Molecular genetic description of the cryptic wheat–*Aegilops geniculata* introgression carrying rust resistance genes *Lr57* and *Yr40* using wheat ESTs and synteny with rice

Vasu Kuraparthy, Shilpa Sood, and Bikram S. Gill

Abstract: The cryptic wheat–alien translocation T5DL·5DS-5M^{g}S(0.95), with leaf rust and stripe rust resistance genes *Lr57* and *Yr40* transferred from *Aegilops geniculata* (U^{g}M^g) into common wheat, was further analyzed. Molecular genetic analysis using physically mapped ESTs showed that the alien segment in T5DL·5DS-5M^{g}S(0.95) represented only a fraction of the wheat deletion bin 5DS2-0.78-1.00 and was less than 3.3 cM in length in the diploid wheat genetic map. Comparative genomic analysis indicated a high level of colinearity between the distal region of the long arm of chromosome 12 of rice and the genomic region spanning the *Lr57* and *Yr40* genes in wheat. The alien segment with genes *Lr57* and *Yr40* corresponds to fewer than four overlapping BAC or PAC clones of the syntenic rice chromosome arm 12L. The wheat–alien translocation breakpoint in T5DL·5DS-5M^{g}S(0.95) was further localized to a single BAC clone of the syntenic rice genomic sequence. The small size of the terminal wheat–alien translocation, as established precisely with respect to Chinese Spring deletion bins and the syntenic rice genomic sequence, further confirmed the escaping nature of cryptic wheat–alien translocations in introgressive breeding. The molecular genetic resources and information developed in the present study will facilitate further fine-scale physical mapping and map-based cloning of the *Lr57* and *Yr40* genes.

Key words: wheat, rust resistance, alien introgression, comparative mapping, synteny.

Résumé : La translocation cryptique T5DL·5DS-5M^{g}S(0.95), laquelle confère la résistance à la rouille brune et à la rouille striée suite au transfert des gènes *Lr57* et *Yr40* de l'*Aegilops geniculata* (U^{g}M^g) au blé tendre, a été étudiée. Des analyses moléculaires au moyen d'EST de position connue sur la carte physique ont montré que le segment d'ADN étranger présent au sein de T5DL·5DS-5M^{g}S(0.95) ne représente qu'une fraction de la délétion 5DS2-0.78-1.00 chez le blé et mesurait moins de 3,3 cM sur la carte génétique du blé diploïde. Des analyses génomiques comparées ont indiqué une importante colinéarité entre la région distale du bras long du chromosomes 12 du riz et la région incluant les gènes *Lr57* et *Yr40* chez le blé. Le segment étranger portant les gènes *Lr57* et *Yr40* correspond à une région qui couvre moins de quatre clones BAC/PAC chevauchants chez le bras chromosomique 12L, lequel est synténique chez le riz. Le point de translocation au sein de T5DL·5DS-5M^{g}S(0.95) a été situé dans un seul clone BAC portant la région correspondante chez le riz. La petite taille de la translocation terminale, telle que révélée précisément par l'analyse des délétions chez Chinese Spring et de la région correspondante chez le riz, a de nouveau confirmé la nature élusive des translocations impliquant de la chromatine étrangère suite à des introgressions chez le blé. Les ressources moléculaires et l'information obtenue dans ce travail vont faciliter une cartographie physique plus fine et le clonage positionnel des gènes *Lr57* et *Yr40*.

Mots-clés : blé, résistance à la rouille, introgression de chromatine étrangère, cartographie comparée, synténie.

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Introduction

Wild relatives are an important source of genes for broadening the genetic basis of disease resistance in wheat. Owing to suppressed and restricted homoeologous recombination between the chromosomes of wheat and a wild relative (often referred to as an alien species), the transfer of a target gene is difficult and often accompanied by linked unacceptable traits (linkage drag). Various procedures for chromosome manipulation, generally referred to as "chromosome engineering", have been developed to overcome linkage drag and reduce the size of alien chromosome segments transferred to wheat. The identification and characterization of cytologically undetectable primary recombinants between chromosomes of wheat and alien species with rust resistance suggests that it is possible to achieve transfer of small alien segments without linkage drag (Kuraparthy et al. 2007*a*, 2007*b*).

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Alien transfers to wheat include centric fusions and translocations of variable size. Small wheat–alien translocations, especially terminal exchanges, are the most desirable for transferring foreign traits to wheat (Kuraparthy et al. 2007*a*, 2007*b*) because most of the genes controlling agronomic traits are located in the terminal gene-rich regions of grass chromosomes (Leister et al. 1998; Dilbirligi et al. 2004; Qi et al. 2004). Recombination is unevenly distributed along wheat chromosomes. Ninety percent of the recombination events occur in the distal chromosomal regions (Gill et al. 1993; Lukaszewski and Curtis 1993; Lukaszewski 1995). Furthermore, homoeologous recombination appears to be highly localized, and wheat–alien transfers are most often derived from single crossover events (Luo et al. 2000; Lukaszewski et al. 2003, 2005).

Characterization of a wheat–alien chromosome translocation includes identifying the translocated chromosome, localizing the breakpoint, and estimating the amount of transferred alien chromatin. C-banding has been applied to determine the amount of alien chromatin based on banding polymorphism relative to the standard wheat pattern (Lukaszewski and Gustafson 1983; Lapitan et al. 1984; Friebe and Larter 1988). Genomic in situ hybridization (GISH) using total genomic DNA, in combination with either enzymatic color reactions (Rayburn and Gill 1985) or fluorescent conjugates (Schwarzacher et al. 1989), provides a direct and precise method of physical mapping. However, the resolution of GISH is too low to reveal the presence of some distally located breakpoints (Lukaszewski et al. 2005). DNA marker methods can more precisely identify and characterize small terminal alien introgressions, but the resolution and saturation of the chromosomal regions with molecular markers in existing wheat maps is not adequate (Dubcovsky et al. 1998).

As of December 2006, more than 600 000 wheat expressed sequence tag (EST) sequences representing 128 000 unique transcripts were deposited in public databases (http://www.ncbi.nlm.nih.gov/dbEST/dbEST_summary.html). A set of wheat deletion lines was used to map more than 16 000 EST loci to specific chromosome bins as part of the National Science Foundation's wheat EST project (Qi et al. 2004). The EST sequences and mapping data provide a valuable resource for genome analysis, identifying candidate genes, predicting the biological function of genes, and comparative genomic analyses in wheat.

Comparative mapping studies using restriction fragment length polymorphism (RFLP) markers have revealed extensive synteny or colinearity of gene content and order among the genomes of cereal crops such as rice, wheat, barley, rye, oat, maize, and sorghum (Ahn et al. 1993; Moore et al. 1995; Van Deynze et al. 1995; Keller and Feuillet 2000; Devos and Gale 2000). The conservation of gene order permits the transfer of information from the completely sequenced genome of rice to other grass species. Syntenic relationships of bin-mapped ESTs with the rice genome have been reported in wheat (Sorrells et al. 2003; Conley et al. 2004; Francki et al. 2004; Hossain et al. 2004; La Rota and Sorrells 2004; Linkiewicz et al. 2004; Munkvold et al. 2004; Peng et al. 2004). Colinear regions of rice can be a useful source of markers for saturation and high-resolution mapping of target genes in wheat (Liu and Anderson 2003; Distelfeld et al. 2004; Valárik et al. 2006; Mateos-Hernandez et al. 2005).

Previously, we identified and characterized a wheat–*Aegilops geniculata* translocation line, T5DL·5DS-5M^{g}S(0.95), with the leaf and stripe rust resistance genes *Lr57* and *Yr40* (Kuraparthy et al. 2007*a*). The *Ae. geniculata* segment in T5DL·5DS-5M^{g}S(0.95) was estimated to be less than 3.5% of chromosome arm 5DS (Kuraparthy et al. 2007*a*). In the present study, we report genomic targeting and mapping of the alien chromatin using wheat ESTs and comparative genomic analysis. Our long-term goal is to develop genetic and molecular resources for map-based cloning of the leaf and stripe rust resistance genes *Lr57* and *Yr40*.

Materials and methods

Plant material

Leaf and stripe rust resistant introgression lines were derived by crossing the disomic substitution line DS5M^{g}(5D) with the Chinese Spring (CS) *Ph*^{I} stock and crossing the F_1 with cultivar WL711 (Aghaee-Sarbarzeh et al. 2002). Four BC_2F_5 lines (TA5599, TA5600, TA5601, TA5603) and one BC_3F_6 line (TA5602) resistant to leaf and stripe rust were developed by backcrossing, selecting, and selfing (Kuraparthy et al. 2007*a*). Three rust-resistant wheat–*Ae. geniculata* introgression lines (TA5599 [T5M^{g}S·5M^{g}L-5DL], TA5601 [T5DL·5DS-5M^{g}S(0.75)], and TA5602 [T5DL·5DS-5M^{g}S(0.95)]), the substitution line TA6675 (DS5M^{g}(5D)), the susceptible backcross derivative TA5604, the susceptible backcross parent WL711, the original rust-resistant donor accession (TA10437) of *Ae. geniculata* Roth. ($2n$ = 28, U^{g}U^{g}M^{g}M^{g}), and CS were used for cytogenetic and molecular characterization using ESTs and synteny with rice.

