AJDAj2 and three of the four probes detected a *Th. junceum*-specific fragment in AJDAj3. AJDAj4 was found to contain *Th. junceum*-specific fragments only from group 2 (Table 2). Thus, we concluded that AJDAj2, AJDAj3, and AJDAj4 all contained a *Th. junceum* chromosome in group 2. The *Th. junceum* chromosomes in AJDAj2 and AJDAj3 were different from each other and also different from the one in AJDAj4 according to the hybridization patterns of these three addition lines with all the probes (Table 2). The *Th. junceum*-specific fragments detected by the probe BCD589 (3L) in both AJDAj2 and AJDAj3 and the one by BCD734 (4L) in AJDAj2 might result from non-homologous recombination or interchromosomal duplication with the *Th. junceum* chromosomes involved (Qi et al. 2004).

Disomic addition line AJDAj11

The probes on the short and long arms of the group 5 chromosomes, CDO344 (5S) and BCD1087 (5L), each detected a *Th. junceum*-specific fragment in AJDAj11 (Table 2). Most likely, the *Th. junceum* chromosome in AJDAj11 belonged to group 5. The *Th. junceum*-specific fragment detected by BCD1262 (4L) might be due to non-homologous recombination or interchromosomal duplication involving the group 5 and 4 *Th. junceum* chromosomes. The *Th. junceum*-specific fragment detected by CDO344 (5S) in AJDAj11 was different from those detected by the same probe in AJDAj 5 and AJDAj6, indicating the group 5 *Th. junceum* chromosome or chromosomal fragment in AJDAj11 was different from the one in AJDAj5 and AJDAj6 (Fig. 5c).

Disomic addition line A1026

The disomic Fuku-*E. rectisetus* addition line A1026 was detected to have *E. rectisetus*-specific fragments by two group 1 probes BCD98 (1S) and CDO393 (1L) (Table 2). This result suggested that the *E. rectisetus* chromosome in A1026 belonged to homoeologous group 1. The additional *E. rectisetus*-specific fragment detected by the group 5 probe BCD9 (5S) in A1026 probably resulted from non-homologous recombination or interchromosomal duplication involving the group 1 and 5 *E. rectisetus* chromosomes.

Disomic addition lines AJDAj1 and HD3515

Only one *Th. junceum*-specific fragment was detected by the probe from each of two different homoeologous groups in the CS-*Th. junceum* addition lines AJDAj1 and HD3515, i.e. groups 4 and 6 for AJDAJ1 and groups 2 and 5 for HD3515 (Table 2). The homoeologous group of the *Th. junceum* chromosome in these two addition lines could not be explicitly determined

based on the RFLP results. Also, diagnostic results were not obtained for these two addition lines from storage protein analysis. Thus, we were unable to determine the homoeologous group of the *Th. junceum* chromosome in AJDAj1 and HD3515 based on the RFLP and protein analysis results obtained in this study.

Partial amphiploids

Restriction fragment length polymorphism analysis was performed on six partial CS-*Th. junceum* amphiploids, including AJAP2, AJAP3, AJAP4, AJAP7, AJAP8, and AJAP9, with the probes from homoeologous groups 1, 2, and 3. *Th. junceum*-specific fragments were detected by the probes in all six amphiploids, indicating they all contained *Th. junceum* chromosomes or chromosomal fragments in these three homoeologous groups (data not shown).

In conclusion, AJDAj5, AJDAj7, AJDAj8, AJDAj9, and HD3508 all contained a *Th. junceum* chromosome belonging to homoeologous group 1. AJDAj7 and AJDAj9 appeared to contain the same *Th. junceum* chromosome, which was different from the one in the other three addition lines. AJDAj2, AJDAj3, and AJDAj4 all contained a *Th. junceum* chromosome in homoeologous group 2, but the *Th. junceum* chromosomes in these three addition lines were different from each other. Very likely, these three *Th. junceum* chromosomes were from the three *Th. junceum* sub-genomes, i.e. J_1, J_2, and E, respectively. The *Th. junceum* chromosome in HD3505 fell into group 4. Both AJDAj6 and AJDAj11 carried a *Th. junceum* chromosome in group 5 derived from different sub-genomes of *Th. junceum*. AJDAj1 might carry a *Th. junceum* chromosome from group 4 or group 6 and HD3515 from group 2 or group 5. An explicit determination of the homoeology for the *Th. junceum* chromosome in these two addition lines could not be achieved according to the RFLP and glutenin analyses in this study. The Fuku-*E.*