An F_2 population of 118 plants developed from a cross between *Triticum monococcum* L. subsp. *monococcum* (TA4342-96) and *T. monococcum* subsp. *aegilopoides* (Link) Thell. (TA4342-95) was used for genetic mapping of the wheat ESTs and STS (sequence tagged site) markers developed based on wheat–rice synteny.

Molecular and comparative genomic analysis of the wheat–alien translocation in TA5602

Physically mapped wheat ESTs of the deletion bin 5DS2-0.78-1.00 of CS wheat (GrainGenes-SQL database, http://wheat.pw.usda.gov/cgi-bin/westsql/map_locus.cgi) and markers developed based on synteny with rice were used for molecular characterization of the translocation lines. The ESTs and STS markers were mapped by RFLP. DNA isolation and Southern hybridization were conducted as reported in Kuraparthy et al. (2007*c*). DNA of the parents of the diploid wheat mapping populations was digested with 6 restriction enzymes (*Dra*I, *Eco*RI, *Eco*RV, *Hin*dIII, *Sca*I, and *Xba*I) for polymorphism screening.

The terminal region of the short arm of homoeologous group 5 chromosomes is syntenic to the distal region of rice chromosome arm 12L (Sorrells et al. 2003; La Rota and Sorrells 2004) and was targeted for comparative genomic analysis. Thirty-one unique wheat EST sequences that mapped in deletion bin 5DS2-0.78-1.00 were used to query the rice genome databases available at the Rice Genome An-

Table 1. Characterization of introgression lines using physically mapped ESTs.

		Wheat–*Ae. geniculata* introgression lines			
EST	Marker	TA6675 (DS5M^g(5D))	TA5599 (T5M^{g}S·5M^{g}L-5DL)	TA5601 (T5DL·5DS-5M^{g}S(0.75))	TA5602 (T5DL·5DS-5M^{g}S(0.95))
BE444854	*XBE444854*	+	+	+	+
BE404135	*XBE404135*	+	+	+	–
BE591279	*XBE591279*	+	+	+	–
BF473571	*XBF473571*	+	+	+	–
BE637485	*XBE637485*	+	+	+	+
BE636954	*XBE636954*	+	+	+	+
BE499184	*XBE499184*	+	+	+	–
BF293016	*XBF293016*	+	+	+	+
BE499835	*XBE499835*	+	+	+	–
BF474606	*XBF474606*	+	+	+	+
BE443842	*XBE443842*	+	+	+	–
BE606637	*XBE606637*	+	+	+	–
BG314328	*XBG314328*	+	+	+	–
BF201102	*XBF201102*	+	+	+	–
BE606535	*XBE606535*	+	+	+	–
BG262914	*XBG262914*	+	+	+	–
BF293305	*XBF293305*	+	+	+	+
BF146054	*XBF146054*	+	+	+	–
BF200555	*XBF200555*	+	+	+	+
TC259123	*XSTS-5S2*	+	+	+	–
TC238022	*XSTS-5S11*	+	+	+	–

Note: "+" and "–" indicate the presence and absence of diagnostically polymorphic bands between wheat and chromosome 5M^g of *Ae. geniculata*.

notation Project (http://tigrblast.tigr.org/euk-blast/index.cgi?project=osa1) and Gramene (http://www.gramene.org/Multi/blastview) by BLASTn (Altschul et al. 1997) and tBLASTx (Ware et al. 2002) searches. Sequences in the target region of rice chromosome arm 12L were also used as queries in BLASTn and tBLASTx searches of the wheat EST databases. The Institute for Genomic Research (TIGR) wheat gene index (TaGI) release 10.0 (http://compbio.dfci.harvard.edu/cgi-bin/tgi/gimain.pl?gudb=wheat) was used to assess the level of synteny and homology of the unmapped wheat ESTs with the syntenic rice genomic sequences physically spanning the *Lr57–Yr40* genomic region. Wheat EST sequences with high levels of homology (*E* values less than e–15) to sequences selected from the corresponding region of rice chromosome arm 12L were used to design primers for EST-based STS markers. Primer design was done with Primer3 software (Rozen and Skaletsky 2000), and amplicons of 160–400 bp were targeted.

In the BLASTn searches, a significant match was declared when there was at least 65% nucleotide identity for at least half of the query sequence but not less than 150 bases, and an *E* value of less than e–20. For tBLASTx searches, significance was declared when there was at least 50% amino acid identity over at least half of the TC (tentative consensus) or EST sequence and an *E* value of less than –11. Whenever there were several significant matches for a single predicted rice gene sequence, only the best match was reported.

Primer design, polymerase chain reaction (PCR) amplification, plasmid cloning, and RFLP probe development of the STS markers were as described in Kuraparthy et al. (2007*d*). MAPMAKER (Lander et al. 1987) version 2.0 for Macintosh was used to calculate linkage distances using the Kosambi mapping function (Kosambi 1944) with a logarithm of odds (LOD) threshold of 3.00.

Results

Characterization of wheat–*Ae. geniculata* introgression lines using ESTs

ESTs physically mapped in CS deletion bin 5DS2-0.78-1.00 were used to identify markers that diagnostically tagged the *Ae. geniculata* segment in T5DL·5DS-5M^{g}S(0.95) and assisted in genomic targeting of the alien segment with respect to the deletion bins of wheat and rice genomic sequence. ESTs mapped in the CS deletion bin 5DS2-0.78-1.00 were selected because the alien segment size was physically less than 3.5% of chromosome arm 5DS (Kuraparthy et al. 2007*a*). Of the 31 ESTs used, 12 were either monomorphic or produced multiple bands and 19 were polymorphic between wheat and *Ae. geniculata* with one or more of the 6 restriction enzymes used in the RFLP experiments. All polymorphic EST markers diagnostically identified the *Ae. geniculata* chromatin in translocations T5M^{g}S·5M^{g}L-5DL (TA5599) and T5DL·5DS-5M^{g}S(0.75) (TA5601) (Table 1). This confirmed the previous observation that the *Ae. geniculata* introgression in translocation line TA5601 was at least 25% of wheat chromosome arm 5DS (Kuraparthy et al. 2007*a*) because the ESTs used in the present study were mapped in the distal 19% of wheat chromosome arm 5BS. Only 7 of the 19 polymorphic wheat ESTs diagnostically identified the *Ae. geniculata* chromatin in translocation T5DL·5DS-5M^{g}S(0.95) (TA5602), suggesting that the alien segment was smaller than 19% of chromosome arm 5DS (Table 1, Fig. 1, Fig. 2).

Fig. 1. Molecular and comparative genomic analysis of translocation T5DL·5DS-5M^{g}S(0.95) in TA5602 using mapped ESTs and colinear rice genomic sequence. In both the genetic map and the inferred physical map, markers that diagnostically identify the *Ae. geniculata* segment in T5DL·5DS-5M^{g}S(0.95) are indicated in green and those that could not detect the alien segment are in red. The STS markers were developed based on synteny with rice. In the genetic map, markers that are syntenic to the colinear rice genomic sequence are indicated in bold. Syntenic rice BAC or PAC clones spanning the *Ae. geniculata* segment in T5DL·5DS-5M^{g}S(0.95) are indicated in green fill. The rice BAC clone encompassing the wheat–*Ae. geniculata* translocation breakpoint in T5DL·5DS-5M^{g}S(0.95) is indicated in bold. Wheat–rice syntenic positions are indicated with arrows.

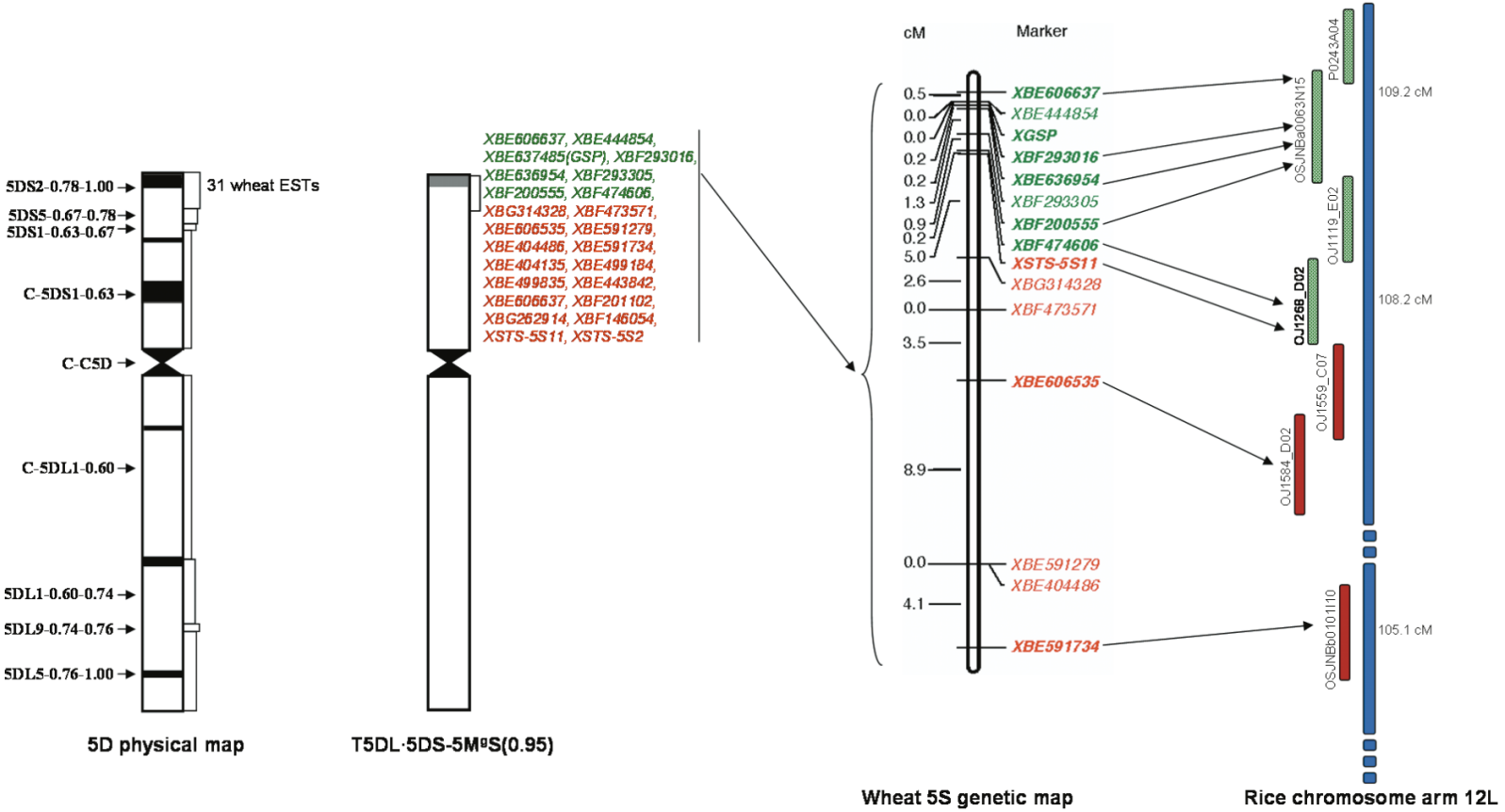
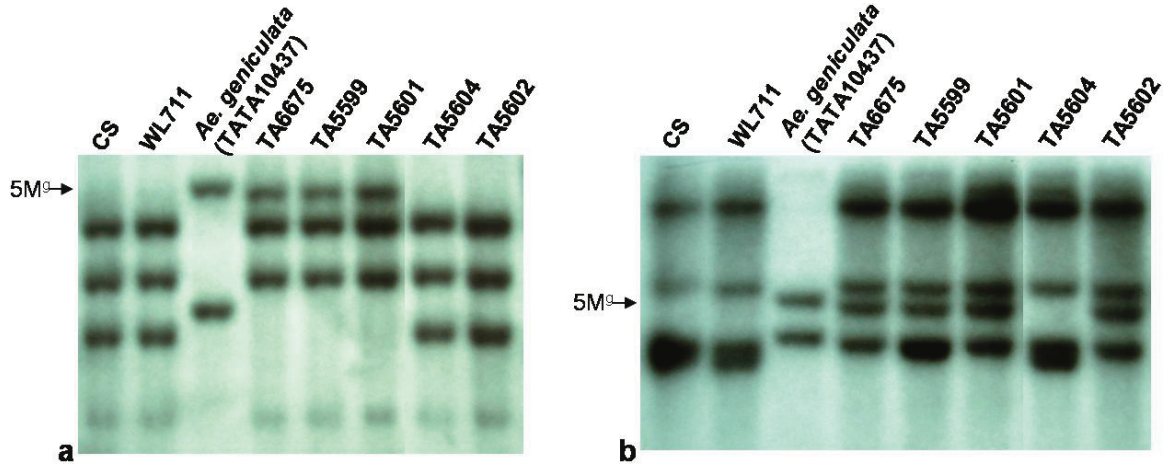


Fig. 2. RFLP analysis of introgression lines using physically mapped ESTs. (*a*) Southern hybridization pattern of *Dra*I-digested genomic DNA of parents and introgression lines probed with BE499835. (*b*) Southern hybridization pattern of *Dra*I-digested genomic DNA of parents and introgression lines probed with BF200555.



The problem of the limited polymorphism available in polyploid wheat was overcome by using diploid wheat, because the A-genome parental species of the mapping population were highly polymorphic (Kuraparthy et al. 2007*c*). Because of the lower ploidy level and less complex DNA hybridization patterns, a greater number of codominant markers could be mapped with a high degree of confidence in *T. monococcum* than was possible in polyploid wheat. Of the 31 ESTs surveyed, 25 were polymorphic between *T. monococcum* subsp. *monococcum* (TA4342-96) and *T. monococcum* subsp. *aegilopoides* (TA4342-95) with one or more restriction enzymes. Only 17 were mapped in the segregating F_2 population, where 15 showed linkage and the polymorphic fragments of 2 EST markers (*XBE443842* and *XBF201102*) (data not shown) were unlinked at a LOD score of 3.0 using the Kosambi mapping function. The genetically mapped EST markers yielded a genetic map of 27.4 cM (Fig. 1). Of the 15 genetically mapped ESTs, only 8 diagnostically detected the *Ae. geniculata* segment in T5DL·5DS-5M^{g}S(0.95) (Fig. 1), suggesting that the alien introgression was only a fraction of the distal deletion bin 5DS2-0.78-1.00. ESTs that diagnostically identified the *Ae. geniculata* segment in T5DL·5DS-5M^{g}S(0.95) mapped distal to those markers that were not diagnostically polymorphic (Fig. 1), indicating that the rust resistance introgression in T5DL·5DS-5M^{g}S(0.95) was terminally located. Genetic mapping further indicated that the ESTs diagnostically identifying the *Ae. geniculata* segment in T5DL·5DS-5M^{g}S(0.95) spanned 3.3 cM of the genetic length in *T. monococcum* (Fig. 1).

Wheat–rice comparative genomic analysis

Comparative genomic analysis using physically or genetically mapped ESTs of deletion bin 5DS2-0.78-1.00 was used to target the rust resistance genes in the alien segment and study the macrocolinearity in the *Lr57* and *Yr40* genomic region. Of the 31 unique wheat ESTs previously mapped in deletion bin 5DS2-0.78-1.00 (Qi et al. 2004), 12 (38.7%) had significant homology to sequences in the terminal region of rice chromosome 12 (Table 2, Fig. 1), 7 had no obvious homology to sequences in the rice genome database, and the remaining 12 had significant hits elsewhere in the rice genome (Table 2). For 3 of the 12 ESTs that showed a high level of sequence similarity with genomic sequences of chromosome 12 of rice, the homologous sequences in rice were not annotated because no predicted function was assigned (Table 2, Table 3).

Of the initial 14 genetically resolved markers, 8 showed high homology with genomic sequence of rice chromosome 12 (Fig. 1, Table 2). The order of the mapped ESTs with high homology to rice chromosome 12 was consistent with the colinear rice sequences on chromosome arm 12L (Fig. 1). Furthermore, the orientation of the telomeric end of wheat chromosome arm 5DS corresponded well with that of the telomeric end of rice chromosome arm 12L (Fig. 1). Five of the 8 ESTs that were colinear with the syntenic rice genomic sequence diagnostically identified the *Ae. geniculata* segment in T5DL·5DS-5M^{g}S(0.95). Three EST markers (*XBE606637*, *XBF293016*, and *XBF200555*) were homologous to rice BAC clone OSJNBa0063N15 and one (*XBE636954*) was homologous to the overlapping region of rice BAC clones OSJNBa0063N15 and OJ1119_E02. The other EST marker, *XBF474606*, showed a high level of homology to rice BAC clone OJ1268_D02 (Fig. 1). Although *XBF200555* mapped proximal to marker *XBE636954* in the genetic map, its homology only to clone OSJNBa0063N15 suggests that the region spanning these two markers could have been rearranged in wheat relative to the syntenic rice sequence (Fig. 1). The most proximal EST marker diagnostically identifying the *Ae. geniculata* segment in T5DL·5DS-5M^{g}S(0.95) showed homology to sequences in clone OJ1268_D02. There were three overlapping syntenic BAC clones distal to OJ1268_D02, and the syntenic region of the alien segment in rice is more than three BAC clones in size (Fig. 1). Hence the wheat–*Ae. geniculata* breakpoint in T5DL·5DS-5M^{g}S(0.95) is located in either OJ1268_D02 or the proximal region of clone OJ1559_C07.

To further localize the TA5602 breakpoint to a specific BAC clone, we selected three coding sequences from OJ1268_D02 and one from OJ1559_C07 to identify wheat ESTs or TCs showing significant homology. Primer pairs were designed for each selected wheat EST or TC (Table 4),

Table 2. Wheat ESTs of deletion bin 5BS6-0.81-1.00 used in the BLASTn and tBLASTx searches of the rice genome sequence, and the similarity level of the wheat ESTs with rice BAC or PAC clones.