rectisetus addition lines A1026 and A1057 each contained an *E. rectisetus* chromosome in group 1 derived from different *E. rectisetus* sub-genomes. A1034 had an *E. rectisetus* chromosome in group 5. A1048 was a mixture of Fuku-*E. rectisetus* addition, substitution, and translocation lines involving *E. rectisetus* chromosomes from group 1 through 6.

Disease resistance

Two disomic Fuku-*E. rectisetus* addition lines (A1026 and A1034), three partial CS-*Th. junceum* amphiploids (AJAP3, AJAP4, and AJAP7), and three disomic CS-*Th. junceum* addition lines (AJDAj2, AJDAj3, and AJDAj6) exhibited resistance to FHB. Since the spikes of the addition lines and partial amphiploids were morphologically quite different from each other, FHB disease was scored in the percentage of infected spike (PIS) as well as the number of infected spike (NIS). The partial amphiploid AJAP3 showed significantly lower NIS and PIS than its wheat parent CS at three weeks. The other two partial amphiploids AJAP4 and AJAP7 also exhibited significantly lower NIS than CS, but their PIS was marginal at the significance level (Table 3). This indicated that these three partial amphiploids contained the *Th. junceum* chromosomes harboring FHB resistance genes. Of the three resistant disomic CS-*Th. junceum* addition lines, only AJDAj3 showed significantly lower NIS than the wheat parent CS, although all three showed a resistance level similar to Sumai 3 (Table 3). It seemed apparent that the group 2 *Th. junceum* chromosome in AJDAj3 contained the gene(s) for resistance to FHB. The *Th. junceum* chromosome in the other two addition lines (AJDAj2 and AJDAj6) might also carry the FHB resistance gene(s). The disomic Fuku-*E. rectisetus* addition lines A1026 and A1034 both exhibited a resistance level comparable to Sumai 3 (Table 3). However, FHB disease data

was not obtained for the wheat parent, Fuku. Thus, we were unable to determine whether the *E. rectisetus* chromosomes in these two addition lines carried the resistance gene(s) to FHB.

Table 3. Mean FHB severity of Fuku-*E. rectisetus* and CS-*Th. junceum* addition lines and partial CS- *Th. junceum* amphiploids in three greenhouse seasons*

Genotypes	**2WS**	**2W%**	**3WS**	**3W%**
Sumai 3	1.64	9.01	2.61	14.53
Russ	2.89	19.1	4.23	28.76
CS	2.75	13.72	4.04	20.78
A1026	1.19	7.61	1.5	9.72
A1034	1.21	8.11	1.89	12.25
A1048	1.5	9.86	2.49	16.17
A1057	1.78	11.03	3.33	21
AJAP1	-	-	-	-
AJAP2	3.67	15.98	4.67	20.53
AJAP3	1.13	6.08	1.13	6.08
AJAP4	1.31	7.82	1.62	9.56
AJAP7	1.18	7.03	1.55	9.04
AJAP8	2.41	16.24	3.73	24.7
AJAP9	3.14	17.45	4.64	26.88
AJDAj1	2.47	12.52	3.35	16.74
AJDAj2	1.38	8.82	2.09	14.15
AJDAj3	1.58	9.36	1.96	11.86
AJDAj4	2.35	13.33	3.65	20.34
AJDAj5	2.17	13.04	3.39	20.34
AJDAj6	1.6	9.22	2.29	13.03
AJDAj7	3.36	21.74	4	23.95
AJDAj8	2.65	13.34	3.65	18.28
AJDAj9	4.55	23.73	6.97	36.72
AJDAj11	3.3	24.54	4.5	34.19
HD3505	2.29	13.04	3.73	21.05
HD3508	2.95	31.53	4.3	43.13
HD3515	3.91	19.81	4.64	23.7
$LSD_{0.05}$	**1.56**	**9.55**	**2.04**	**12.9**

* 2WS and 3WS refer to average numbers of infected spikelets (NIS) at 2 wk (14 d) and 3 wk (21 d), respectively. 2W% and 3W% refer to average percentage of infected spikelets (PIS) at 2 and 3 wk, respectively.