		Syntenic relationship with rice					
		E value		BLASTn			
EST or TC	Marker	tBLASTx	BLASTn	Rice BAC or PAC clone(s)	Rice chromosome	Genetic position on chromosome 12 (cM)	Physical position on chromosome 12 (bp)
BE444854	*XBE444854*[a]	1.5e–60	3.4e–162	OSJNBa0064G16	2	NA	NA
BE404135	*XBE404135*	1.2e–62	1.7e–76	P0043B10	1	NA	NA
BE591279	*XBE591279*[a]	6.9e–13	5.3e–21	P0421H07	1	NA	NA
BF473571	*XBF473571*[a]	1.9e–106	5.0e–119	OSJNAb0015J03	10	NA	NA
BE637485	*XBE637485*[a]	No hits	No hits	NA	NA	NA	NA
BE636954[b]	*XBE636954*[a]	5.7e–285	1.2e–273	OSJNBa0063N15	12	109.2	27263817–27386548
			5.8e–273	OJ1119_E02			
BE499184	*XBE499184*	1.0e–52	3.7e–42	OJ1249_F12	2	NA	NA
BF293016[b]	*XBF293016*[a]	2.3e–40	1.9e–41	OSJNBa0063N15, OSJNBb0011N16	12	109.2	27263817–27386548
BE499835	*XBE499835*	2.3e–89	3.0e–96	P0605D08	2	NA	NA
BF474606	*XBF474606*[a]	1.4e–275	3.0e–264	OJ1268_D02	12	~108.2	27137180–27263816
BE443842	*XBE443842*	7.0e–52	1.7e–33	OSJNBb0101I10	12	105.1	26302833–26350254
BE606637[b]	*XBE606637*[a]	2.6e–39	1.5e–53	OSJNBa0063N15, OJ1119_E02, OSJNBb0011N16	12	109.2	27263817–27386548
BG314328	*XBG314328*[a]	8.5e–20	NS	P0436E04	1	NA	NA
BF201102	*XBF201102*	9.4e–218	1.2e–211	OJ1122_G07	12	107.4	26622882–26703968
BE606535	*XBE606535*[a]	3.5e–49	4.9e–45	OJ1584_D02	12	108.2	26967477–27086498
BG262914	*XBG262914*	NS	NS	OSJNBa0091J19	NA	NA	NA
BF293305	*XBF293305*[a]	1.6e–78	3.7e–37	OSJNBa0014K08	1	NA	NA
BF200555	*XBF200555*[a]	1.8e–105	6.3e–91	OJ1300_E01	8	109.2	27263817–27386548
		1.6e–53	6.1e–30	OSJNBa0063N15	12		
BE404486	*XBE404486*[a]	1.9e–103	6.8e–109	OJ1005_A08	5	NA	NA
BE494952	*XBE494952*	NS	NS	OSJNBa0077J22	5	NA	NA
BE403857	*XBE403857*	No hits	No hits	NA	NA	NA	NA
BE499622	*XBE499622*	2.7e–60	1.9e–73	OJ1323_A06	8	NA	NA
BE443751	*XBE443751*	2.5e–17	NS	OSJNBa0016C14	12	90.6	23287763–23434975
BG263064	*XBG263064*	NS	NS	OSJNBb0013K10	9	NA	NA
BG604620	*XBG604620*	4.7	5.7e–28	OJ2056_H01	2	NA	NA
BG312568	*XBG312568*	No hits	NS	OJ1202_D10	NA	NA	NA
BE591734	*XBE591734*[a]	3.7e–46	3.1e–50	OSJNBb0101I10	12	105.1	26302833–26350254
BF474459	*XBF474459*	2.4e–176	9.0e–180	P0498H04	8	NA	NA
BG263797	*XBG263797*	NS	NS	OSJNAa0064E16	NA	NA	NA
TC259123	*XSTS-5S2*	2.6e–132	1.5e–149	OJ1584_D02, OJ1559_C07	12	108.2	26967477–27137179
TC238022	*XSTS-5S11*[a]	1.3e–102	9.4e–113	OJ1268_D02	12	~108.2	27137180–27263816

Note: NA, not applicable; NS, not significant.

[a]Markers used only in the genetic mapping.

[b]Shows very high sequence similarity with rice BAC/PAC sequence, but the homologous sequence in rice was not annotated and no function was predicted.

and PCR products from genomic DNA were cloned, sequenced, and used as probes in the RFLP analysis. Except for *XSTS-5S12*, all STS markers developed were single-copy genes in each wheat genome and were used for molecular characterization and mapping. Marker *XSTS-5S8*, although polymorphic between wheat and *Ae. geniculata*, did not show the *Ae. geniculata*–specific alleles in the substitution line or in all the introgression lines, suggesting that this marker mapped elsewhere in the wheat genome. Although both *XSTS-5S2* and *XSTS-5S11* diagnostically identified *Ae. geniculata*–specific alleles in the translocation lines T5M^{g}S·5M^{g}L-5DL and T5DL·5DS-5M^{g}S(0.75), they could not detect the *Ae. geniculata* segment in T5DL·5DS-5M^{g}S(0.95) (Table 1, Fig. 1). Marker *XSTS-5S11*, developed based on rice gene sequences from OJ1268_D02, genetically mapped immediately proximal to the EST marker *XBF474606* (Fig. 1), which diagnostically identified the *Ae. geniculata* segment in T5DL·5DS-5M^{g}S(0.95). Because *XBF474606* showed significant homology with sequences from OJ1268_D02 (Table 2, Fig. 1) and was the most proximally mapped diagnostic marker in the T5DL·5DS-5M^{g}S(0.95) genetic map (Fig. 1), the wheat–*Ae. geniculata* translocation breakpoint is located in clone OJ1268_D02. Furthermore, because the homologous rice sequences of

Table 3. Annotated rice sequences in the syntenic rice BAC clones that span the alien segment in the translocation T5DL·5DS-5M^{g}S(0.95) and their wheat homologues based on the best BLASTn and tBLASTx hits in the wheat gene indices.

	Syntenic relation with wheat			
		E value		
Locus identifier	Homologous wheat EST or TC[a]	BLASTn	tBLASTx	Putative function
BAC OJ1268_D02				
LOC_Os12g43750	CK195153	2.7e–18	1.4e–08	Expressed protein
LOC_Os12g43770	CV772140[b]	7e–104	2.1e–39	Hypothetical protein
LOC_Os12g43780	CK210549	1.9e–07	2.3	Expressed protein
LOC_Os12g43790	CK207076	9.7e–11	0.20	Ocs element-binding factor 1, putative, expressed
LOC_Os12g43810	TC268335	6.7e–15	No hits	Expressed protein
LOC_Os12g43820	CV779162	1.4e–23	2.0e–09	Expressed protein
LOC_Os12g43830	TC273537	1.3e–69	4.0e–49	Expressed protein
LOC_Os12g43840	TC258118	3.6e–66	2.0e–67	Ankyrin-1, putative, expressed
LOC_Os12g43870	TC269522	4.6e–12	1.1e–10	Hypothetical protein
LOC_Os12g43880	TC241039	3.6e–78	2.5e–84	Expressed protein
LOC_Os12g43890	TC239321	3.9e–31	1.6e–11	GNS1/SUR4 membrane protein, putative, expressed
LOC_Os12g43930	TC267961[b]	2.2e–96	1.1e–79	RING finger protein 5, putative, expressed
LOC_Os12g43940	TC258118	4.6e–89	3.8e–78	Ankyrin repeat and protein kinase domain-containing protein 1, putative, expressed
LOC_Os12g43950	TC252302[b]	1.2e–285	1.0e–179	BEL1-related homeotic protein 30, putative, expressed
LOC_Os12g43960	No hits	—	—	Hypothetical protein
LOC_Os12g43970	TC238022[b]	3.1e–120	3.4e–104	Epoxide hydrolase 2, putative, expressed
LOC_Os12g43990	TC248023	0.00020	9.9	Expressed protein
LOC_Os12g44000	TC266536	5.5e–94	1.4e–69	Ubiquitin-conjugating enzyme E2 W, putative, expressed
BAC OJ1119_E02				
LOC_Os12g44010	No hits	—	—	Purple acid phosphatase precursor, putative, expressed
LOC_Os12g44020	TC254670	2.1e–176	3.2e–119	Purple acid phosphatase precursor, putative, expressed
LOC_Os12g44030	TC254670	5.9e–97	6.7e–105	Purple acid phosphatase precursor, putative, expressed
LOC_Os12g44050	CK209539	3.2e–37	3.0e–24	Purple acid phosphatase precursor, putative, expressed
LOC_Os12g44060	TC268714	2.6e–88	1.6e–69	Nitrate and chloride transporter, putative, expressed
LOC_Os12g44070	TC268714	1.2e–79	5.0e–68	Nitrate and chloride transporter, putative, expressed
LOC_Os12g44080	TC232140	0.00071	7.5e–12	p8MTCP1, putative, expressed
LOC_Os12g44090	TC270164	1.9e–283	1.3e–164	ATP binding protein, putative, expressed (putative receptor like protein kinase-in wheat)
LOC_Os12g44100	TC251798	4.4e–135	5.2e–115	Peptide transporter PTR2, putative, expressed
LOC_Os12g44110	TC251798	5.2e–84	1.1e–89	LigA, putative, expressed
LOC_Os12g44130	TC268048	8.1e–31	1.1e–35	Collagen protein 50
LOC_Os12g44140	TC267879	7.6e–168	2.4e–160	Expressed protein
LOC_Os12g44150	TC248557[b]	0.0	0.0	Plasma membrane ATPase 1, putative, expressed
LOC_Os12g44160	TC273639	2.7e–15	8.4e–15	Oxidoreductase, putative, expressed
LOC_Os12g44170	CK209505	5.2e–80	6.3e–52	ATP binding protein, putative, expressed
LOC_Os12g44180	TC262067	2.0e–95	3.4e–76	Nodulin-like protein, putative, expressed
LOC_Os12g44190	TC235454	3.1e–189	3.6e–172	ATPase 3, putative, expressed
LOC_Os12g44000	TC266536	5.5e–94	1.4e–69	Ubiquitin-conjugating enzyme E2 W, putative, expressed
BAC OSJNBa0063N15				
LOC_Os12g44210	TC235450	1.7e–133	7.6e–126	Cell division protein AAA ATPase family, putative, expressed
LOC_Os12g44220	TC235454	1.8e–101	4.6e–86	Cell division protein AAA ATPase family, putative, expressed
LOC_Os12g44230	TC254862	6.5e–91	1.4e–89	Expressed protein
LOC_Os12g44240	TC267569	1.5e–130	3.8e–98	BGGP beta-1-3-galactosyl-*O*-glycosyl-glycoprotein, putative, expressed
LOC_Os12g44250	TC262074	2.4e–48	4.6e–34	Synaptobrevin family protein, expressed
LOC_Os12g44260	TC240750	1.1e–05	4.2	DnaJ domain containing protein
LOC_Os12g44270	TC262527	1.0e–07	No hits	Glycine-rich cell wall protein precursor, putative
LOC_Os12g44280	TC241432	2.1e–22	2.7	Conserved hypothetical protein

Table 3 (*concluded*).

Locus identifier	Syntenic relation with wheat: Homologous wheat EST or TC[a]	*E* value: BLASTn	*E* value: tBLASTx	Putative function
LOC_Os12g44290	TC271022	7.8e–88	1.3e–96	Cytochrome P450 71D7, putative, expressed
LOC_Os12g44300	TC253012	3.2e–05	4.5e–05	Monovalent cation proton antiporter, putative, expressed
LOC_Os12g44310	TC235550	7.9e–269	5.8e–233	9,10-9,10 carotenoid cleavage dioxygenase 1, putative, expressed
LOC_Os12g44320	TC262267	6.0e–58	5.0e–94	ATP binding protein, putative, expressed
LOC_Os12g44330	TC257773	2.1e–125	7.5e–125	Serine/threonine-protein kinase PRP4, putative, expressed
LOC_Os12g44340	TC276165	1.0e–101	7.4e–47	ATMAP70-2, putative, expressed
LOC_Os12g44350	TC264048	9.4e–197	1.8e–184	Actin-1, putative, expressed
LOC_Os12g44360	TC242797	2.3e–151	0.0	Sodium/hydrogen exchanger 7, putative, expressed
BAC P0243A04				
LOC_Os12g44370	CA658897	1.2e–14	1.3e–12	Expressed protein
LOC_Os12g44380	TC252950	1.2e–133	6.8e–119	Sucrose transport protein SUC4, putative, expressed
LOC_Os12g44390	TC252752	0.0	4.9e–283	TTN8, putative, expressed
LOC_Os12g44360	TC242797	2.3e–151	0.0	Sodium/hydrogen exchanger 7, putative, expressed

[a]Designations of ESTs (GenBank) and TCs (TIGR) as of February 2008.
[b]Closest EST-based STS markers placed in the linkage map.

markers *XBF474606* and *XSTS-5S11* are 9.4 kb apart in OJ1268_D02 (http://rice.plantbiology.msu.edu/cgi-bin/pseudoBAC_view.pl?BAC=OJ1268_D02), the translocation breakpoint in T5DL·5DS-5M^{g}S(0.95) is actually located within this interval (Fig. 1). Consistent with this observation, markers *XBF474606* and *XSTS-5S11*, flanking the translocation breakpoint, were 0.2 cM apart in the wheat genetic map (Fig. 1). Considering the high level of wheat–rice synteny in the *Lr57* and *Yr40* genomic region, all these results suggest that the *Ae. geniculata* introgression is less than 3.3 cM in genetic length and physically spans fewer than 4 overlapping syntenic rice BAC or PAC clones of chromosome arm 12L (Fig. 1). Because the cumulative size of these 4 overlapping syntenic rice BAC or PAC clones is 459 kb, the physical size of the introgressed alien segment is estimated to be at least 459 kb in T5DL·5DS-5M^{g}S(0.95).

Comparative genomic analysis involving similarity searches of the predicted rice gene sequences from the syntenic BAC or PAC clones against the wheat gene index showed that, of the 56 predicted rice genes, 54 had hits in the wheat gene index and 40 showed significant homology with wheat sequences. Of the 54 rice genes that showed hits in the wheat gene index, 42 had known function, 10 were expressed proteins, and 2 were hypothetical proteins (Table 3). This showed that about 74% of the predicted rice genes had significant homologues in wheat, although the physical localization of the corresponding ESTs or tentative contigs in wheat is unknown (Table 3). The putative functions of the rice genes showing high homology with wheat are given in Table 3.

Discussion

The wheat homoeologous group 5 chromosomes were shown to be the least conserved of all the homoeologous groups when compared with rice chromosomes (Sorrells et al. 2003; La Rota and Sorrells 2004). However, close colinearity between wheat and rice in the genomic region spanning the alien segment in translocation T5DL·5DS-5M^{g}S(0.95) was observed in the present study (Table 2, Fig. 1). A similar high level of conserved synteny between wheat and rice at the micro level was also observed in the same genomic region containing the *Ha* locus of wheat (Chantret et al. 2004), but frequent breaks occurred in the colinearity between wheat homoeologous group 5 chromosomes and the rice genome at both the macro and micro levels (Lu and Faris 2006). A high level of wheat–rice microcolinearity was observed in the genomic region containing the wheat *vernalization1* gene (*Vrn1*), enabling map-based cloning of *Vrn1* (Yan et al. 2003). The high level of colinearity observed at the *Ha* locus region (in the present study and by Chantret et al. 2004) and the *Vrn1* region (Yan et al. 2003) and the low level of colinearity at the *Tsn1* region (Lu and Faris 2006) suggest that complex macro- and microcolinearity exists between the wheat homoeologous group 5 chromosomes and the rice genomic sequence.

Genome synteny is more complex than previously thought (for a review, see Delseny 2004). Colinearity among the wheat genomes is higher in the proximal chromosomal regions than in the distal regions (Akhunov et al. 2003*a*). The ends of chromosomes seem to be particularly rich in exceptions to colinearity. Such perturbations in colinearity seem to be associated with higher gene density and higher rates of recombination in the telomeric regions of the large genomes of the Triticeae species (Akhunov et al. 2003*b*). High recombination rates also were associated with a higher frequency of colinearity interruption among wheat homoeologous chromosomes in the distal regions relative to the centromeric regions (Akhunov et al. 2003*a*). In agreement with this general trend at the macro level, most wheat–rice microcolinearity studies have also shown good conservation in the

Table 4. Wheat STS markers developed based on wheat–rice synteny that were used for characterization and genomic targeting of the introgression line T5DL·5DS-5M^{g}S(0.95).

Marker	Source[a]	Forward primer (5′→3′)	Reverse primer (5′→3′)	T_A (°C)	Fragment size (bp)
XSTS-5S2	TC259123	CTTCCAACAGCCGAGATCAT	CTGGTATCTCGCCGTAGAGC	60	202
XSTS-5S8	CV772140	CTTCAGGATGGGCCAGTTTA	GAGCACGAGAAGCCCAATAG	55	175
XSTS-5S11	TC238022	TTGGATGTCGGAGGAAGAAC	GCTTGACTCCAAAGGACTCG	60	197
XSTS-5S12	TC267961	GAGGTGTGCTTCCTCTTTGC	CCCACTCGATCATTCATCCT	60	208

[a]GenBank accession number or TC identifier (TIGR) as of December 2006.

proximal regions of wheat chromosomes (Roberts et al. 1999; SanMiguel et al. 2002; Yan et al. 2003; Distelfeld et al. 2004). Breaks in wheat–rice microcolinearity have been frequently observed in studies involving the distal regions of the wheat genome, such as the *Lrk–Tak* region (Feuillet and Keller 1999), the *Sh2–X1–X2–A1* region (Li and Gill 2002), and the *Rpg1* region (Kilian et al. 1997). Comparative genomic analysis at the whole-genome level between wheat and rice also indicated increased divergence of gene sequences physically located at or near the telomeric ends of wheat chromosomes (See et al. 2006). The *Lr57* and *Yr40* region analyzed in this study, however, does not follow this general pattern and shows good colinearity with rice despite its distal location on chromosome arm 5DS (Fig. 1). Except for the duplication events, conserved wheat–rice microcolinearity at the *Ha* genomic region of wheat also was observed (Chantret et al. 2004). Furthermore, the relative sizes of the intergenic regions in wheat and rice showed good conservation (Chantret et al. 2004), unlike the general trend of considerable expansion of intergenic regions in wheat relative to rice. This surprisingly high level of wheat–rice synteny in the distal region of 5DS is of considerable interest for understanding the biological and evolutionary processes underlying exceptional colinearity among grasses.