DISCUSSION

Wheat-alien species addition lines partition the genome of alien species into individual chromosomes in the wheat genetic background. They are important genetic materials for the identification, characterization, and utilization of desirable genes from specific alien chromosomes for wheat improvement. In the present study, we clearly identified the *Th. junceum* and *E. rectisetus* chromosomes and chromosomal segments in the CS-*Th. junceum* partial amphiploids and addition lines and Fuku-*E. rectisetus* addition lines and determined their chromosome constitutions by FGISH. Results from the FGISH analysis confirmed the presence of one pair of *Th. junceum* or *E. rectisetus* chromosome in the addition lines, except A1048 that segregated for multiple *E. rectisetus* chromosomes. These were the groundwork for RFLP analysis and determination of the homoeology of the individual *Th. junceum* and *E. rectisetus* chromosomes in the addition lines. Without confirmation of physical appearance of the alien chromosome in the addition lines, it would be very difficult or even impossible in some cases to interpret molecular marker data and to determine the homoeology of alien chromosomes with wheat. A1048 in the present study was a typical example that demonstrated the importance of understanding chromosome constitution of the material prior to molecular marker analysis. We would not be able to properly interpret the RFLP and protein analysis data if we did not have knowledge of the chromosome constitution of A1048 revealed by FGISH.

Southern blot hybridization has been a reliable method to detect chromosome-specific DNA fragments and to determine the homoeology of alien chromosomes to wheat (Qi et al. 1997; Kishii et al. 2004). *Th. junceum*, *E. rectisetus*, and bread wheat are all allo-hexaploids with three homoeologous sub-genomes. Most of the RFLP probes used in this study detected

three distinct DNA fragments in *Th. junceum*, *E. rectisetus*, and the wheat parents of the addition lines. The original *Th. junceum* accession used for developing the CS-*Th. junceum* addition lines was not available, instead another *Th. junceum* accession (D3668) was employed in this study. High polymorphisms were observed between these two wild species and bread wheat. Also, polymorphic homoeologous alleles were identified at several RFLP loci on the homoeologous *Th. junceum* chromosomes from different sub-genomes, including *BCD1072* (1S), *BCD446* (1L), *BCD433* (2S), *BCD855* (2S), and *CDO344* (5S). These results allowed for the distinction of the *Th. junceum* chromosomes within the same homoeologous group from each other in terms of their sub-genomic origins. In this study, 3-7 RFLP probes distributed on the short and long arms from each of the seven homoeologous groups were used to genotype the *Th. junceum* and *E. rectisetus* chromosomes in the addition lines. The overall Southern blot hybridization patterns of the addition lines with all 29 RFLP probes provided inclusive information to determine the homoeologous relationships of the *Th. junceum* and *E. rectisetus* chromosomes with wheat in most of the addition lines.

Most of the addition lines involved in this study were found to contain *Th. junceum*- or *E. rectisetus*-specific DNA fragments from 2-3 different homoeologous groups by RFLP analysis. Previous studies (Charpentier, 1992; Xue and Wang, 1999) and FGISH analysis in this study indicated each of these addition lines, except A1048, carried only one pair of *Th. junceum* or *E. rectisetus* chromosomes. Thereby, those additional RFLP alleles from other homoeologous groups must result from structural rearrangements between non-homologous chromosomes. About 30% of RFLP loci were observed to be duplicated in einkorn wheat (*T. monococcum* L.) and barley (*Hordeum vulgare* L.) (Dubcovsky et al. 1996). Evidently, interchromosomal

duplications of DNA sequences have widely occurred in the wheat genome (Akhunov et al. 2003; Qi et al. 2004). Interchromosomal structural rearrangements, including translocations and duplications, in the *Th. junceum* and *E. rectisetus* genomes would be expected at least as prevalent as in the wheat genome.