Identification of *Ae. geniculata* chromatin in T5DL·5DS-5M^{g}S(0.95) by only a fraction of the 31 physically mapped ESTs and the fact that the alien segment with a genetic length of 3.3 cM mapped distally towards the telomeric end of chromosome arm 5DS suggest that the *Ae. geniculata* segment is a very small terminal alien introgression. This confirms the previous analysis (Kuraparthy et al. 2007*a*) suggesting the size of the *Ae. geniculata* segment in T5DL·5DS-5M^{g}S(0.95) is less than 3.5% of chromosome arm 5DS. The genetic length of 3.3 cM found in the present study corresponds to 561 kb because 1 cM of genetic length at the *Ha* locus region accounts for about 170 kb (Tranquilli et al. 1999; Chantret et al. 2004). The breakpoint of the TA5602 translocation is located in rice BAC clone OJ1268_D02 and the orientation of the telomeric end of wheat chromosome arm 5DS corresponds to that of the telomeric end of rice chromosome arm 12L, indicating that the *Ae. geniculata* segment in T5DL·5DS-5M^{g}S(0.95) corresponds to 4 BAC or PAC clones of rice if we consider the existence of synteny between wheat and rice in this region. Because the syntenic rice contig spans 4 BAC or PAC clones and the total size of these 4 clones (OSJNBa0063N15 = 109.22 kb, P0243A04 = 78.6 kb, BAC OJ1119_E02 = 124.6 kb, and OJ1268_D02 = 146.58 kb) is 459.00 kb, the *Ae. geniculata* segment in T5DL·5DS-5M^{g}S(0.95) corresponds to 459.00 kb of rice sequence. Although the sequenced wheat BAC clone 109N23 (101 kb) corresponded well in terms of size and colinearity with rice BAC clone OSJNBa0063N15 (109.22 kb) in the comparative genomic analysis, Chantret et al. (2004) predicted an approximately 75% increase in the number of genes present in wheat relative to the orthologous region in rice owing to duplication events. Since the genomes of Triticeae species are amplified by the insertion of repetitive sequences and regions colinear to rice are frequently amplified in the Triticeae chromosomes (Li and Gill 2002; SanMiguel et al. 2002), and there is a possibility that the distal end of chromosome 5M^{g} includes segments that are non-colinear with rice chromosome 12, the *Ae. geniculata* segment in T5DL·5DS-5M^{g}S(0.95) could be larger than the predicted 459.00 kb based on syntenic rice BAC clones.

Disease resistance genes are known to evolve faster than other genes (Michelmore and Meyers 1998). In cereals, resistance genes are organized in rapidly reorganizing genomic regions (Leister et al. 1998). Because these rapidly reorganizing regions are in the high-recombination, gene-rich distal regions of wheat chromosomes, the decay in colinearity may limit synteny-based cloning of disease resistance genes in cereals. Three disease resistance genes have been cloned in wheat. No rice genes are homologous to *Lr10* (Feuillet et al. 2003) or *Lr21* (Huang et al. 2003) at the nucleotide level. Rice genes homologous to *Pm3* (Yahiaoui et al. 2004) were located in nonsyntenous regions. No clear candidate gene was identified for barley stem rust resistance gene *Rpg1* (Kilian et al. 1997). We are optimistic that the candidate gene approach could be useful for cloning the rust resistance genes because the genomic region spanning the alien segment with *Lr57* and *Yr40* showed a high level of colinearity between wheat and rice at both macro (present study) and micro levels (Chantret et al. 2004). Within the 4 syntenic rice BAC clones spanning *Lr57* and *Yr40*, at least 2 annotated rice genes coding for protein kinases, which are involved in disease resistance, showed high homology with wheat ESTs (Table 3). A reverse-genetics approach using virus-induced gene silencing in combination with mutagenesis is being used to test the candidate gene approach in cloning the *Lr57* and *Yr40* genes.

A major difficulty in map-based cloning of alien genes in wheat is that the alien chromatin does not recombine with the wheat homoeologues. Consequently, the development of genetic stocks to facilitate the cloning of such genes is difficult. Nevertheless, several methods could be used for molecular cloning of target genes in alien segments transferred to wheat, but they demand considerable time and resources. Recombination between alien translocations and a wheat ho-

moeologous chromosome can be achieved to some extent by inducing recombination in a *ph1* mutant background. Lukaszewski (2000) induced recombination between wheat and rye segments in a *ph1* mutant background, allowing limited mapping of DNA markers and the rust resistance genes on chromosome arm 1RS (Mago et al. 2002). Data from a mapping population involving the 'Petkus' rye T1BL·1RS translocation (*Sr31*, *Lr26*, *Yr9*) and 1R from 'King II' rye (*sr31*, *lr26*, *yr9*) failed to separate the rust resistance loci (Singh et al. 1990). Recombination and mutagenesis established that the rust resistance genes *Sr31*, *Lr26*, and *Yr9* are independent and are located in the distal region of chromosome arm 1RS (Mago et al. 2005). The lack of synteny between wheat and rice in this region and the relatively large deletions posed challenges to cloning of these genes using map-based methods (Mago et al. 2005). Cloning *Lr57* and *Yr40* in TA5602 may be more feasible for the following reasons: (1) the alien segment is a small wheat–alien terminal translocation, (2) the translocated *Ae. geniculata* segment is submicroscopic and estimated to be approximately 0.56 Mb, and (3) wheat–rice synteny is well conserved in the region that spans the alien genes. This should enable the use of rice sequence information for candidate gene analysis and for saturation mapping in the map-based cloning of the resistance genes. Because the total number and organization of rust resistance genes in wild species is unknown, genetic mapping in the wild donor species, combined with use of mutants and candidate gene analysis in the targeted genomic region, could be an efficient alternative for cloning rust resistance genes *Lr57* and *Yr40* in wheat. Such a methodology, where mapping and cloning are done at different ploidy levels, is called "shuttle mapping" and was used successfully to clone leaf rust resistance gene *Lr21* (Huang et al. 2003). We have identified and used a leaf rust susceptible *Ae. geniculata* accession to develop an $F_{2:3}$ population for the molecular genetic mapping of the *Lr57* and *Yr40* genes (our unpublished results).

Most wheat–alien translocations are likely to have limited value to agriculture because of linkage drag. A small wheat–alien terminal translocation with less linkage drag was produced in wheat (Kuraparthy et al. 2007*a*, 2007*b*). Such "cryptic" translocations might be useful for accessing the disease resistance genes in wheat because they are located mostly in the distal regions (Leister et al. 1998; Dilbirligi et al. 2004; Qi et al. 2004) and homoeologous recombination is highly localized towards telomeric ends of the wheat chromosomes (Luo et al. 2000; Lukaszewski et al. 2003, 2005), where wheat–alien transfers are mostly derived from single crossover events (Rogowsky et al. 1993; Qi et al. 2007). Such small alien transfers with disease resistance genes were also detected in rice, where a nonconventional recombination mechanism was postulated to explain the events (Jena et al. 1992). Molecular characterization of the alien segment in T5DL·5DS-5M^{g}S(0.95) using physically and genetically mapped ESTs and targeted genomic mapping with highly syntenic rice genomic sequence might provide evidence for similar events leading to small wheat–alien translocations in wheat.

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References

Aghaee-Sarbarzeh, M., Ferrahi, M., Singh, S., Singh, H., Friebe, B., Gill, B.S., and Dhaliwal, H.S. 2002. *Ph^{I}*-induced transfer of leaf and stripe rust-resistance genes from *Aegilops triuncialis* and *Ae. geniculata* to bread wheat. Euphytica, **127**(3): 377–382. doi:10.1023/A:1020334821122.

Ahn, S., Anderson, J.A., Sorrells, M.E., and Tanksley, S.D. 1993. Homoeologous relationships of rice, wheat and maize chromosomes. Mol. Gen. Genet. **241**(5–6): 483–490. doi:10.1007/BF00279889. PMID:7903411.

Akhunov, E.D., Akhunova, A.R., Linkiewicz, A.M., Dubcovsky, J., Hummel, D., Lazo, G., et al. 2003*a*. Synteny perturbations between wheat homoeologous chromosomes caused by locus duplications and deletions correlate with recombination rates. Proc. Natl. Acad. Sci. U.S.A. **100**(19): 10836–10841. doi:10.1073/pnas.1934431100. PMID:12960374.

Akhunov, E.D., Goodyear, A.W., Geng, S., Qi, L.L., Echalier, B., Gill, B.S., et al. 2003*b*. The organization and rate of evolution of wheat genomes are correlated with recombination rates along chromosome arms. Genome Res. **13**(5): 753–763. doi:10.1101/gr.808603. PMID:12695326.

Altschul, S.F., Madden, T.L., Schäffer, A.A., Zhang, J., Zhang, Z., Miller, W., and Lipman, D.J. 1997. Gapped BLAST and PSI-BLAST: a new generation of protein database search programs. Nucleic Acids Res. **25**(17): 3389–3402. doi:10.1093/nar/25.17.3389. PMID:9254694.