We differentiated native RFLP loci from duplicated or translocated loci to determine the homoeologous groups of the *Th. junceum* or *E. rectisetus* chromosomes in most of the addition lines according to their overall hybridization patterns with all the probes. However, we were unable to determine the native loci in the four disomic CS-*Th. junceum* addition lines (AJDAj1, AJDAj5, HD3508, and HD3515), where two RFLP loci were detected by the probes from two different homoeologous groups. Glutenin and gliadin analysis provided additional information to resolve the RFLP puzzle for two of these four addition lines, i.e. AJDAj5 and HD3508. The native RFLP locus remained undetermined in the other two addition lines (AJDAj1and HD3515) based on the protein analyses because they did not contain a group 1 or group 6 *Th. junceum* chromosome where the genes for glutenins and gliadins reside (Payne et al. 1984; Gupta and Shepherd 1990). We detected two *Th. junceum*-specific fragments from group 4 and 6, respectively, in AJDAj1 by RFLP analysis. Wang et al. (2010) determined that AJDAj1 carried a group 6 *Th. junceum* chromosome using EST-SSR markers. Thus, the RFLP locus we detected from the group 4 *Th. junceum* chromosome was probably a duplicated or translocated locus on the group 6 *Th. junceum* chromosome in this addition line. In addition, Wang et al. (2010) identified HD3515 to contain a group 3 *Th. junceum* chromosome based on the results from the EST-SSR analysis. Our RFLP data, however, showed that HD3515 contained two *Th. junceum*-specific fragments from group 2 and 5, respectively, but not from group 3. Further RFLP

analysis with additional probes may provide a more explicit identification of the *Th. junceum* chromosome in this addition line.

Of the 16 disomic addition lines, only AJDAj7 and AJDAj9 were identified to contain the same *Th. junceum* chromosome according to the RFLP and protein analyses, chromosome morphology, and their reactions to the diseases. All other addition lines contained different *Th. junceum* or *E. rectisetus* chromosomes either from different homoeologous groups or from different sub-genomes within the same homoeologous group. Three partial CS-*Th. junceum* amphiploids (AJAP3, AJAP4, and AJAP7) exhibited significant resistance to FHB. Also, three disomic CS-*Th. junceum* addition lines (AJADj2, AJADj3, and AJADj6) showed resistance to FHB, but their resistance levels were lower than the amphiploids and not significantly different from their wheat parent CS. This demonstrated a quantitative nature of inheritance for FHB resistance conferred by the *Th. junceum* chromosomes in the addition lines and amphiploids. Also, these disease data suggested that some other *Th. junceum* chromosomes, besides the one in AJADj2, AJADj3, and AJADj6, might contain additional genes for FHB resistance.

All the disomic CS-*Th. junceum* addition lines included in this study, except HD3505, exhibited resistance to tan spot and four of them (AJDAj1, AJDAj2, AJDAj3, and AJDAj4) showed moderate resistance to Stagonospora nodorum blotch (SNB) comparing to the resistant and susceptible controls in a previous study by Oliver et al. (2008). However, resistance levels of these addition lines were not significantly higher than their wheat parent CS (Oliver et al. 2008). Therefore, the *Th. junceum* chromosomes in these addition lines might not contain resistance genes to tan spot and SNB. Of the three disomic Fuku-*E. rectisetus* addition lines, A1057 was moderately resistant to both tan spot and SNB and its resistance levels to both

diseases were significantly higher than its wheat parent Fuku (Oliver et al. 2008). This indicated the group 1 *E. rectisetus* chromosome in A1057 contained the gene(s) for resistance to tan spot and SNB. In addition, the disomic CS-*Th. junceum* addition line, HD3505, was moderately resistant to the stem rust Ug99 races and its wheat parent CS was susceptible to the Ug99 races (Xu et al. 2009). Thus, the group 4 *Th. junceum* chromosome in HD3505 contained the resistance gene(s) to Ug99. Determination of the homoeologous relationships of the *Th. junceum* and *E. rectisetus* chromosomes with wheat in this study will facilitate introgression of the disease resistance genes into the wheat genome and utilization in wheat breeding.

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