Chantret, N., Cenci, A., Sabot, F., Anderson, O., and Dubcovsky, J. 2004. Sequencing of the *Triticum monococcum hardness* locus reveals good microcolinearity with rice. Mol. Genet. Genomics, **271**(4): 377–386. doi:10.1007/s00438-004-0991-y. PMID:15014981.

Conley, E.J., Nduati, V., Gonzalez-Hernandez, J.L., Mesfin, A., Trudeau-Spanjers, M., Chao, S., et al. 2004. A 2600-locus chromosome bin map of wheat homoeologous group 2 reveals interstitial gene-rich islands and colinearity with rice. Genetics, **168**(2): 625–637. doi:10.1534/genetics.104.034801. PMID:15514040.

Delseny, M. 2004. Re-evaluating the relevance of ancestral shared synteny as a tool for crop improvement. Curr. Opin. Plant Biol. **7**(2): 126–131. doi:10.1016/j.pbi.2004.01.005. PMID:15003211.

Devos, K.M., and Gale, M.D. 2000. Genome relationships: the grass model in current research. Plant Cell, **12**(5): 637–646. doi:10.1105/tpc.12.5.637. PMID:10810140.

Dilbirligi, M., Erayman, M., Sandhu, D., Sidhu, D., and Gill, K.S. 2004. Identification of wheat chromosomal regions containing expressed resistance genes. Genetics, **166**(1): 461–481. doi:10.1534/genetics.166.1.461. PMID:15020436.

Distelfeld, A., Uauy, C., Olmos, S., Schlatter, A.R., Dubcovsky, J., and Fahima, T. 2004. Microcolinearity between a 2-cM region encompassing the grain protein content locus *Gpc-6B1* on wheat chromosome 6B and a 350-kb region on rice chromosome 2. Funct. Integr. Genomics, **4**(1): 59–66. doi:10.1007/s10142-003-0097-3. PMID:14752608.

Dubcovsky, J., Lukaszewski, A.J., Echaide, M., Antonelli, E.F.,

and Porter, D.R. 1998. Molecular characterization of two *Triticum speltoides* interstitial translocations carrying leaf rust and greenbug resistance genes. Crop Sci. **38**: 1655–1660.

Feuillet, C., and Keller, B. 1999. High gene density is conserved at syntenic loci of small and large grass genomes. Proc. Natl. Acad. Sci. U.S.A. **96**(14): 8265–8270. doi:10.1073/pnas.96.14.8265. PMID:10393983.

Feuillet, C., Travella, S., Stein, N., Albar, L., Nublat, A., and Keller, B. 2003. Map-based isolation of the leaf rust disease resistance gene *Lr10* from the hexaploid wheat (*Triticum aestivum* L.) genome. Proc. Natl. Acad. Sci. U.S.A. **100**(25): 15253–15258. doi:10.1073/pnas.2435133100. PMID:14645721.

Francki, M., Carter, M., Ryan, K., Hunter, A., Bellgard, M., and Appels, R. 2004. Comparative organization of wheat homoeologous group 3S and 7L using wheat-rice synteny and identification of potential markers for genes controlling xanthophyll content in wheat. Funct. Integr. Genomics, **4**(2): 118–130. doi:10.1007/s10142-004-0110-5. PMID:15105995.

Friebe, B., and Larter, E.N. 1988. Identification of a complete set of isogenic wheat/rye D-genome substitution lines by means of Giemsa C-banding. Theor. Appl. Genet. **76**(3): 473–479. doi:10.1007/BF00265353.

Gill, K.S., Gill, B.S., and Endo, T.R. 1993. A chromosome region-specific mapping strategy reveals gene-rich telomeric ends in wheat. Chromosoma, **102**(6): 374–381. doi:10.1007/BF00360401.

Hossain, K.G., Kalavacharla, V., Lazo, G.R., Hegstad, J., Wentz, M.J., Kianian, P.M., et al. 2004. A chromosome bin map of 2148 expressed sequence tag loci of wheat homoeologous group 7. Genetics, **168**(2): 687–699. doi:10.1534/genetics.104.034850. PMID:15514045.

Huang, L., Brooks, S.A., Li, W., Fellers, J.P., Trick, H.N., and Gill, B.S. 2003. Map-based cloning of leaf rust resistance gene *Lr21* from the large and polyploid genome of bread wheat. Genetics, **164**(2): 655–664. PMID:12807786.

Jena, K.K., Khush, G.S., and Kochert, G. 1992. RFLP analysis of rice (*Oryza sativa* L.) introgression lines. Theor. Appl. Genet. **84**(84): 608–616. doi:10.1007/BF00224159.

Keller, B., and Feuillet, C. 2000. Colinearity and gene density in grass genomes. Trends Plant Sci. **5**(6): 246–251. doi:10.1016/S1360-1385(00)01629-0. PMID:10838615.

Kilian, A., Chen, J., Han, F., Steffenson, B., and Kleinhofs, A. 1997. Towards map-based cloning of the barley stem rust resistance genes *Rpg1* and *rpg4* using rice as an intergenomic cloning vehicle. Plant Mol. Biol. **35**(1–2): 187–195. doi:10.1023/A:1005768222615. PMID:9291972.

Kosambi, D. 1944. Estimation of map distances from recombination values. Ann. Eugen. **12**: 172–175.

Kuraparthy, V., Chhuneja, P., Dhaliwal, H.S., Kaur, S., Bowden, R.L., and Gill, B.S. 2007*a*. Characterization and mapping of cryptic alien introgression from *Aegilops geniculata* with new leaf rust and stripe rust resistance genes *Lr57* and *Yr40* in wheat. Theor. Appl. Genet. **114**(8): 1379–1389. doi:10.1007/s00122-007-0524-2. PMID:17356867.

Kuraparthy, V., Sood, S., Chhuneja, P., Dhaliwal, H.S., Kaur, S., Bowden, R.L., and Gill, B.S. 2007*b*. A cryptic wheat–*Aegilops triuncialis* translocation with leaf rust resistance gene *Lr58*. Crop Sci. **47**(5): 1995–2003. doi:10.2135/cropsci2007.01.0038.

Kuraparthy, V., Sood, S., Dhaliwal, H.S., Chhuneja, P., and Gill, B.S. 2007*c*. Identification and mapping of a tiller inhibition gene (*tin3*) in wheat. Theor. Appl. Genet. **114**(2): 285–294. doi:10.1007/s00122-006-0431-y. PMID:17115129.

Kuraparthy, V., Sood, S., and Gill, B.S. 2007*d*. Genomic targeting and mapping of tiller inhibition gene (*tin3*) of wheat using ESTs and synteny with rice. Funct. Integr. Genomics, **8**(1): 33–42. doi:10.1007/s10142-007-0057-4. PMID:17891549.

La Rota, M., and Sorrells, M.E. 2004. Comparative DNA sequence analysis of mapped wheat ESTs reveals the complexity of genome relationships between rice and wheat. Funct. Integr. Genomics, **4**(1): 34–46. doi:10.1007/s10142-003-0098-2. PMID:14740255.

Lander, E.S., Green, P., Abrahamson, J., Barlow, A., Daly, M.J., Lincoln, S.E., et al. 1987. MAPMAKER: an interactive computer package for constructing primary genetic linkage maps of experimental and natural populations. Genomics, **1**(2): 174–181. doi:10.1016/0888-7543(87)90010-3. PMID:3692487.

Lapitan, N.L.V., Sears, R.G., and Gill, B.S. 1984. Translocations and other karyotypic structural changes in wheat × rye hybrids regenerated from tissue culture. Theor. Appl. Genet. **68**(6): 547–554. doi:10.1007/BF00285012.

Leister, D., Kurth, J., Laurie, D.A., Yano, M., Sasaki, T., Devos, K., et al. 1998. Rapid reorganization of resistance gene homologues in cereal genomes. Proc. Natl. Acad. Sci. U.S.A. **95**(1): 370–375. doi:10.1073/pnas.95.1.370. PMID:9419382.

Li, W., and Gill, B.S. 2002. The colinearity of the *Sh2/A1* orthologous region in rice, sorghum and maize is interrupted and accompanied by genome expansion in the Triticeae. Genetics, **160**(3): 1153–1162. PMID:11901130.

Linkiewicz, A.M., Qi, L.L., Gill, B.S., Ratnasiri, A., Echalier, B., Chao, S., et al. 2004. A 2500-locus bin map of wheat homoeologous group 5 provides insights on gene distribution and colinearity with rice. Genetics, **168**(2): 665–676. doi:10.1534/genetics.104.034835. PMID:15514043.

Liu, S., and Anderson, J.A. 2003. Targeted molecular mapping of a major wheat QTL for Fusarium head blight resistance using wheat ESTs and synteny with rice. Genome, **46**(5): 817–823. doi:10.1139/g03-066. PMID:14608398.

Lu, H., and Faris, J.D. 2006. Macro- and microcolinearity between the genomic region of wheat chromosome 5B containing the *Tsn1* gene and the rice genome. Funct. Integr. Genomics, **6**(2): 90–103. doi:10.1007/s10142-005-0020-1. PMID:16372189.

Lukaszewski, A.J. 1995. Physical distribution of translocation breakpoints in homoeologous recombination induced by the absence of the *Ph1* gene in wheat and triticale. Theor. Appl. Genet. **90**(5): 714–719. doi:10.1007/BF00222138.

Lukaszewski, A.J. 2000. Manipulation of the 1RS·1BL translocation in wheat by induced homoeologous recombination. Crop Sci. **40**: 216–225.

Lukaszewski, A.J., and Curtis, C.A. 1993. Physical distribution of recombination in B-genome chromosomes of tetraploid wheat. Theor. Appl. Genet. **86**(1): 121–127. doi:10.1007/BF00223816.

Lukaszewski, A.J., and Gustafson, J.P. 1983. Translocations and modifications of chromosomes in triticale × wheat hybrids. Theor. Appl. Genet. **64**(3): 239–248. doi:10.1007/BF00303771.

Lukaszewski, A.J., Rybka, K., Korzun, V., Malyshev, S.V., Lapinski, B., and Whitkus, R. 2003. Genetic and physical mapping of homoeologous recombination points involving wheat chromosome 2B and rye chromosome 2R. Genome, **47**(1): 36–45. doi:10.1139/g03-089.

Lukaszewski, A.J., Lapinski, B., and Rybka, K. 2005. Limitations of *in situ* hybridization with total genomic DNA in routine screening for alien introgressions in wheat. Cytogenet. Genome Res. **109**(1-3): 373–377. doi:10.1159/000082422. PMID:15753599.

Luo, M.C., Yang, Z.L., Kota, R.S., and Dvořák, J. 2000. Recombination of chromosomes *3A*^{m} and *5A*^{m} of *Triticum monococcum* with homeologous chromosomes *3A* and *5A* of wheat: the distri-

bution of recombination across chromosomes. Genetics, **154**(3): 1301–1308. PMID:10757771.

Mago, R., Spielmeyer, W., Lawrence, J., Lagudah, S., Ellis, G., and Pryor, A. 2002. Identification and mapping of molecular markers linked to rust resistance genes located on chromosome 1RS of rye using wheat-rye translocation lines. Theor. Appl. Genet. **104**(8): 1317–1324. doi:10.1007/s00122-002-0879-3. PMID: 12582587.

Mago, R., Miah, H., Lawrence, G.J., Wellings, C.R., Spielmeyer, W., Bariana, H.S., et al. 2005. High-resolution mapping and mutation analysis separate the rust resistance genes *Sr31, Lr26* and *Yr9* on the short arm of rye chromosome 1. Theor. Appl. Genet. **112**(1): 41–50. doi:10.1007/s00122-005-0098-9. PMID: 16283230.

Mateos-Hernandez, M., Singh, R.P., Hulbert, S.H., Bowden, R.L., Huerta-Espino, J., Gill, B.S., and Brown-Guedira, G. 2005. Targeted mapping of ESTs linked to the adult plant resistance gene *Lr46* in wheat using synteny with rice. Funct. Integr. Genomics, **6**(2): 122–131. doi:10.1007/s10142-005-0017-9. PMID: 16374594.

Michelmore, R.W., and Meyers, B.C. 1998. Clusters of resistance genes in plants evolve by divergent selection and a birth-and-death process. Genome Res. **8**(11): 1113–1130. PMID:9847076.

Moore, G., Devos, K.M., Wang, Z., and Gale, M.D. 1995. Cereal genome evolution. Grasses, line up and form a circle. Curr. Biol. **5**(7): 737–739. doi:10.1016/S0960-9822(95)00148-5. PMID:7583118.

Munkvold, J.D., Greene, R.A., Bermudez-Kandianis, C.E., La Rota, C.M., Edwards, H., Sorrells, S.F., et al. 2004. Group 3 chromosome bin maps of wheat and their relationship to rice chromosome 1. Genetics, **168**(2): 639–650. doi:10.1534/genetics.104.034819. PMID:15514041.

Peng, J.H., Zadeh, H., Lazo, G.R., Gustafson, J.P., Chao, S., Anderson, O.D., et al. 2004. Chromosome bin map of expressed sequence tags in homoeologous group 1 of hexaploid wheat and homoeology with rice and *Arabidopsis*. Genetics, **168**(2): 609–623. doi:10.1534/genetics.104.034793. PMID:15514039.

Qi, L.L., Echalier, B., Chao, S., Lazo, G.R., Butler, G.E., Anderson, O.D., 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**(2): 701–712. doi:10.1534/genetics.104.034868. PMID:15514046.

Qi, L.L., Friebe, B., Zhang, P., and Gill, B.S. 2007. Homoeologous recombination, chromosome engineering and crop improvement. Chromosome Res. **15**(1): 3–19. doi:10.1007/s10577-006-1108-8. PMID:17295123.

Rayburn, A.L., and Gill, B.S. 1985. Use of biotin-labeled probes to map specific DNA sequences on wheat chromosomes. J. Hered. **76**: 78–81.

Roberts, M.A., Reader, S.M., Dalgliesh, C., Miller, T.E., Foote, T.N., Fish, L.J., et al. 1999. Induction and characterization of *Ph1* wheat mutants. Genetics, **153**(4): 1909–1918. PMID: 10581295.

Rogowsky, P.M., Sorrels, M.E., Shepherd, K.W., and Langridge, P. 1993. Characterisation of wheat-rye recombinants with RFLP and PCR probes. Theor. Appl. Genet. **83**: 489–494.

Rozen, S., and Skaletsky, H.J. 2000. Primer3 on the WWW for general users and for biologist programmers. *In* Bioinformatics methods and protocols: methods in molecular biology. *Edited by* S. Krawetz and H.J. Skaletsky. Humana Press, Totowa, N.J. pp. 365–386.

SanMiguel, P.J., Ramakrishna, W., Bennetzen, J.L., Busso, C.S., and Dubcovsky, J. 2002. Transposable elements, genes and recombination in a 215-kb contig from wheat chromosome $5A^m$. Funct. Integr. Genomics, **2**(1–2): 70–80. doi:10.1007/s10142-002-0056-4. PMID:12021852.

Schwarzacher, T., Leitch, A.R., Bennett, M.D., and Heslop-Harrison, J.S. 1989. *In situ* localization of parental genomes in a wide hybrid. Ann. Bot. **64**: 315–324.

See, D.R., Brooks, S., Nelson, J.C., Brown-Guedira, G., Friebe, B., and Gill, B.S. 2006. Gene evolution at the ends of wheat chromosomes. Proc. Natl. Acad. Sci. U.S.A. **103**(11): 4162–4167. doi:10.1073/pnas.0508942102. PMID:16537502.

Singh, N.K., Shepherd, K.W., and McIntosh, R.A. 1990. Linkage mapping of genes for resistance to leaf, stem and stripe rusts and ω-secalins on short arm of rye chromosome 1R. Theor. Appl. Genet. **80**(5): 609–616. doi:10.1007/BF00224219.

Sorrells, M.E., La Rota, M., Bermudez-Kandianis, C.E., Greene, R.A., Kantety, R., Munkvold, J.D., et al. 2003. Comparative DNA sequence analysis of wheat and rice genomes. Genome Res. **13**(8): 1818–1827. PMID:12902377.

Tranquilli, G., Lijavetzky, D., Muzzi, G., and Dubcovsky, J. 1999. Genetic and physical characterization of grain texture-related loci in diploid wheat. Mol. Gen. Genet. **262**(4–5): 846–850. doi:10.1007/s004380051149. PMID:10628869.

Valárik, M., Linkiewicz, A.M., and Dubcovsky, J. 2006. A microcolinearity study at the earliness *per se* gene *Eps-A^m1* region reveals an ancient duplication that preceded the wheat–rice divergence. Theor. Appl. Genet. **112**(5): 945–957. doi:10.1007/s00122-005-0198-6. PMID:16432738.

Van Deynze, A.E., Dubcovsky, J., Gill, K.S., Nelson, J.C., Sorrells, M.E., Dvořák, J., et al. 1995. Molecular-genetic maps for group 1 chromosomes of Triticeae species and their relation to chromosomes in rice and oat. Genome, **38**(1): 45–59 doi:10.1139/g95-006.. PMID:18470151.

Ware, D.H., Jaiswal, P., Ni, J., Yap, I.V., Pan, X., Clark, K.Y., et al. 2002. Gramene, a tool for grass genomics. Plant Physiol. **130**(4): 1606–1613. doi:10.1104/pp.015248. PMID:12481044.

Yahiaoui, N., Srichumpa, P., Dudler, R., and Keller, B. 2004. Genome analysis at different ploidy levels allows cloning of the powdery mildew resistance gene *Pm3b* from hexaploid wheat. Plant J. **37**(4): 528–538. doi:10.1046/j.1365-313X.2003.01977.x. PMID:14756761.

Yan, L., Loukoianov, A., Tranquilli, G., Helguera, M., Fahima, T., and Dubcovsky, J. 2003. Positional cloning of the wheat vernalization gene *VRN1*. Proc. Natl. Acad. Sci. U.S.A. **100**(10): 6263–6268. doi:10.1073/pnas.0937399100. PMID:12730378.